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Ferdowsi University of Mashhad,
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P.O. Box: 1793; Postal Code: 9177948974

Tel: +98 51 3880 3742 ***Fax:*** +98 51 3876 3852
Web: ijvst.um.ac.ir ***Email:*** ijvst@um.ac.ir

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Iranian Journal of Veterinary Science and Technology (IJVST) publishes important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, large and small animal medicine, poultry diseases, diseases of equine species, and aquaculture. Articles can comprise research findings in basic sciences, as well as applied veterinary findings and experimental studies and their impact on diagnosis, treatment, and prevention of diseases. IJVST publishes four kinds of manuscripts: Research Article, Review Article, Short Communication, and Case Report.

ON THE COVER

Ringworm appearance of alopecia with mild erythematous lesions above the left eye in a 50-days-old Persian cat due to Microspurum canis infection (Dermatophytosis) which was confirmed by Wood's lamp examination, direct microscopy and culture results.; *see page 53.*

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RESEARCH ARTICLE

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Biochemical and Haematological Evaluation of the Replacement of Ensiled Cassava Pulp with Cocoa Pod in the Diet of West African Dwarf Goats

Christie Oluwatosin Raimi[a], Abiodun Adefunmilayo Adeloye[b]

[a] Department of Agricultural Technology, School of Agriculture and Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria.
[b] Department of Animal Production, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State, Nigeria.

ABSTRACT

This experiment was conducted to evaluate the effects of supplementing cassava pulp with cocoa pod and acacia leaf on the blood metabolites of WAD goats. Twenty-eight WAD bucks aged 5 months with the mean body weight of 7 ± 0.2 kg were used in this completely randomized experiment. The goats were randomly assigned to seven dietary treatments in different ratios of 0:60:40 (T1), 10:50:40 (T2), 20:40:40 (T3), 30:30:40 (T4), 40:20:40 (T5), 50:10:40 (T6), and 60:0:40 (T7) g/kg DM. The collected data were analyzed by the analysis of variance using SPSS. The ob-tained results showed that the highest PCV was obtained from treatment 1 (26.83%), followed by treatments 2 (23.40%) and 3 (22.27%). Haemoglobin concentration was the highest in treatment 1 (11.4 g/dl), followed by treatments 2 (11.15 g/dl) and 3 (10.37 g/dl). At the end of the experiment, there was a sharp decline in the PCV and haemoglobin values of the goats in treatments 5, 6, and 7. RBC values significantly ($p < 0.05$) decreased as the levels of cocoa pod increased. Total protein and albumin had the ranges of 7.23-5 and 3.7-2.1 g/dl, respectively and Total protein were significantly ($p < 0.05$) different among the groups. The hepatic enzymes ALT, ALP, and AST were within the normal range. Our study revealed that supplementing cassava pulp with cocoa pod and acacia leaf at the combinations of 0% cocoa pod, 60% cassava pulp, and 40% acacia leaf to 20% cocoa pod, 40% cassava pulp, and 40% acacia leaf had no negative effects on the blood profile of WAD goats.

Keywords

Cassava pulp, Cocoa pod, Acacia leaf, Haematology, Serum biochemistry, WAD goats

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Abbreviations

WAD: West African Dwarf
PCV: Packed cell volume
RBC: Red blood cell
WBC: White blood cell
Hb: Haemoglobin
MCHC: Mean corpuscular haemoglobin concentration
MCV: Mean corpuscular volume
MCH: Mean corpuscular haemoglobin
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
Ph: Phosphorus

Corresponding author:
Christie Oluwatosin Raimi
Email: taiwo.raimi@eksu.edu.ng
Tel: +234(81)0225-6197

Introduction

The livestock sector plays a significant economic role in most developing countries and is essential for the survival of the population. The productivity of animals is low due to inadequacy and poor

quality of the feeds, which in turn influences the feedintake by the animals. Some agroindustrial byproducts can be processed into valuable livestock feeds, such as cocoa pod and cassava pulp, which can beserved as a substitute for maize in formulating rationsfor chickens, pigs, and small ruminants. However,these ingredients must be included at optimal levels that will not pose any risk to the animal [1].

Cocoa pod contains flavonoids, which are antiox

idants needed by animal for the proper functioing of the heart and brain. The nitrogen content of cocoa and cassava are made of water-soluble alkaloids, namely theobromine, caffeine, and cyanide which can be tolerated by the animals to some extent. Alkaloids exist in byproducts in small quantities. As a result, there is a need to subject the byproducts to different treatments before utilizing them as animals' feed [2]. Cocoapod theobromine can be minimized or removed by chemical or biological means [3]. Cyanide in cassava wastes could also be treated with chemical or physi cal methods, such as sundry or air dried. These prod-ucts are served as panacea to feed challenges becauseof their availability at all seasons [4]. Many browsesare also used as feeds for ruminants due to availability throughout the year [5]. Agro-industrial by products such as cocoa pod and cassava pulp served as panacea to feed challenges because of their availability at all seasons [4]. are readily available at low costs and are accepted for usage by most farmers after thorough processing.

An early study showed that the inclusion of 9%cocoa shell in the diets of lambs/kids stimulated feed intake and growth. However, higher inclusion rates caused a reduction in feed intake and weight gain.

Others observed a reduction in body weight when cocoa shell was included in the daily ration of sheep and goats. This phenomenon was reversed when cocoa materials were excluded from the diet [6]. According to Olugosi [7], the dietary inclusion of biologically up-graded cocoa pod husk (BPCHM) up to 10% supports the performance and stability of the haemato-biochemical indices of broiler chickens. After the delivery of pups, no abnormal litter characteristics or tera togenic effects were observed relative to the control, suggesting further that the feeds with 30% Talaromyces verruculosus-treated cocoa pod substitution had no adverse reproductive or genotoxic effects [8].

Evaluating blood profile may suggest the potentials of dietary treatment to meet the metabolic needs of animals and their effects on blood constituents which help draw conclusion on the nutritive quality of the feeds [9] and the health status of the animals [10]. Mostly, blood profile is influenced by the qualty, quantity, toxicity, and anti-nutritional factors of the feeds [11]. Sometimes, reduction in PCV and haemoglobin suggests feed toxicity which would have effects on blood indices. Likewise, decrease in RBC and PCV might result from low nutritional feed intake or mild anaemia [12]. Generally, the haematological and biochemical indices of an animal suggest their physiological disposition to the nutritional composition of the feeds.The aim of this study was to evaluate the effects of replacing ensiled cassava pulpwith cocoa pod on haematological and serum biochemical profile in WAD goats. We hypothesized that the combinations of cassava pulp and cocoa pod at the optimal level would not pose detrimental effects on animal health.

Result

The results of the haematological parameters are shown in Table 1. All the measured parameters, except monocyte count, were significantly ($p < 0.05$) different between dietary treatments. The results of the serum biochemical indices are summarized in Table 2. The evaluated biochemical parameters, except total cholesterol, albumin, potassium, urea, and creatinine, were significantly ($p < 0.05$) different between treatments. At the end of the experiment, sodium content (136.13 mmol/l) was significantly ($p < 0.05$) higher in the control group compared to other groups, followed by the combination of 10% cocoa pod, 50% cassava pulp, and 40% acacia leaf (Diet 2) (121.17 mmol/l).

On the other hand, the lowest value was found in T7 group with 60% cocoa pod, 0% cassava pulp, and 40% acacia leaf (99.7 mmol/l). Total protein value was higher in the control group (7.23 g/dl) than in the group with the high concentration of cocoa pod group (Diet 7). Creatinine level was higher in T7, which had a high concentration of cocoa pod (2.3 mg/dl) compared to the control group (0.9 mg/dl).

Discussion

The diets in this study caused significant differences ($p < 0.05$) in the blood profile of the animals. Most of the erythrocyte indices remained in the normal range reported for haematological factors by Daramola et al. [12], Blood et al. [13], and Arash [14].

It is believed that haematological indices reveal the physiological state and health status of the animals which help in diagnosing the suspected toxicant in feed given [15].

The results of the analysis showed that PCV val-

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Table 1.
Effect of ensiled combinations of cocoa pod and cassava pulp and acacia leaf on Haematological Parameters of West African Dwarf Goat

Parameters	Range	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
At the start of experimen								
PCV (%)	22-38	26.30^{a} ± 1.20	*22.40^{b} ± 1.35*	21.80^{b} ± 1.73	20.70^{c} ± 1.84	20.30^{d} ± 1.23	19.10^{e} ± 1.35	19.80^{e} ± 0.80
RBC (106/UL)	8-18	7.50^{a} ± 0.89	*6.20^{b} ± 0.65*	6.00^{b} ± 0.57	5.60^{c} ± 0.63	5.20^{c} ± 0.87	5.30^{c} ± 0.76	5.10^{c} ± 0.67
WBC (109/L)	4-13	4.80^{e} ± 0.68	*5.35^{e} ± 0.38*	6.30^{d} ± 0.43	7.60c ± 0.46	8.40^{b} ± 0.56	8.90^{b} ± 0.34	9.30^{a} ± 0.47
HB (g/dl)	7-15	11.70^{a} ± 0.43	*11.20^{a} ± 0.67*	10.50^{b} ± 0.58	9.70c ± 0.45	9.50^{c} ± 0.48	8.90^{c} ± 0.54	7.60^{d} ± 0.75
MCHC (g/L)	30-36	33.20 ± 0.06	*33.20 ± 0.48*	33.40 ± 0.52	33.60 ± 0.66	33.83 ± 0.67	33.85 ± 0.48	33.90 ± 0.53
MCH (pg	5.2-8	12.82^{a} ± 1.04	*12.51^{a} ± 0.23*	11.90^{b} ± 0.83	11.40^{b} ± 0.57	10.81^{c} ± 0.34	10.64^{c} ± 0.83	10.53^{c} ± 0.67
MCV (fl)	16-25	33.72^{a} ± 2.45	*30.14^{b} ± 2.13*	29.56^{c} ± 2.00	29.43^{c} ± 2.02	29.23^{c} ± 2.57	28.64^{d} ± 2.54	27.63^{e} ± 2.56
Lymphocyte (%)	50-70	58.36^{a} ± 2.23	*57.83^{a} ± 2.33*	56.82^{b} ± 2.54	53.85^{c} ± 2.43	48.82^{d} ± 2.46	45.13^{e} ± 2.34	45.00^{e} ± 2.14
Monocyte (%)	0-4	3.30 ± 1.37	*3.32* ± 1.34	3.50 ± 1.46	3.44 ± 1.62	3.32 ± 1.53	3.23 ± 1.66	3.20 ± 1.54
Neutrophils (%)	30-48	33.82^{a} ± 1.32	*33.00^{a} ± 1.53*	29.87^{b} ± 1.45	26.02^{b} ± 1.68	25.83^{c} ± 1.47	22.50^{d} ± 1.58	22.00^{d} ± 1.63
Eosinophil (%)	1-8	3.83^{a} ± 0.31	*3.75^{a} ± 0.43*	3.00^{b} ± 0.23	3.23^{b} ± 0.43	2.74^{c} ± 0.53	2.45^{c} ± 0.64	2.35^{d} ± 0.37
After the experiment								
PCV (%)	22-38	24.70^{a} ± 1.44	21.50^{ab} ± 1.55	20.70^{ab} ± 1.84	20.50^{b}±1.88	18.70^{b} ± 1.10	17.50^{b} ± 1.31	18.70^{b} ± 0.78
RBC (106/UL)	7-18	7.20^{a} ± 0.91	5.90^{b} ± 0.55	6.80^{b} ± 0.46	5.00^{b} ± 0.55	4.60^{c} ± 0.76	4.20^{c} ± 0.86	4.50^{c} ± 0.57
WBC (109/L)	4-13	5.60^{e} ± 0.75	*6.02^{d} ± 0.73*	6.90^{d} ± 0.66	8.20^{c} ± 0.50	8.70^{c} ± 0.40	9.40^{b} ± 0.60	10.20^{a} ± 0.64
HB (g/dl)	7-15	10.60^{a} ± 0.92	*10.60^{a} ± 0.78*	9.80^{a} ± 0.51	9.00^{b} ± 0.70	8.70^{c} ± 0.85	8.10^{c} ± 0.75	6.50^{d} ± 0.74
MCHC (g/L)	30-36	33.40^{a} ± 0.30	*33.50^{a} ± 0.30*	33.50^{a} ± 0.60	33.80^{a} ±0.10	33.93^{a} ± 0.23	34.00^{b} ± 0.57	34.10^{b} ± 0.35
MCH (pg)	5.2-8	11.70^{a} ± 1.05	*11.40^{a} ± 0.10*	10.97^{b} ± 0.14	10.77^{b}± 0.71	10.40^{b} ± 0.46	10.13^{c} ± 0.57	10.00^{c} ± 0.71
MCV (fl)	16-25	33.13^{a} ± 2.59	*32.12^{a} ± 2.16*	31.27^{c} ± 2.80	31.13^{c} ± 2.03	31.00^{c} ± 2.61	30.53^{d} ± 2.01	30.25^{d} ± 1.90
Lymphocyte(%)	50-70	55.67^{a} ± 2.65	*55.50^{a} ± 2.08*	55.03^{a} ± 2.64	51.90^{ac} ± 2.55	46.47bc± 2.84	43.57^{b} ± 2.00	43.85$^{b\,c}$± 2.63
Monocyte (%)	0-4	2.44 ± 1.62	*3.17 ± 1.17*	3.43 ± 1.25	3.23 ± 1.40	3.17 ± 1.26	3.16 ± 1.40	3.14 ± 1.13
Neutrophils (%)	30-48	30.70^{ab} ± 1.69	*32.00^{a} ± 1.56*	27.03^{ab} ± 1.75	24.83^{ab} ±1.04	24.27^{ab} ± 1.24	21.67^{b} ±1.27	21.65^{ab} ± 0.49
Eosinophil (%)	1-8	3.20^{a} ± 0.53	*3.13^{a} ± 0.51*	2.90^{ab} ± 0.17	3.00^{ab} ± 0.00	2.30^{ab} ± 0.36	2.17^{b} ± 0.20	2.30^{ab} ± 0.00

abcde = means within the same row with different superscripts are significantly (P<0.05) different.
T1: 0% cocoa pod, 60% cassava pulp and 40% acacia leaf; T2: 10% cocoa pod, 50% cassava pulp and 40% acacia leaf; T3: 20% cocoa pod, 40% cassava pulp and 40% acacia leaf; T4: 30% cocoa pod, 30% cassava pulp and 40% acacia leaf; T5: 40% cocoa pod, 20% cassava pulp and 40% acacia leaf; T6: 50% cocoa pod, 10% cassava pulp and 40% acacia leaf; T7: 60% cocoa pod, 0% cassava pulp and 40% acacia leaf
*Reference ranges by Daramola et al. (2005); **Blood, et al. (2007).

Table 2.
Serum Biochemical Parameters of West African Dwarf Goats fed combinations of cocoa pod and cassava pulp and Acacia leaves

Parameters	Range	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	*P-value*
At the start of experiment									
Total Protein (g/dl)	51.0-74.5	7.30a±0.52	6.90b±0.45	6.73b±0.06	6.52b±0.27	6.13bc±0.42	5.85c±0.35	5.70c±0.54	0.001
Albumin (g/dl)	2.8-4.3	4.00±0.01	3.92±0.34	3.58±0.64	3.45±0.05	3.33±0.57	3.17±0.45	2.64±0.63	0.210
Total Cholesterol (mg/dl)	65.0-136.0	80.90±3.57	92.84±3.46	102.22±4.78	114.00±5.54	121.5±4.34	125.87±5.49	127.35±5.55	0.465
Sodium (mmol/L)	124.0-146.0	136.00a±5.34	131.7a±5.47	118.83b±4.36	111.78b±4.42	108.37c±4.30	102.35c±4.56	101.10c±4.38	0.000
Potassium(mmol/L)	0.8-9.7	4.97±0.25	5.33±0.65	5.50±0.80	5.63± 1.01	5.83±0.90	6.00±0.85	6.50± 0.71	0.758
Urea (mg/dl)	12.6-27.0	16.90±2.00	17.43±2.11	18.00±2.71	20.43± 3.44	23.47± 3.45	25.60± 3.56	26.85±3.69	0.885
Glucose(mg/dl)	48.2-76.0	38.20d±3.45	45.80c±4.30	51.40b±4.52	52.25b±4.65	58.11b±5.32	63.73a±5.67	68.70a±5.43	0.040
Creatinine(mg/dl)	0.7-1.50	1.00±0.12	1.82±0.35	2.33±0.54	2.46±0.48	2.63±0.52	2.70±0.12	2.85±0.54	0.180
AST (IU/L)	66-230	95.30e±5.54	112.52d±5.43	115.33d±5.46	138.90b±6.45	143.3a±6.51	146.70a±6.23	148.70a±6.50	0.001
ALT(IU/L)	2.0-221	53.70d±3.45	74.71d±3.43	83.80c±3.54	95.52c±4.35	104.1b±5.42	115.40a±5.56	122.60a±5.67	0.002
ALP (U/L)	61-283	56.80e±3.67	61.81d±3.54	77.52c±3.45	85.55c±3.76	94.33b±3.45	105.20a±3.87	117.50a±3.89	0.003
Inorganic Phosphorus (mg/dl)	3.38-5.70	4.30c±0.05	4.53c±0.42	4.60c±0.54	7.50b±0.48	7.80b±0.32	7.92b±0.54	8.82a±0.43	0.003
After the experiment									
Total Protein (g/dl)	51.0-74.5	7.23a0.06	6.67ab±0.40	6.50abc±0.20	5.90abc±0.56	5.60bcd±0.44	5.20cd±0.51	5.00d±0.99	0.001
Albumin (g/dl)	2.8-4.3	3.70±0.36	2.97±0.83	2.77±0.70	2.67±0.67	2.40±0.79	2.17± 0.81	2.10± 0.71	0.206
Total Cholesterol (mg/dl)	65.0-136.0	82.60±3.99	*97.77±3.72*	108.27±5.32	123.00±6.84	125.07±6.25	128.70±6.23	130.55±5.35	0.460
Sodium (mmol/L)	124.0-146.0	136.13a±5.61	*121.17b±5.38*	114.6bc±5.77	110.43cd±1.17	106.04d±2.3	100.40d±4.55	99.70d± 2.98	0.000
Potassium(mmol/L)	0.8-9.7	5.20±0.65	*6.00±0.65*	6.34±0.50	6.75± 1.01	6.73±0.90	6.80±0.85	6.91± 0.71	0.753
Urea (mg/dl)	12.6-27.0	12.27±0.33	*14.25±0.43*	14.80±0.50	17.40±0.46	20.45±0.34	22.20±0.46	23.30±0.05	0.883
Glucose(mg/dl)	48.2-76.0	35.60a±3.15	*41.60ab±4.89*	48.47ab±4.82	50.77ab±5.34	55.53ab±5.37	60.50b±6.76	65.30ab±6.47	0.035
Creatinine(mg/dl)	0.7-1.50	0.90±0.17	*1.47±0.49*	1.73±0 .67	1.77±0.71	2.10±0.72	2.20±0.70	2.30±0.71	0.189
AST (IU/L)	66-230	91.57a±6.12	*104.67ab±7.8*	111.73b±7.14	130.80c±7.31	136.1c±7.52	141.37c±8.30	143.20c±8.78	0.000
ALT(IU/L)	2.0-221	45.23a±3.16	*64.93ab±4.39*	73.96ab± 4.42	86.70ab±5.91	94.77b± 6.92	106.03b±6.44	104.60ab±6.4	0.024
ALP (U/L)	61-283	60.90a±3.63	*79.63ab± 4.41*	93.77abc±5.82	99.73abc±6.63	111.53c±6.79	130.07c±6.30	131.10c±6.23	0.001
Inorganic Phosphorus (mg/dl)	3.38-5.70	5.43a±0.21	*5.52ab± 0.59*	5.63abc±0.65	8.47bc±0.87	8.77bc±0.95	9.37bc±1.50	9.45d±0.91	0.001

abcde= means within the same row with different superscripts are significantly (P<0.05) different.
T1: 0% cocoa pod, 60% cassava pulp and 40% acacia leaf; T2: 10% cocoa pod, 50% cassava pulp and 40% acacia leaf T3: 20% cocoa pod, 40% cassava pulp and 40% acacia leaf; T4: 30% cocoa pod, 30% cassava pulp and 40% acacia leaf T5: 40% cocoa pod, 20% cassava pulp and 40% acacia leaf; T6: 50% cocoa pod, 10% cassava pulp and 40% acacia leaf T7: 60% cocoa pod, 0% cassava pulp and 40% acacia leaf
*Reference ranges by Daramola et al. (2005); **Blood, et al. (2007).

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ues corroborated the reports of Swati and Varsha [16]. Such high PCV values had been regarded as healthy state and high productivity according to Addass et al. [17]. It was observed that feeding WAD goats with 0%-20% cocoa pod supplementation could probably return PCV to normal level as goat was the only animal with PCV higher than 22% [12, 13]. The low PCV values reported in combinations T4-T7 in the present study could have resulted from hepatic toxicity caused by high cocoa pod intake and high theobromine in the diets according to Adeyina [18]. The Hb values (7.33-11.15 g/dl) of the treatment groups were in the normal range (7-15 g/dl) reported by Tambuwal et al.[19] for WAD goats.

Recently, research indicated that cocoa pod can be developed and processed to be used in highly valuable feed stuffs [20]. At the end of the experiment, the major limitation of cocoa pod in this respect was the alkaloid and theobromine that had cumulative effect on livestock production system as in T4-T7 groups. The RBC count (4.9-7.87 × 106/µl) was also in the normal range of 7-18 × 106/µl. MCHC in this study (33.47-34.27 g/dl) was in the range of 30-36 g/dl reported by Blood et al. [13] and Daramola et al. [12]. The high MCV and MCH values recorded in the present study compared well with the values reported by-Anya [1]. MCH values recorded in the present study compared well with the values reported by Anya [1] who described high MCV as an indication of regenerative anaemia emanating from high destruction which led to erythropoiesis in the tissues.

WBC count of 5.57-6.9 × 109/l in 0%-20% cocoa pod inclusion corroborates the findings of Daramola et al. [12] and Anya [1]. This implied that goats on diets T1-T3 remained clinically healthy as indicated by researchers [21] and animals had good immune system against any foreign body in the circulatory system. WBC played a prominent role in disease resistance, especially regarding antibody generation. High WBC values in T4-T7 have been associated with the toxicity of diets or poor detoxification process as the WBC is responsible for fighting foreign substances in the body [22]. In addition, lymphocytes and neutrophils in this study fell within the broad range of 50%-70% and 30%-48% as reported by Daramola et al. [12], respectively.

Biochemical indices contributed to the knowledge of metabolic profile in feedlots performance o WAD goats and their possible disorders according to Oloche [23]. Total protein and albumin showed a consistently raising trend from the first to the last treatment level (T1-T7). The difference was significant for the former and non-significant for the later parameter, showing it to be healthy to add ensiled cocoa pod to the diet of goats instead of cassava pulp. Total protein and albumin decreased with increasing cocoa pod and differences in the values were significant at widely variable ratios of the feed inputs. Total protein in diets T1-T3 (6.5-7.23 g/dl) were comparable to the normal protein range for WAD goats (6.4-7.5 g/dl) as reported by Dhanotiya [24]. The differences in protein values were suggestive of the influence of feeds on the feed intake of goats according to Anya [1]. Albumin in this study (2.1-3.7 g/dl) was similar to 3.3 g/dl reported by Ibrahim et al. [25]. Therefore, it can be affirmed that protein on combinations T1-T3 was of good quality to meet the nutritional needs of the animals.

The cholesterol content was the highest and lowest in T7 (127.55 mg/dl) and T1 with no cocoa podinclusion (82.6 mg/dl), respectively. It can be attributed to the cholesterol-reducing ability of protein supplement in T1 used in the present study. These comparable values of cholesterol suggested that the meat from the experimental animals of the T1-T3 groups was safe for consumption according to Igwebuike et al. [26] who reported that serum cholesterol is associated with the quantity and quality of protein supplied in the diet. The results about glucose agreed with thenormal range of 50-75 mg/dl for goats reported byDhanotiya [24]. The glucose concentrations rose sig-nificantly showing a consistent upward trend with the increase in the cocoa pod from 0% (T1) to 60% (T7). Consequently, it appeared plausible to infer that the observed higher serum glucose concentrations in diet combinations T4 (5.77 mg/dl) to T7 were due to cocoa pod intoxication. ALT, AST, and ALP levels increased steadily across T1-T7. The high values in T5-T7 suggested severe liver injury.

Based on the haematological indices and serum-biochemistry, it may be concluded that ensiled cocoa pod, cassava pulp, and acacia leave up to a ratio of 20:40:40 can serve as a sustainable feedstuff for dwarf goats, especially during the dry season without adverse effects. These diets would be rich in nutrients and highly digestible and could meet the nutrient requirements for the growth and maintenance of these animals.

Materials and Methods

Experimental Site

The current study was carried out at the Teaching and Research Farm of the Department of Animal Sciences, Landmark University, Omu Aran, Kwara State, Nigeria.

Animals and Study Design

A total of 28 WAD goats (bucks) aged 4-5 months with an average body weight of 7 ± 0.2 kg were prepared from the local livestock market in Ekiti. Previously, the nutritional properties of 30 silage samples prepared from the combinations of cocoa pod, cassava pulp, and acacia leaf had been evaluated. Based on the ob-

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tained results, the best seven dietary combinations of cocoa pod, cassava pulp, and acacia leaf were chosen for the present experiment. They were designated as T1, T2, T3, T4, T5, T6, and T7 as presented in Table 1. Diet T1 was a positive control and contained no cocoa pod, while T7 was the negative control with no cassava pod. Diets T2, T3, T4, T5, and T6 contained 10%, 20%, 30%, 40%, and 50% of cocoa pod and 50%, 40%, 30%, 20%, 10% of cassava pulp, respectively. The animals were allotted to seven dietary treatments after 14 days of acclimatization in a completely randomized design with four animals per treatment under an intensive management system. One goat was penned individually and replicated four times.

Experiment Procedure

Experimental Diets

Silage Preparation

Theobroma cacao (cocoa pods) was collected from a reputable cocoa farm and was sundried to reach a moisture content of 37% and pounded (using mortar and pestle) to an average size of 0.6 cm2. Cassava pulp was obtained from a cassava processing farm and was sundried to a moisture content of 37% as described by Olawoye [27]. Moreover, acacia leaves were harvested from the pasture plants of the Teaching and Research Farm of the institution. The legume was allowed to wilt in the open air for a day and thereafter chopped at 2-3 cm. The purpose of chopping and compacting the diets for silage was to ensure that all the air was pushed out of the plant material so that when the bag was sealed, the ensiled materials would be free of air. The wilted chopped acacia leaf, cocoa pod, and cassava pulp were mixed with overripe banana (Musa spp.) slurry at the rate of 5% of the weight of the diets. Uniform compaction was ensured until the bags were filled and tightly tied packed in a polythene bag and were put inside plastic bags of 20 liters and ensiled at 37°C as described by Olawoye [27]. Afterwards, each plastic was compacted with a 20 kg weight to remove air and create an anaerobic condition until the expiration of fermentation (7 weeks).

Feeding Trials and Laboratory analysis

The animals were housed in well-ventilated pens in an open-sided housing system with corrugated aluminum roofing sheets and concreted slatted floors. The pen was fumigated with Izal solution two weeks prior to the experiment. All the goats were weighed and randomly allotted to different dietary groups individually (Table 1). The animals were dewormed by anthelmintic (super ivermectin) according to their body weight and were sprayed with acaricide (parannex) against external parasites. The goats were fed experimental diets early in the morning (8:00 am) and had access to fresh drinkable water ad libitum during the experimental period. Daily feed offered and refusals were recorded to compute feed intake. The weight parameters were already published in one article under growth parameters. The feeding trial was carried out for 45 days due to the toxicity of the high concentration of cocoa pod which led to the mortality of some animals on diets T4-T7. The possible cause was increased toxic substance due to high theobromine concentration in the diets. In these groups, about 50% of animals died, while there was no record of death in animals on diets T1-T3 because of the minimal inclusion of cocoa pod.

Blood was collected two weeks after dietary adaptability as a baseline sample and experiment termination to determine the effects of the diets at the beginning of the experiment and after the ingestion of diets. Two sets of jugular vein blood samples (10 ml) were taken from each animal per treatment using a syringe and needle into clean bottles. One set was introduced in tubes containing anticoagulant ethylene diamine tetraacetate to evaluate haematological parameters, while the second set of blood samples was in clean bottles devoid of anticoagulant for assessing serum biochemical parameters. All haematological and serum biochemical factors were measured in triplicates using the methods of Al-Eissa and Alkahtani [28]. The PCV was determined by the Hawskey microhematocrit method [29]. The Hb concentration was measured spectrophotometrically by the cyanmethemoglobin method [30] using an SP6-500UV spectrophotometer (PYE, UNICAM, England). RBC, as well as total and differential WBC

Table 3.
Dietary Composition and Calculated Nutrients of combinations of cocoa pod, cassava pulp and Acacia leaf to WAD Goats (%)

Feedstuffs (%) Control							
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**	**Diet 7**
Cocoa pod	00.0	10.0	20.0	30.0	40.0	50.0	60.0
Cassava pulp	60.0	50.0	40.0	30.0	20.0	10.0	00.0
Acacia leaf	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Acacia leaf	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical Composition of the experimental diets (% Dry matter)							
Dry matter	65.08	60.43	73.19	79.07	71.00	82.58	60.23
Moisture Content	34.92	39.57	26.81	20.93	29.00	17. 42	39.77
Crude Protein	12.51	11.23	11.91	13.17	12.07	12.05	13.20
Crude fibre	5.18	7.85	15.66	12.23	11.68	9.91	5.07
Ether Extract	18.10	13.31	18.53	23.12	17.61	24.58	16.30
Ash	2.46	2.75	6.14	8.77	5.78	8.50	3.44
Nitrogen free extract	39.31	36.49	35.05	36.72	35.90	39.55	35.39

counts were assessed by the hemocytometer method [29] using improved Hawskey hemocytometer. MCV, MCHC, and MCH were calculated based on PCV, Hb, and RBC [29].

The serum biochemical factors were measured using commercial kits (Randox, England) and a UV spectrophotometer (Jenway Spectrophotometer 6305, England). Serum ALT activity, ALP activity, total protein, albumin, urea, creatinine, andcholesterol were measured by the Reitman-Frankel [31], phenolphthalein monophosphate [32], direct Biuret [33], Bromocresol green [34], modified Berthelot-Searcy [35], modified Jaffe methods [36], and cholesterol oxidase-peroxidase method [37], respectively. Furthermore, sodium and potassium concentrations were measured using the flame photometer (Corning model 400, Corning Scientific Ltd, England) [38] and and phosphorus was determined using spectrophotometer (Biokom, Warsaw, Poland)according to Bauer [39].

Data Analysis

The data obtained from the blood parameters were subjecte to standard methods of statistical analysis using windows based SPSS (Version 20.0) [40]. The one-way analysis of variance was used and the level of significance was set at $p < 0.05$.

Declarations

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Animal Welfare Statement

Ethics approval

The authors confirm that the ethical policies ofthe journal, as noted in the journal authors guide lines, have been adhered to. Approval to perform the research and use animals was obtained from the Ethics Committee of the University of Ilorin, Kwara State, Nigeria.

Authors' Contributions

C.O.R and A.A.A conceived and planned the experiments. Both authors participated in design and coordination. C.O.R performed the experiments, contributed to sample preparation, interpreted the results, and took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analyze, and write the manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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Serial blood gas analysis during fluid resuscitation of hypovolemic dogs

Reza Avizeh[a], Reza Azargoun[b], Alireza Ghadiri[a], Mahdi Pourmahdi Boroujeni[c], Mohammad Razi Jalali[a], Hadi Imani Rastabi[a]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.
[b] Department of Internal Medicine and Clinical Pathology, Faculty of Veterinary Medicine, Urmia University, Iran.
[c] Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.

ABSTRACT

It has been documented that hemodynamic disturbances occur in hypovolemic patients. Therefore, the early management of hypovolemia is essential to achieve optimal outcomes. Blood gas, which changes rapidly during hemodynamic instability, can be used as a diagnostic approach for monitoring emergency patients. The objective of the current study was to evaluate the results of resuscitation with hydroxyethyl starch (HES) or lactated Ringer's solution (LR) on venous and arterial blood gas. In addition, the difference between venous and arterial blood gas parameters is investigated to assess the possibility of using venous blood gas analysis as a successor for arterial blood gas analysis in the resuscitation of hypovolemic dogs. Venous and arterial pH, PO_2, PCO_2, HCO_3-, and base excess were analyzed at the end of each study stage as follow: 1) Establishment of anesthesia, 2) Blood collection to an arterial mean pressure of 40-50 mm Hg, 3) Maintaining dogs in a hypovolemic state, 4) Resuscitation with LR (group A) or HES (group B) in four steps, and 5) One hour after the final resuscitation step. Hypovolemia decreased the studied parameters, except venous PCO_2, which showed a significant increase ($p < 0.05$). Fluid resuscitation returned the studied parameters to the control values as venous PCO_2 in group A and HCO_3- in group B showed a significant change in comparison with the control values ($p < 0.05$). We found that venous pH, HCO_3-, and PCO_2 can be used as less invasive and safer alternatives to similar arterial parameters to monitor the fluid resuscitation of hypovolemic dogs.

Keywords

Arterial blood gas, Venous blood gas, Hydroxyethyl starch, Ringer's Lactate

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Abbreviations

LR: Lactated ringer
HES: Hydroxyethyl starch
PO_2: Partial pressures of oxygen
PCO_2: Partial pressures of carbon dioxide
HCO_3-: Bicarbonate
BE: Base excess

Corresponding author: *Reza Azargoun*
Email: R.azargoun@urmia.ac.ir
Tel: +98 (91)1393-7797

Introduction

About half of traumatic deaths are associated with hemorrhage. As a result, they are essential to be diagnosed early and well-resuscitated to prevent the noxious effects of hypovolemic shocks, such as cellular hypoperfusion, anaerobic metabolism, and metabolic acidosis [1]. Fluid resuscitation is the cornerstone of hypovolemic shock treatment because adequate fluid therapy is necessary to maintain cellular perfusion. However, there is no consensus on the best type of fluid resuscitation in terms of safety and effectiveness [2].

Monitoring hypovolemia is a daily challenge in the emergency department. Following acute and severe hemorrhage, robust compensatory physiological responses are activated, which have traditionally been used to diagnose hypovolemic shock and to guide fluid resuscitation. However, studies have demonstrated that monitoring these physiologic criteria, including heart rate, blood pressure, and respiratory rate may not be proportional to the hypovolemic states, and when the above-mentioned criteria are close to the reference range, occult hypoxemia is probable at the cellular level [1].

It is well-known in human medicine that blood gas changes rapidly during hypovolemic shock. Furthermore, the information provided by venous and arterial blood gas analysis can reflect the patient's true oxygenation and metabolic status at the cellular level. Sánchez-Díaz and colleagues have shown that blood gas parameters can be used as a tool to monitor shock severity and fluid resuscitation adequacy [3]. However, the effects of different types of fluids (e.g., crystalloid and colloidal solutions) on the blood gas parameters of veterinary patients are not well understood.

Although in the previous studies blood gas and acid-base status of healthy dogs were assessed [4, 5], according to our knowledge, changes in blood gas during resuscitation of hypovolemic dogs have not been investigated. Consequently, the primary objective of this study was to evaluate the effects of resuscitation with LR solution and HES on venous and arterial blood gas in dogs with experimental hypovolemic shock. The secondary objective of this study was to evaluate the difference between venous and arterial blood gas parameters and the possibility of using venous blood gas analysis as a less invasive and safer surrogate method for arterial blood gas analysis in the resuscitation of hypovolemic dogs.

Result

The mean ± SD of the collected blood volume was 57.11 ± 5.17 and 52.86 ± 8.22 ml/kg in the animals of groups A and B, respectively, which represent almost 61% of the blood volume (90 ml/kg) on average. There was no significant difference between the groups in this regard ($p > 0.05$). In either group, the dogs were successfully resuscitated with fluid therapy without death. Animals in groups A and B in each of the resuscitation steps, received 385.08 ± 97.85 ml LR and 89.5 ± 23.17 ml HES, respectively. Moreover, mean arterial pressure did not have a significant difference in any of the study stages between the two groups ($p > 0.05$).

In the hypovolemic stage, only venous PCO2 increased significantly ($p < 0.05$), while other studied parameters decreased compared to the control stage. However, the decline in venous BE in both groups and venous bicarbonate in group A was not significant ($p > 0.05$, Tables 1 and 2). During the resuscitation stage, venous and arterial blood gas parameters in both groups almost returned to the control stage, so that in the eighth step of the study, no significant change was found among the control and post-resuscitation stages, except for venous PCO2 in group A and HCO3- in group B. The statistical analysis of the data did not show a significant difference between the two types of solutions used ($p > 0.05$).

Statistical analysis revealed that in some stages of the research, there was a significant difference between the venous and arterial blood gas parameters (Figure 1). However, in all stages of the study, the venous and arterial partial pressures of oxygen were significantly different ($p < 0.05$), while the venous and arterial bicarbonate concentration and pH did not have a significant difference ($p > 0.05$).

Discussion

In this study, changes in venous and arterial blood gas parameters during endpoint resuscitation by LR solution and HES, as the most frequently used crystalloid and colloidal solutions, were analyzed in dogs with experimental hypovolemic shock.

Trauma-induced massive hemorrhage results in hypovolemia, hypotension, and reduced cardiac output. During a hypovolemic shock state, poor cellular oxygenation due to insufficient red cell mass and hemoglobin concentration causes anaerobic metabolism and subsequent metabolic acidosis [6, 7]. In the present study, pH, PO_2, and HCO_3- declined during the hypovolemic stage, which confirms metabolic acidosis and hypoxia, and can be attributed to decreased oxygen delivery, increased lactate, and excess hydrogen ions. The BE represents the combined effects of HCO_3- and hydrogen ion concentrations. Depletion of BE in the background of metabolic acidosis may indicate hypovolemia-induced pre-hepatic dysfunction in restoring the pH levels. In this situation, lactate removal is reduced as a result of sym-

Table 1.
Mean ± SD of venous blood gas parameters during different steps of the study

Factor	pH		PO_2 (mm Hg)		PCO_2 (mm Hg)		HCO_3– (mmol/L)		BE (mmol/L)	
Steps	LR	HES	LR	HES	LR	HES	LR	HES	LR	HES
A1	7.31 ± 0.01^{a}	7.32 ± 0.04^{a}	93.80 ± 37.47^{a}	108.20 ± 36.12^{a}	41.60 ± 10.31^{c}	43.10 ± 5.75^{bc}	20.72 ± 5.58^{ab}	21.90 ± 3.16^{a}	-5.52 ± 5.72^{abc}	-6.46 ± 3.21^{b}
A2	7.24 ± 0.07^{b}	7.20 ± 0.03^{b}	28.20 ± 12.47^{c}	31.60 ± 12.91^{c}	49.40 ± 0.11^{b}	45.98 ± 10.88^{bc}	21.10 ± 3.76^{a}	16.80 ± 1.93^{b}	-6.22 ± 4.17^{abc}	-11.58 ± 2.21^{bc}
A3	7.13 ± 0.10^{c}	7.11 ± 0.08^{c}	32.61 ± 11.02^{b}	36.92 ± 13.68^{b}	55.63 ± 8.05^{a}	51.52 ± 10.02^{a}	20.14 ± 3.96^{b}	16.03 ± 1.67^{c}	-8.07 ± 3.62^{c}	-11.85 ± 2.13^{bc}
A4	7.22 ± 0.08^{b}	7.19 ± 0.06^{b}	57.80 ± 16.87^{b}	63.80 ± 19.61^{b}	50.50 ± 14.97^{ab}	48.22 ± 16.27^{ab}	20.26 ± 5.35^{b}	17.04 ± 1.21^{b}	-7.44 ± 5.97^{bc}	-11.00 ± 1.05^{bc}
A5	7.27 ± 0.04^{ab}	7.27 ± 0.05^{a}	132.20 ± 54.28^{a}	104.34 ± 50.66^{a}	47.43 ± 13.03^{bc}	46.05 ± 14.17^{abc}	21.44 ± 4.22^{a}	19.28 ± 1.41^{a}	-4.68 ± 4.45^{ab}	-0.86 ± 8.07^{a}
A6	7.28 ± 0.05^{a}	7.22 ± 0.07^{b}	213.80 ± 102.79^{a}	184.60 ± 142.10^{a}	46.74 ± 14.01^{bc}	46.48 ± 14.31^{abc}	21.52 ± 5.79^{a}	19.75 ± 3.88^{a}	-5.18 ± 5.84^{abc}	-7.26 ± 3.17^{b}
A7	7.31 ± 0.02^{a}	7.18 ± 0.06^{bc}	231.40 ± 145.46^{a}	189.20 ± 99.39^{a}	45.00 ± 11.50^{bc}	43.84 ± 11.22^{bc}	22.32 ± 4.13^{a}	16.60 ± 3.24^{bc}	-3.72 ± 3.71^{a}	-12.16 ± 2.55^{c}
A8	7.25 ± 0.04^{ab}	7.25 ± 0.06^{a}	155.40 ± 102.54^{a}	124.40 ± 105.91^{a}	55.98 ± 15.11^{a}	39.65 ± 6.31^{c}	23.90 ± 5.60^{a}	17.80 ± 3.24^{b}	-3.30 ± 5.42^{a}	-9.66 ± 2.30^{bc}

The different letters in each column represent significant differences ($p < 0.05$)

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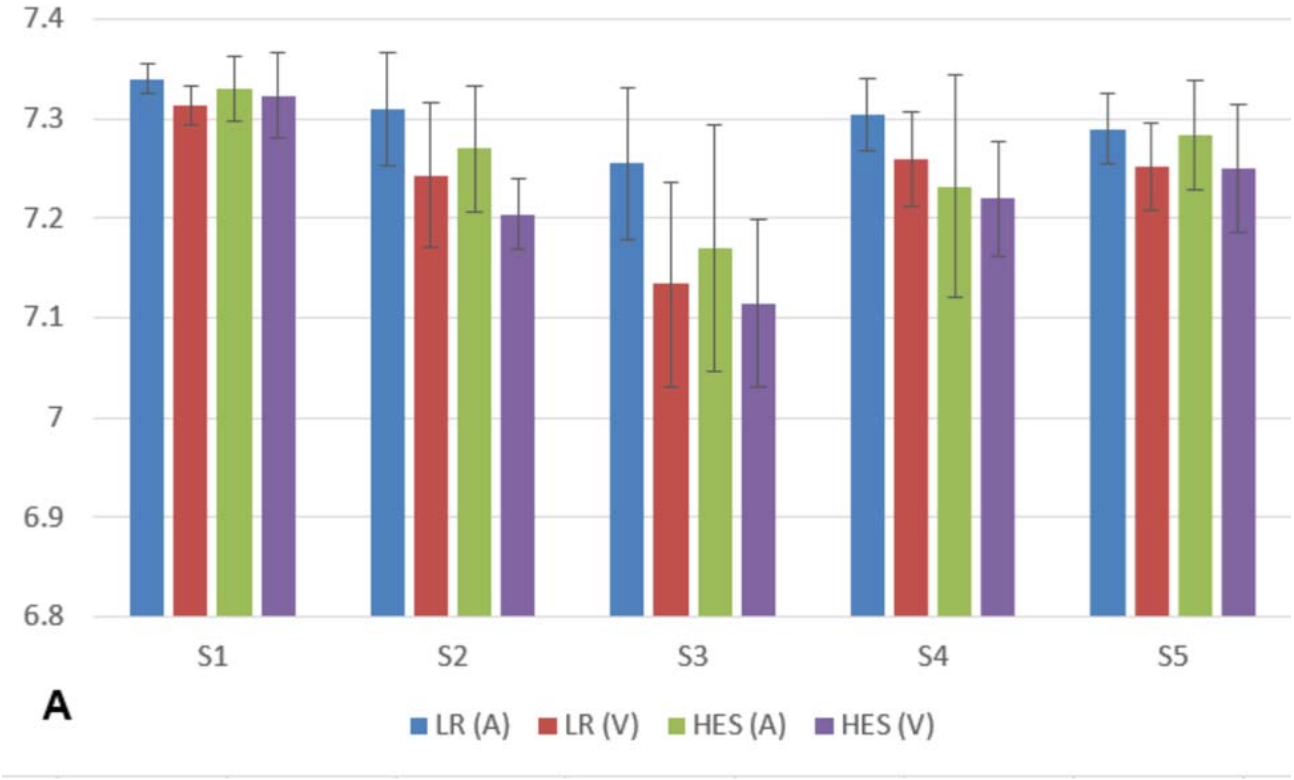
Table 2.
Mean ± SD of arterial blood gas parameters during different steps of the study

Factor	pH		PO_2 (mm Hg)		PCO_2 (mm Hg)		HCO_3– (mmol/L)		BE (mmol/L)	
Steps	LR	HES	LR	HES	LR	HES	LR	HES	LR	HES
A1	7.34 ± 0.01^{a}	7.33 ± 0.03^{a}	669.00 ± 13.76^{a}	581.40 ± 90.77^{a}	41.24 ± 5.89^{a}	37.94 ± 9.90^{a}	20.28 ± 1.81^{a}	19.56 ± 4.86^{a}	-5.65 ± 2.60^{a}	-7.40 ± 4.07^{a}
A2	7.31 ± 0.05^{a}	7.27 ± 0.06^{ab}	650.00 ± 34.87^{ab}	509.20 ± 117.25^{a}	37.64 ± 7.63^{a}	31.16 ± 6.03^{b}	18.32 ± 3.84^{ab}	13.34 ± 3.77^{ab}	-7.26 ± 5.34^{ab}	-14.28 ± 6.27^{b}
A3	7.25 ± 0.07^{b}	7.17 ± 0.12^{b}	485.20 ± 128.77^{c}	442.80 ± 73.81^{c}	29.63 ± 9.02^{b}	29.52 ± 6.34c	15.45 ± 3.30^{c}	12.73 ± 3.31^{bc}	-9.17 ± 2.32^{c}	-15.41 ± 5.84^{b}
A4	7.33 ± 0.04^{a}	7.21 ± 0.1^{b}	606.80 ± 138.29^{ab}	417.20 ± 161.93^{c}	36.74 ± 9.87^{a}	39.26 ± 11.34^{a}	20.14 ± 2.16^{a}	10.52 ± 6.27^{c}	-6.64 ± 4.03^{a}	-15.68 ± 5.37^{b}
A5	7.29 ± 0.0^{ab}	7.21 ± 0.1^{b}	612.60 ± 80.88^{ab}	463.20 ± 190.61^{bc}	40.40 ± 6.74^{a}	36.30 ± 21.33^{ab}	17.90 ± 4.70^{bc}	12.86 ± 8.94^{ab}	-8.10 ± 2.78^{bc}	-13.98 ± 7.66^{b}
A6	7.27 ± 0.0^{ab}	7.23 ± 0.12^{ab}	509.80 ± 159.42^{bc}	583.00 ± 172.64^{a}	42.28 ± 17.03^{a}	43.71 ± 21.87^{a}	16.98 ± 7.03^{bc}	15.88 ± 4.76^{ab}	-11.78 ± 10.39^{c}	-11.46 ± 4.28^{ab}
A7	7.32 ± 0.05^{a}	7.26 ± 0.10^{ab}	661.40 ± 23.33^{ab}	617.20 ± 137.76^{a}	45.18 ± 11.92^{a}	43.94 ± 19.28^{a}	21.48 ± 2.70^{a}	17.90 ± 5.18^{a}	-5.54 ± 1.98^{a}	-9.38 ± 5.12^{a}
A8	7.29 ± 0.0^{ab}	7.28 ± 0.05^{a}	547.20 ± 162.66^{ab}	495.20 ± 170.97^{ab}	45.26 ± 8.60^{a}	43.12 ± 11.04^{a}	21.44 ± 4.53^{a}	18.54 ± 2.84^{a}	-5.08 ± 4.81^{a}	-9.14 ± 3.09^{a}

The different letters in each column represent significant differences ($p < 0.05$)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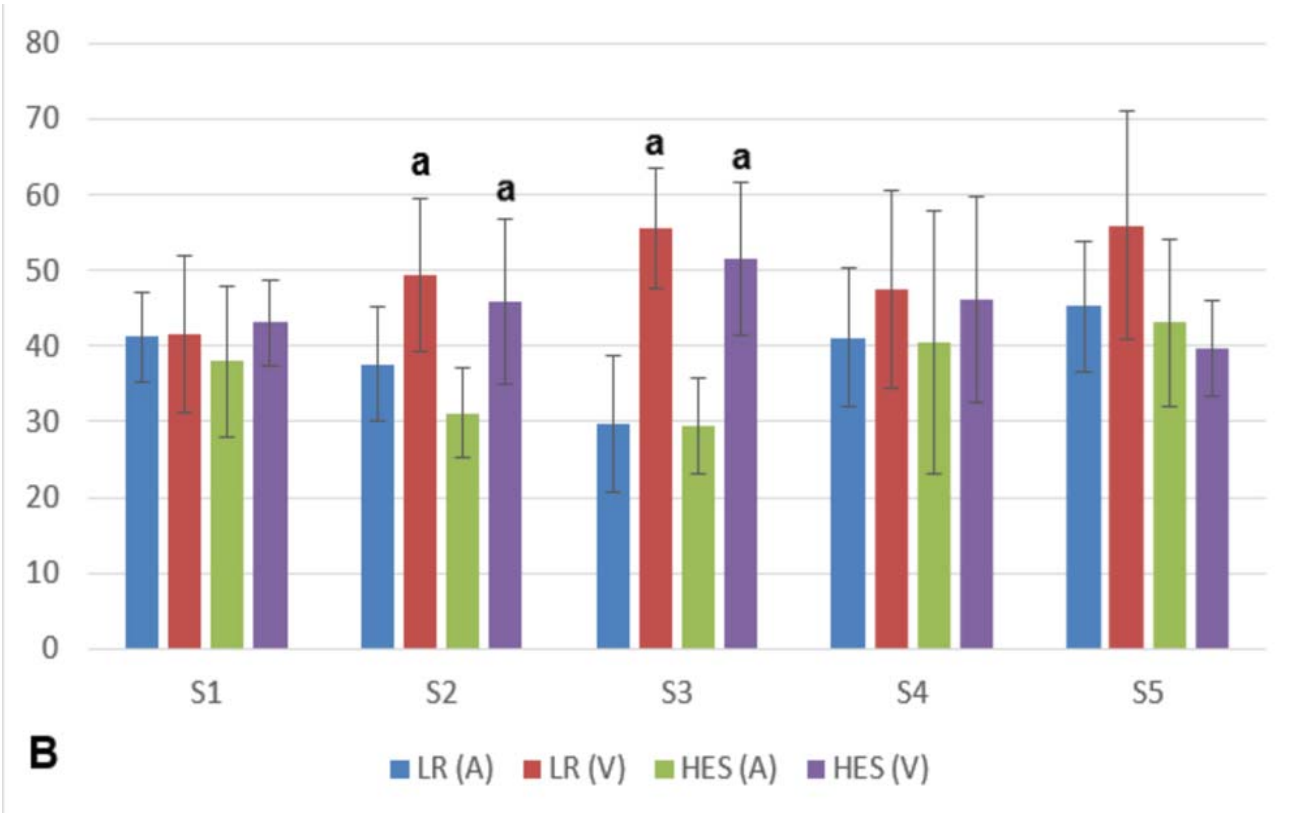
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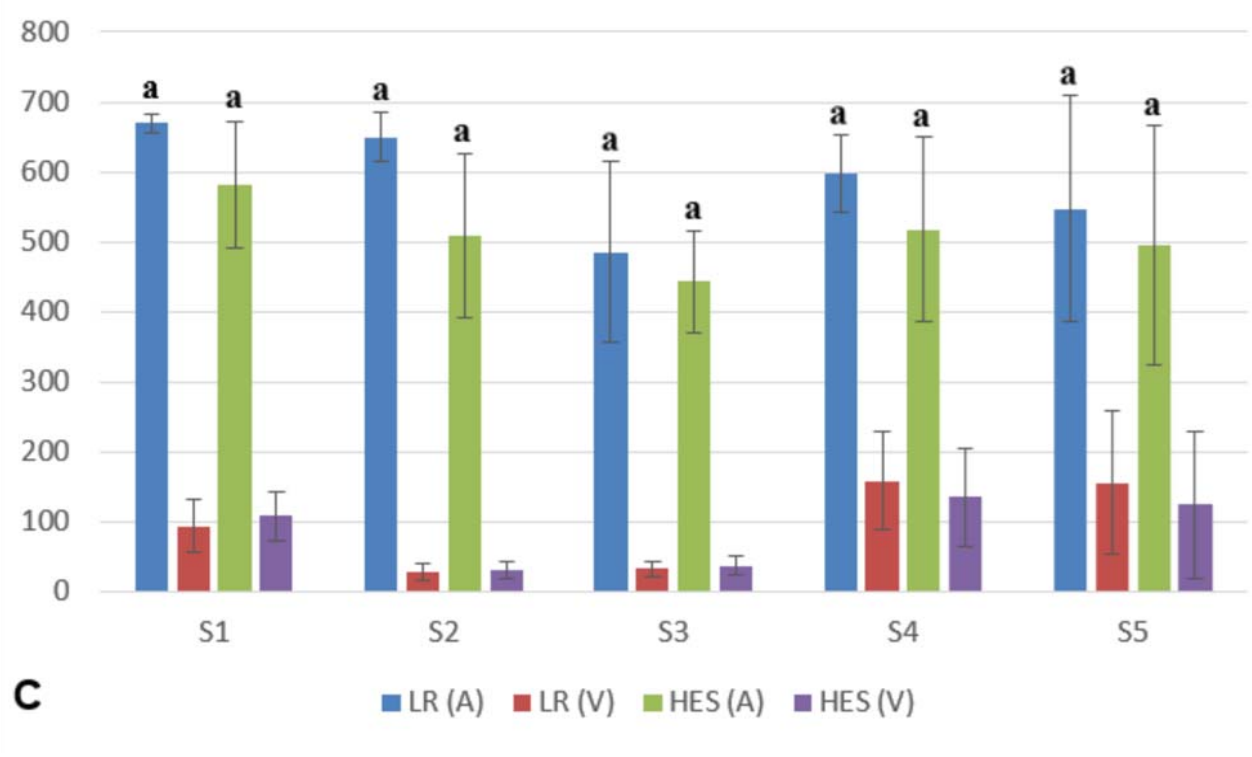
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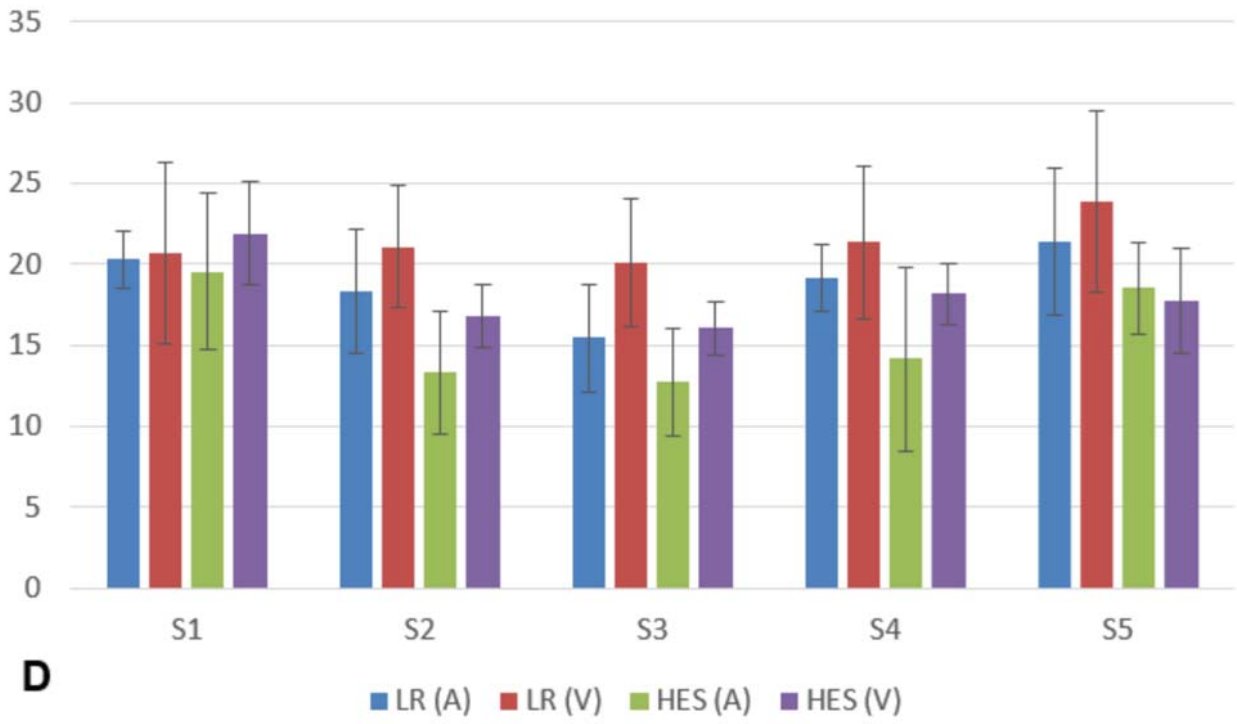
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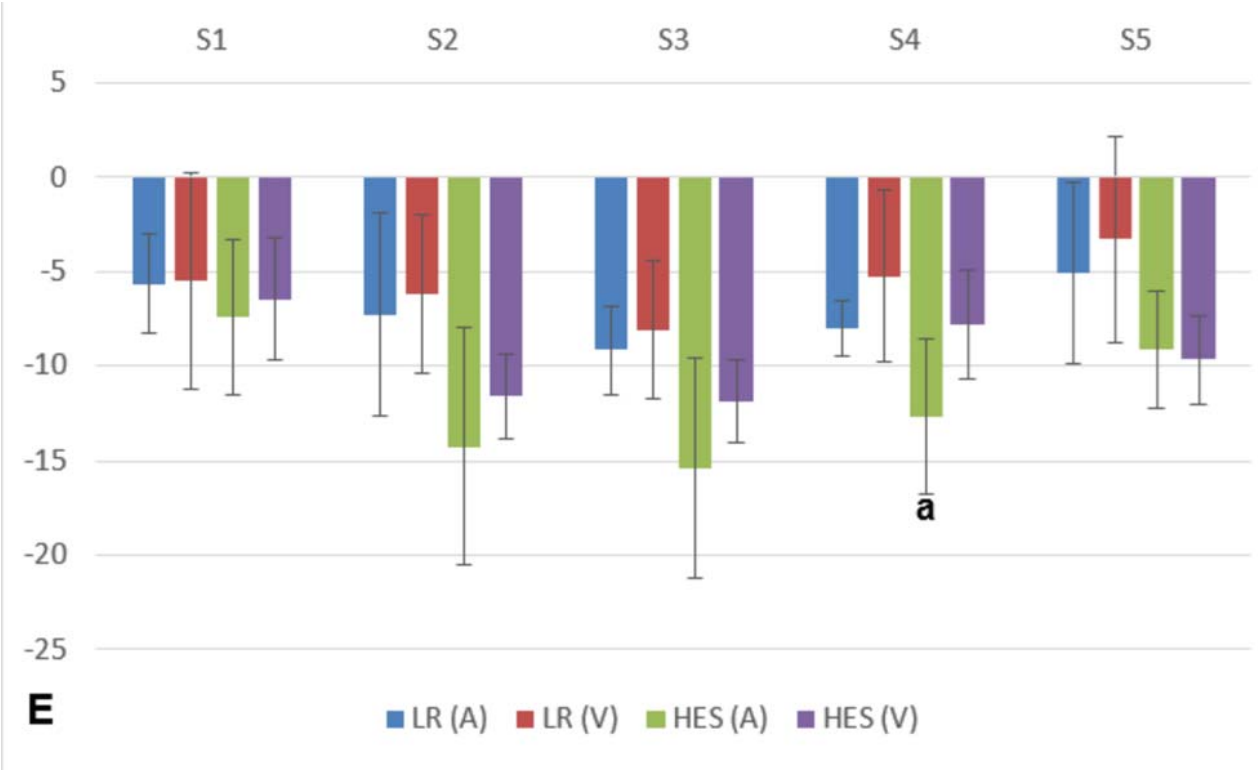

Figure 1.
Mean ± SD of venous (V) and arterial (A) blood gas parameters in the studied stages.
A) pH, B) PCO_2, C) PO_2, D) HCO_3-, E) BE. S1: Control stage, S2: Hemorrhagic stage, S3: Hypovolemic stage, S4: Resuscitation stage, S5: Post-resuscitation stage. [a]$p < 0.05$

pathoadrenal vasoconstriction and decreased blood flow to the liver as the primary consumer of lactate. It is worth noting that excess hydrogen ions can reduce the sensitivity of cardiomyocytes to calcium and consequently reduce cardiac contractility. Poor cardiac contractility leads to decreased cardiac output and worsening cellular oxygenation [6, 8-10]. Following diminished cardiac output, venous CO_2 delivery and elimination in the pulmonary alveoli are reduced and venous hypercarbia is exacerbated [7]. This probably explains the rise in venous PCO_2 in the hypovolemic dogs investigated in the present research. These findings are confirmed by other studies as similar changes in the blood gas parameters of different species with hemorrhagic shock (e.g., cats, rabbits, and pigs) [7, 9, 11].

Given that metabolic acidosis in hypovolemic patients is usually due to tissue hypoperfusion, the best approach to correcting acidosis is fluid resuscitation [8]. We used the endpoint resuscitation method to serially evaluate the blood gas parameters during the fluid therapy of hypovolemic dogs. Crystalloid and colloid solutions can affect the acid-base status differently. Compared to LR, HES has more chloride (109 vs. 154 mmol/l) and a lower pH (6.5 vs. 5.4) [2]. This difference caused a decline in pH during the resuscitation stage in group B compared to A. However, this difference was not statistically significant.

Although most of the blood gas parameters in both groups returned to baseline values by the end of the fluid resuscitation steps, venous PCO_2 and HCO_3- showed a significant difference between the last and first stages of the study in the LR and HES resuscitated groups, respectively. The main reason for this significant difference might be discrepancies in the mechanism of action and durability of the solutions used. About 80% of an isotonic crystalloid solution, such as LR, is transferred to the interstitial space within 1 h after infusion [12]. Accordingly, continued fluid therapy or a change in the resuscitation protocol in group A could be necessary because despite fluid therapy with about a quarter of the full shock dose (80-90 ml/kg) in four 15-minute intervals, venous PCO_2, as a reliable indicator of tissue perfusion [7], was slightly beyond the baseline value in the post-resuscitation stage.

A significant decrease in HCO_3- concentration in the post-resuscitation stage compared to the control stage in group B could be related to the lower pH of HES and renal dysfunction in producing bicarbonate

[9]. As a result, in human medicine, serious concerns about the occurrence of acute kidney injury because of HES administration, especially in critically ill patients, have increased. Bae et al. reported that following the administration of HES to a Golden retriever, acute kidney injury remarkably developed, which is probably due to the nephrotoxicity of HES [13].

Serial arterial blood gas analyses, known as the gold standard method to evaluate the acid-base status and blood oxygenation in critically ill patients is a helpful method in monitoring resuscitation efforts [7]. However, arterial puncture can have serious complications, including arterial injury, hemorrhage, hematoma formation, thrombosis, pseudoaneurysm, and limb ischemia [14]. Venous blood gas analysis is usually less painful and easier. In addition, most critically ill patients require venipuncture as a part of clinical evaluations. Hence, many researchers have recently focused on evaluating the accuracy and reliability of venous blood gas as an alternative to arterial blood gas.

In human medicine, Kelly showed in a review article that in patients who are not in a shock state, venous and arterial pH, BE, and bicarbonate had a good agreement to be an alternative for arterial parameters. However, venous and arterial PCO_2 agreement was poor and unpredictable to be useful in emergency medical care [15]. Rudkin et al. demonstrated that in hypovolemic patients, due to peripheral vasoconstriction and blood shunting to vital organs, peripheral venous pH, HCO_3-, and PCO_2 do not agree sufficiently to surrogate their arterial equivalents [16].

In veterinary medicine, Tamura and colleagues documented good agreement between BE in arterial and venous samples. However, there were considerable differences in the pH, PCO_2, PO_2, and HCO_3- between central venous and arterial blood gas in conscious dogs. These authors suggested that the analysis of both venous and arterial blood gas is indispensable for hemodynamic evaluation in dogs [5]. Another study on rabbits with hemorrhagic shock showed a significant difference for arteriovenous PCO_2, while the statistical difference for pH was not significant [7].

Blood gas is principally analyzed to assess acid-base status (typically pH and bicarbonate) and respiratory function (typically PCO2 and to some extent pH) in the emergency department [15]. Our results demonstrated no difference between arterial and venous pH, HCO_3-, and PCO_2 during fluid resuscitation of hypovolemic dogs. Therefore, these parameters can be used interchangeably. The partial pressure of oxygen is an indicator of efficient oxygenation of the lungs and BE is considered the best marker of changes in blood volume. A decrease in BE indicates an increased oxygen debt [4, 6]. According to the results of this study, due to statistical differences, venous PO_2 and BE are not valid alternatives to similar arterial parameters in the fluid resuscitation of hypovolemic dogs.

The differences in our results with prior studies and the published reference ranges may be due to discrepancies in the methodology of experience. Blood gas parameters can change in a few seconds with wide ranges. Therefore, there may be some degrees of discrepancy between the parameters obtained from the two standard blood gas analyzers [11]. The composition of the blood gas largely depends on the site of blood collection, so that in a hypovolemic state, the occurrence of compensatory peripheral vasoconstriction uncouples the central and peripheral vascular compartments [16]. Several venous samples (e.g., central vein, jugular vein, and mixed venous blood) can reliably present the acid-base status. Previous human medicine studies have shown central venous blood gas authenticity as a successor to arterial blood gas. In the current study, jugular blood samples were used to evaluate venous blood gas because it was found that the values of jugular vein samples are similar to the central vein [5]. Furthermore, to minimize the errors during the analysis of blood samples, we used the evacuated syringe technique and expelled the air in the syringes immediately. The analysis was completed in 5 min after blood collection.

Our study had two main limitations that need to be highlighted. First, this research was not conducted on conscious dogs. Therefore, the possible compensatory response of the respiratory system in a hypovolemic state was not investigated. However, performing this experiment without the induction of anesthesia was certainly stressful for the animals. Second, the post-resuscitation monitoring period was short. It has been documented that acidosis can reduce myocardial contractility and affect the inflammatory response [17]. Interestingly, higher BE and PCO_2 have been shown to be related to improved survival in patients with hemorrhagic shock [6]. Nonetheless, in this canine model of hypovolemic shock, no casualties were observed following the one-week animal monitoring period.

In conclusion, we analyzed all the commonly used venous and arterial blood gas parameters during resuscitation with LR and HES in hypovolemic dogs. We found that dogs with hypovolemic shock experienced acidosis. Moreover, following fluid resuscitation, most of the parameters, except for venous PCO_2 and HCO_3- in the groups resuscitated with LR and HES, respectively, returned to the control stage values. In addition, our results showed that arterial and venous pH, HCO_3-, and PCO_2 could be used interchangeably to serially monitor the hypovolemic dogs

during fluid resuscitation. Arterial and venous PO_2 and BE had significant differences in the resuscitation stage. However, a clinically acceptable difference between venous and arterial parameters is not known and should be investigated in the future. Given that blood gas parameters should be interpreted according to the individual patient's clinical status, it is recommended that blood gas analysis, as a reliable method to identify and manage critically ill patients, be performed on the other types of shock in small animals.

Materials and Methods

Animals

The present survey was authorized by the Research Ethical Committee of the Shahid Chamran University of Ahvaz with code EE/97.24.3.49872/Scu.ac.ir. Ten male Iranian native dogs with an approximate age of 1.5-3.5 years and body weight of 18.57 ± 4.82 kg were evaluated. The dogs included in the study were healthy based on clinical, electrocardiographic, and echocardiographic parameters, and were excluded in the case of cardiopulmonary disease. The dogs had free access to water, but the food was withheld for 12 h before the experiment. In this study, splenectomy was not performed.

Instrumentation

Initially, the right cephalic vein (for medication administration and fluid resuscitation) and the left external jugular vein (for collecting venous blood samples) were cannulated with an 18-gauge catheter. Next, the induction of anesthesia was performed with an intravenous dosage of 6 mg/kg propofol (Lipuro 1%, Melsungen, Germany) and 5 µg/kg fentanyl (Caspian, Rasht, Iran). Following the intubation of dogs with an 8-8.5 mm cuffed endotracheal tube and immobilization in right recumbency, the maintenance of anesthesia was performed using 1.8% isoflurane in 100% oxygen. The medial surface of the right hind limb of dogs was dissected and the exposed femoral artery was catheterized with a 14-gauge angiocath connected to a three-way stopcock for bleeding, direct blood pressure measurement, and collecting arterial blood samples [18]. Using a multi-parameter monitor system (PM-9000Vet, Burtons, Kent, UK), vital parameters, such as respiratory rate, blood pressure, and heart rate were assessed during the experiment. Furthermore, body temperature was monitored by a rectal probe and was preserved at 37°C-38°C using a heating mattress.

Experimental Protocol

In the present study, the eight-step (A1-A8) analysis of venous and arterial blood gas was performed in five divided stages as below [18]:

Control stage (A1): Base-line blood gas analysis was performed after instrumentation and establishment of anesthesia.

Hemorrhagic stage (A2): Blood was collected from each dog up to an arterial mean pressure of 40-50 mm Hg. This step lasted 30 min and the collected blood was stored in blood packets.

Hypovolemic stage (A3): Dogs were maintained in a hypovolemia state for 30 min during which no fluid was administered. In the case of compensatory physiological responses and an increase in mean arterial pressure, more blood was collected to return the mean arterial pressure to the range of 40-50 mm Hg.

Resuscitation stage (A4-A7): Dogs were accidentally divided into two identical groups and fluid resuscitation was performed in four consecutive 15-min steps. The animals in groups A and B were resuscitated with 20 ml/kg of LR (Shahid Ghazi Pharmaceutical, Tabriz, Iran) and 5 ml/kg of HES (Voluven 6%, Homburg, Germany), respectively.

Post-resuscitation stage (A8): The last step of blood gas analysis was accomplished 60 min after the end of fluid therapy. Afterward, the dogs recovered from the anesthesia.

Blood gas analysis

In each of the mentioned steps, 1 ml of venous and arterial blood samples was taken with pre-heparinized (5000 unit/ml of sodium heparin, Alborz Darou Pharmaceutical, Tehran, Iran) insulin syringes. Next, blood samples were analyzed as soon as possible for pH, PCO_2, PO2, HCO_3-, and BE using a portable blood gas analyzer (EDAN, i15 Blood Gas and Chemistry Analyzer, Shenzhen, China).

Data analysis

The SPSS software version 24 (IBM Corporation, New York, USA) was utilized for statistical analysis. All data are shown as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was applied to evaluate the normal distribution of data. Changes in the studied parameters during the experimental steps were tested by repeated measures analysis of variance. Moreover, in order to evaluate the difference between venous and arterial blood gas parameters, paired sample t-test was used. A p-value of 0.05 or less means that a finding is statistically significant.

Authors' Conributions

S.Y, S.A., E.Ç., B.E.K. and N.S. conceived and planned the experiments. All authors took part in the operations. S.Y. took part in the writing of the play.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Modulatory roles of ergothioneine on heat shock protein-70, tumour necrosis factor-alpha, and rectal temperatures of Arabian stallions following race of 2000 m in a hot-dry environment

Adakole S. Adah[a], Joseph O. Ayo[b], Peter I. Rekwot[c], Tagang Aluwong[b], Deborah A. Adah[d]

[a] Department of Veterinary Physiology and Biochemistry, University of Ilorin, P. M. B. 1515, Ilorin, Nigeria.
[b] Department of Veterinary Physiology, Ahmadu Bello University, P. M. B. 1044, Samaru- Zaria, Nigeria.
[c] National Animal Production Research Institute, P. M. B. 1096, Shika-Zaria, Nigeria.
[d] Department of Veterinary Medicine, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

ABSTRACT

Experiments were performed to determine the effect of ergothioneine on rectal temperature and the serum concentrations of heat shock protein-70 (HSP-70) and tumor necrosis factor-α (TNF-α) in stallions following a race of 2000 m in a hot-dry environment. Eighteen stallions weighing approximately 400 kg each were used for the experiment. They were divided into three groups of six stallions each. Group I (EEX) was the experimental group that was administered ergothioneine (0.5 mg/kg per os), while group II (EEC) did not receive ergothioneine before exercise. The third group (EEN) was neither administered ergothioneine nor exercised. The dry-bulb temperature and the relative humidity of the experiment were determined for six days and on the day of the experiment. The temperature-humidity index was also calculated. Rectal temperature, serum HSP-70, and TNF-α concentrations of all horses were measured before commencement, immediately after, and 2 h after the exercise. The dry-bulb temperature and relative humidity which showed diurnal fluctuations increased significantly ($p < 0.05$) between 06.00 h and 12.00 h (22.6 ± 1.23 and 38.6 ± 6.5, respectively). Serum TNF-α and HSP-70 levels of the stallions in the EEX group were higher than the values obtained in the EEC and EEN groups ($p < 0.05$). The values of rectal temperature obtained were lower ($p < 0.05$) in the EEX group than in the other groups. Therefore, it could be concluded that ergothioneine modulated rectal temperature, as well as TNF-α and HSP-70 concentrations in the stallions, and might be beneficial to horses during exercise.

Keywords

Ergothioneine, hot-dry season, tumor necrosis factor-α, heat shock protein-70, rectal temperature

Number of Figures: 2
Number of Tables: 4
Number of References:: 39
Number of Pages: 7

Abbreviations

HSP-70: Heat shock protein-70
TNF-α: Tumor necrosis factor-α
EEX: Group administered ergothioneine before exercise
EEC: Group was not administered ergothioneine but exercised
EEN: Group not administered ergothioneine and not exercised
THI: Temperature-humidity index
RH: Relative humidity
DBT: Dry-bulb temperature
OCTN-1: Organic cation transporter novel type-1
ELISA: Enzyme-linked immunosorbent assay

Corresponding author: *Adakole S. Adah*
Email: adakole.as@unilorin.edu.ng
Tel: +2348036848157

Introduction

Horses are elite athletes compared to other animals; their respiratory, musculoskeletal, and cardiovascular systems are the most important systems during exercise [1, 2, 3]. Exercise testing is used to evaluate fitness and training status in these animals [4], and it is crucial to understand how exercise influences physiological responses so that the tests can be applied correctly [5, 6].

Determination of rectal temperature is the most accurate method of evaluating the core body temperature and is considered an important index of the body's heat load [7, 8]. In comparison with other animals, the horse appears to have the disadvantage of having a fast metabolic rate and a small surface area for heat dissipation, especially when sweat evaporation is the primary mechanism of heat dissipation. Sweating dissipates at least two-thirds of the metabolic heat load in most workout situations [9].

Rectal temperature is closely linked to heat load following exercise; therefore, it is useful to examine health status under different physiological conditions. It is of value in evaluating the effects of training and also to examine the core body temperature capacity which is an important index of thermoregulation in horses [10, 11]. The work output of muscles and heat dissipation via the skin of horses during exercise depends on the heart size and capacity to deliver large volumes of blood to the tissues and the splenic reserve supply [12, 13]. It also depends on the capacity of the respiratory system to supply sufficient oxygen for cellular respiration [14, 15, 16].

Exposure of body tissues and cells to environmental stressors during exercise can cause serious disruptions in cellular homeostasis, which is primarily caused by harmful protein modifications, such as unfolding, misfolding, or aggregation. Cells produce a set of proteins known as HSPs or stress proteins in response to stress proteins, such as myosin heavy chain. In horses, exercise is a physiological stressor that has been established to affect the levels of HSPs. The HSPs are upregulated in response to cellular stress and are necessary to maintain cellular functions and prevent cellular death [17].

In horses, decreased skeletal muscle mass and strength have been associated with a range of chronic illnesses and higher mortality [18]. Consequently, understanding the underlying adaptability of skeletal muscle is crucial for maximizing health and longevity. Skeletal muscles have an exceptional ability to change phenotype in response to mechanical/metabolic stimuli, with muscle fiber size increasing (i.e., hypertrophy) or decreasing (i.e., atrophy) [19].

When secreted from muscle, TNF-α plays a crucial role in a muscle state change. TNF-α is a pro-inflammatory cytokine that has been linked to muscle tissue loss, particularly in disease processes, such as cachexia [20] and wasting syndrome [21]. However, high levels of circulating TNF-α after exercise, for example, have been linked to higher muscle mass and strength in healthy adult horses due to the anti-inflammatory functions under certain conditions [20].

Ergothioneine is a relatively stable antioxidant because it does not auto-oxidize at physiological pH and does not enhance the production of hydroxyl radicals from H_2O_2 and Fe^{2-} ions as a Fenton reaction [22]. The fast clearance from the circulation into retained tissues with little metabolism and high stability, as well as its long half-life (approximately 30 days) and reduced tendency to auto-oxidize or produce free radicals from peroxide and iron at physiologic pH, are all beneficial physiologic properties of ergothioneine [23]. Ergothioneine requires the specific carrier protein OCTN-1 to cross the cell membrane [24, 25]. OCTN-1 is mainly found in the kidneys, trachea, erythrocytes, lungs, heart, and bone marrow [23]. Unlike other antioxidants, such as glutathione or N-acetyl-L-cysteine, EGT acts as a cation chelator [26], bioenergetics factor [27], the immune regulator [28], and antioxidant [29], and is widely distributed within the body tissues [30].

The aim of this study was to evaluate the effect of ergothioneine on rectal temperature and HSP-70 and TNF-α concentrations in response to exercise in horses in a hot-dry environment.

Results

The thermal environmental parameters obtained during the period of the study are presented in Table 1. The mean DBT increased significantly from 22.6°C ± 0.24°C at 06.00 h to 38.6°C ± 0.53°C at 12.00 h ($p < 0.05$) (Table 1). The mean THI also rose significantly from 76.41°C ± 0.24°C to 83.36°C ± 0.53°C at 06.00 h and 12.00 h, respectively ($p < 0.05$). The rectal temperature responses are shown in Table 2. The rectal temperature obtained in the EEX group (37.82°C ± 1.91°C) was lower ($p < 0.05$) than the value obtained in the EEC group (38.87°C ± 4.61°C) 15 min post-exercise.

Figure 1 shows serum HSP-70 concentrations of the stallions. The HSP-70 concentration of the EEX group was significantly ($p < 0.05$) higher than the values obtained in the EEC and EEN groups.

Figure 2 shows the concentration of TNF-α in the stallions. The TNF-α concentration of the EEX group was significantly ($p < 0.05$) higher than the values obtained in the EEC and EEN groups.

Discussion

The thermal environmental data recorded during the study period were characterized by high temperatue humidity index, ambient temperature, and relative humidity, typical of the hot dry season in the Southern Guinea Savannah zone of Nigeria. The mean AT values recorded during the research period were higher than the established thermoneutral zones of 5°C-25°C for horses [32, 33]. Moreover, the mean RH of 74.3% ± 0.73% and 78.8% ± 0.77% recorded at 12.00 h and 18.00 h, respectively, are clearly above the 70% recommended for horses [34].

The results indicated that the natural meteorological conditions were stressful to the horses and may impair their respiratory and cardiovascular responses and also impair their performance [10]. High ambient temperature and high RH are the most important meteorological indices, that cause heat stress in horses [7]. The heat generated during exercise, which is a byproduct of the inefficient metabolic production of energy, can accumulate when ambient temperature and RH are high because a hot, humid environment as observed in this study imposes added thermal stress on horses during exercise resulting in the inability of horses to dissipate heat effectively [15, 35].

The high THI of 76.41 ± 0.24 to 83.24 ± 0.49 recorded in the present study is higher than the accepted level recommended for horses [34] indicating that meteorological conditions prevailing in the study area were unfavorable for horse performance. Therefore, measures and supplements aimed at alleviating the effects of high THI are necessary to reduce the risks of heat stress and enhance performance. The rectal temperature of the EEX group was lower than the EEC group after exercise suggesting a modulatory role of ergothioneine on the thermoregulatory system of the stallions after exercise. Supplementation with ergothioneine before exercise suggests that the agent helps in a more efficient heat dissipation mechanism by a process that requires further study, thereby reducing the risk of exercise-induced hyperthermia, rhabdomyolysis, and heat stroke [36].

The concentration of HSP-70 was higher in the EEX group compared to the EEC and EEN groups indicating a modulatory role by ergothioneine. Ergothioneine may enhance the synthesis of HSP-70 by a mechanism that requires further study.

Table 1.
Thermal environmental parameters of the experimental site in the hot-dry season

Time of the Day	Dry-Bulb Temperature (°C)	Relative Humidity (%)	Temperature-Humidity Index
06.00	22.6 ± 1.23[a]	64.4 ± 2.34[a]	76.41 ± 0.56[a]
	(22-24)	(63-68)	(68.71-71.65)
12.00	38.6 ± 6.53[b]	74.3± 6.73[b]	83.36 ± 4.53[b]
	(37-39)	(72-78)	(81.32-89.01)
18.00	36.5 ± 0.17[1]	78.8 ± 5.98[2]	83.24 ± 3.49[2]
	(36-37)	(76-81)	(83.21-84.95)
Overall Mean ± SEM	37.22 ± 4.17	75.19 ± 5.98	78.19 ± 5.18

[a, b] Means in the same column with different superscript letters are significantly ($p < 0.05$) different
[1, 2] Means in the same row with different superscript numbers are significantly ($p < 0.05$) different
Values in parentheses are minimum to maximum

Table 2.
Rectal temperatures of stallions

	EEN (°C)	EEC (°C)	EEX (°C)
Pre-exercise	37.76 ± 3.61	37.69 ± 2.97	37.58 ± 2.77
15 min post-exercise	37.44 ± 2.91[a]	38.87 ± 4.61[b]	37.82 ± 1.91[a]
2 h post-exercise	37.43 ± 3.01	37.87 ± 3.13	37.53 ± 2.74

[a, b] Means in the same row with different superscript letters are significantly ($p < 0.05$) different
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX: Treated with ergothioneine before exercise

Table 3.
Heat shock protein-70 concentration in stallions subjected to exercise (values are in ng/ml)

	EEN (Mean ± SEM)	EEC (Mean ± SEM)	EEX (Mean ± SEM)	P values
Pre-exercise	112.32 ± 13.33	114.34 ± 12.76	114.54 ± 13.67	0.257
15 min post-exercise	117.54 ± 13.65[a]	118.54 ± 14.55[a]	137.34 ± 26.55[b]	0.019
2 h post-exercise	109.3 ± 10.76[a]	117.44 ± 11.55[a]	143.52 ± 28.77[b]	0.023

[a, b] Means in the same row with different superscript letters are significantly ($p < 0.05$) different
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX: Treated with ergothioneine before exercise

HSP-70 has been demonstrated to decrease inflammatory processes by suppressing oxidative stress, reducing apoptosis and hyperplasia, as well as inhibiting the expression of adhesion molecules that lead to leukocyte extravasation and inflammatory cytokine production. HSP-70 interacts with several signal transduction pathways that affect cell homeostasis, proliferation, differentiation, and cell death, and is related to less atherosclerotic intima thickening and a lower risk of coronary artery disease [37]. The concentration of TNF-α was also higher in the EEX group than in the other groups. Ergothioneine might have activated TNFR2 receptors by a mechanism not yet understood. TNF-TNFR2 interaction activates the reciprocal PI3K/Akt pathway [38]. The TNFR2–Etk–vascular endothelial growth factor receptor 2 (VEGFR 2) complex, which is important in cell adhesion, migration, survival, and proliferation, is formed by this pathway. The TNF-TNFR2 interaction also activates the PI3K/Akt pathway in the opposite direction. The TNFR2–Etk–VEGFR2 complex is implicated in cell adhesion, migration, survival, and proliferation after exercise [39]. Therefore, it can be inferred that supplementing racing horses with ergothioneine may be beneficial in the modeling, remodeling, and adaptation of the muscles to exercise.

Table 4.
Tumour necrosis factor-α concentration in stallions subjected to exercise (values are in pg/ml)

	EEN (Mean ± SEM)	EEC (Mean ± SEM)	EEX (Mean ± SEM)	P values
Pre-exercise	142.32 ± 16.13	144.34 ± 12.76	146.54 ± 12.67	0.224
15 min post-exercise	144.54 ± 16.25[a]	161.84 ± 14.55a	187.54 ± 26.55[b]	0.027
2 h post-exercise	147.36 ± 15.86[a]	167.74 ± 11.55[a]	183.52 ± 28.77[b]	0.031

[a, b] Means in the same row with different superscript letters are significantly ($p < 0.05$) different
EEN : Not treated, not exercised
EEC : Not treated but exercised
EEX : Treated with ergothioneine before exercise

According to our results, ergothioneine modulated rectal temperature, as well as TNF-α and HSP-70 concentrations in the studied stallions, and could be beneficial to horses during exercise.

Materials and Methods

Experimental Animals

The experiment involved 18 healthy Arabian stallions with a mean body weight of 401 ± 32.11 kg (395-404 kg) and an age of 5.7 ± 0.54 years (5-6 years). The stallions were from a standard stable in Ilorin, Nigeria (8 30 N, 4 33 E) that was exclusively used for pleasure riding. They were kept in a concrete-walled stable with a corrugated iron roof. They were fed hay with concentrate as a supplement. A constant supply of clean, cool water was also made available. Only apparently healthy animals were included in the research after the stallions were screened for gastrointestinal para-

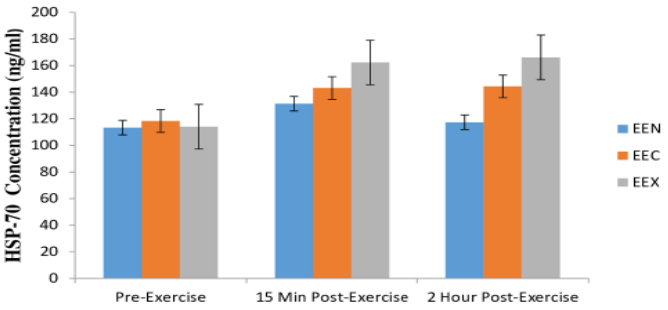

Figure 1.
Heat shock protein-70 (HSP-70) concentration of the stallions
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX: Treated with ergothioneine before exercise

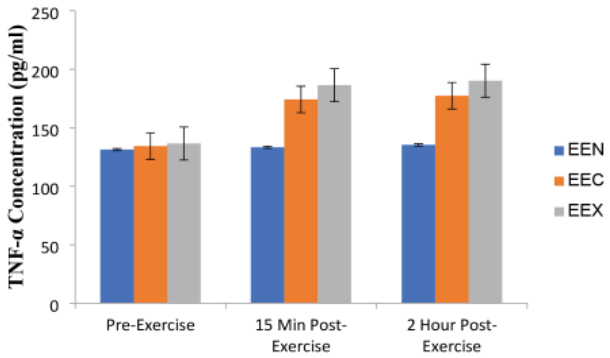

Figure 2.
Tumor necrosis factor-α (TNF-α) concentration of the stallions
EEN: Not treated, not exercised
EEC: Not treated but exercised
EEX:Treated with ergothioneine before exercise

sites, haemoparasites, and ectoparasites.

Experimental Design

The stallions were divided into three groups of six horses each: Group I (EEX) horses were treated with ergothioneine at a dose of 0.02 mg/kg daily for two months before being subjected to exercise, Group II (EEC) were not treated before exercise, and the third group (EEN) neither were administered ergothioneine nor exercised. Prior to the commencement of the experiment, RT was taken using a digital clinical thermometer (Hartman's Company, England). The thermometer was inserted into the rectum of each animal, and the RT value was recorded after a beep sound indicating the end of the reading.

Exercise Protocol

Each of the stallions in the EEX group was saddled by properly dressed and trained riders weighing 70.56 ± 4.23 kg on a conventional horse race track and raced for 2000 m at maximum speed. Horses were kept in the shade immediately after exercising.

Blood Sampling

Blood samples (10 ml) were taken from each stallion using 18-gauge needles before the experiment, as well as 15 min and 2 h after exercise. The collection site was disinfected before sampling with a cotton swab bathed in methylated spirit. The samples were taken from the jugular vein and were placed in plain vacuum containers.

Determination of Thermal Environmental Parameters

The DBT and RH were determined using the wet and dry-bulb thermometer (Mark, England). The THI was calculated using the formula of Hartmann et al. [31]:

$$THI = [DBT \times 0.8] + \{[RH/100 \times [DBT-14.4] + 46.4\}$$

Assessment of Heat Shock protein-70

MyBioSource horse HSP-70 ELISA detection kit (San Diego, California, USA) was used to measure the serum HSP-70 levels. It is an ELISA kit for detecting HSP-70 in microwell, strip plate format. The ELISA analytical biochemical technique used HSP-70 antibody-antigen interactions (immunosorbency) and a colorimetric detection device to detect HSP-70 antigen in serum.

Measurement of Serum Tumor Necrosis Factor-α

The concentration of TNF-α in the serum was evaluated using the TNF-α ELISA kit (Mybiosource.com, San Diego, California, United States). Sandwich enzyme immunoassay is the method used in the kit. The package included a microtiter plate that was pre-coated with an anti-TNF-α antibody. A biotin-conjugated antibody specific for tumor necrosis factor-α was added to the appropriate microtiter plate wells with serum. Afterwards, each microplate well was treated with Avidin coupled with horseradish peroxidase. Only the color of the wells containing TNF-α, biotin-conjugated antibody, and enzyme-conjugated Avidin changed once the TMB substrate solution was added. The color shift was detected spectrophotometrically at a wavelength of 450 nm 10 min after the enzyme-substrate reaction was stopped by adding sulphuric acid solution. By comparing the optical density of the samples to the standard curve, the concentration of TNF-α in the samples was measured.

Data Analysis

The results of this experiment are presented as mean ± SEM and were tested for normality using the Shapiro-Wilk test. All the data were found to be normally distributed. The one-way analysis of variance was used to analyze all the data, followed by the Tukey post-hoc test. The analyses were carried out using the software Graph Pad Prism (version 5.3).

Authors' Contributions

ASA and JOA conceptualized and designed the study. ASA, JOA, PIR, and TA wrote and edited the manuscript. DAA and ASA performed the experiments and analyzed the data.

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Conflicts of Interest

The authors state that they have no competing interests.

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Protective, immunologic, and histopathologic effects of garlic extract (*Allium sativum*) on rainbow trout (*Oncorhynchus mykiss*) exposed to acute toxicity with copper (Cu^{2+})

Mohammad Farhangi, Hossein Adineh, Mohammad Harsij

Department of Fisheries, Faculty of Agricultural Science and Natural Resources, Gonbad Kavous University, Gonbad, Iran.

ABSTRACT

The present study is an attempt to assess the protective and immunity effects of *Allium sativum* in *Oncorhynchus* mykiss to acute exposure to copper .55 rainbow trout fish with an average weight of 51.20 ± 3.73 g were subjected to various densities of copper (0.02, 0.1, 0.3, and 0.4 mg/l). Under stable conditions, the lethal concentration of copper was detected to be 0.40 mg/l. The treatments included a control with no Cu or garlic treatment (T1), feeding with garlic additive and Cu exposure (T2), and exposure to a lethal dose of Cu with no garlic additive (T3). The blood sample was used to designate hematological indices such as white blood cell (WBC) and red blood cell (RBC) count, hematocrit (HCT), hemoglobin (HB) mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes, monocytes, eosinophils percent and Immunological indices (glucose, total protein, lysozyme, IgM). The results indicated significant differences among the treatments when the concentration of copper was increased ($p < 0.05$). According to the results obtained, there were noteworthy differences in MCV, MCHC, and HCT amongst the treatments ($p < 0.05$). The histopathological results indicated that the main lesions were hyperplasia and necrosis of epithelial cells (in gill), enlargement of Bowman's capsule and tubular degeneration (in kidney), hepatocytes necrosis (in liver) in all the fish. Pathologic severity signs in sampled tissues were increased by increasing in concentration and exposure times of copper sulphate However, the results revealed that the use of garlic in dietary can be beneficial to increasing fish resistance to copper.

Keywords

Garlic extract, Histopathology, Immunity, Protective, Rainbow Trout (Oncorhynchus mykiss)

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Number of Tables: 4
Number of References:: 51
Number of Pages: 11

Abbreviations

MCV: Mean Corpuscular Volume,
MCHC: Mean Corpuscular Hemoglobin Concentration
Hct: Hematocrit
WBC: white blood cell
RBC: red blood cell (RBC)
HB: hemoglobin
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
Ph: Phosphorus

Corresponding author: *Mohammad Farhangi*
Email: s.farhangi@yahoo.com
Tel: +98(93)8133-5401

Introduction

Rainbow trout (*Oncorhynchus mykiss*) belongs to the family Salmonidae and is the main cultured freshwater fish species worldwide [38]. Fish require copper (Cu) as a micronutrient and obtain it from either water or diet [1]. Copper sulfate is an effective algaecide, but is toxic to many fish species at or near the concentration necessary for algal control [2]. Toxicity experiments show the sensitivity of an organism to a specific toxin. These experiments are useful for determining the acceptable amount of poison in an environment [3]. Toxicity studies are very necessary to predict the harmless amount of compounds in the environment [4].

Garlic (Allium sativum) is a small underground bulb crop from the family Alliaceae or Liliaceae [5, 6]. Some research has also reported the beneficial effects of garlic and its components on the prevention and treatment of fish diseases [7] and toxicosis [8]. However, to the best knowledge of the authors, there are no reports about the resistance rate of rainbow trout (Oncorhynchus mykiss) fed with Allium sativum. Therefore, this research aimed to evaluate the resistance of rainbow trout fed with garlic to acute exposure to copper.

Result

Toxicity Test

This study aimed to examine the poisonous influences of copper sulfate on rainbow trout (O. mykiss) nourished with A. sativum at various period intervals of 24, 48, 72, and 96 h. Table 1 shows the relationship between the mortality rate and the copper sulfate density of O. mykiss. The results presented that mortality reached 100% at the concentration of 0.4 mg/l. The acute poisonousness of copper sulfate indicated that mortality was directly compared to the level of copper sulfate although mortality was not found in the control group (Table 1).

The results attained from the acute static 96-h poisonousness tests of copper sulfate for rainbow trout and the estimated LC50 values with confidence limits are listed in Table 2. The mean LC50 of the effect of copper sulfate on rainbow trout was found to be 0.186 and 0.207 mg/l based on Finney's Probit analysis technique for T3 and T2, respectively. The blood factors of the treatments are shown in Table 2. No mortality was detected in T1 during the experiment.

Hematological and Immunological Changes

The analysis of hematological indices after 96 h revealed numerous significant variations in blood factors in the treatments ($p < 0.05$) (Table 3). Our results indicated that the highest WBC count was detected in T3. There were no significant differences in the Hct percentage of T2 and T3 compared to T1 ($p > 0.05$). Increases were detected in RBC and WBC counts, and Hct levels. Nonetheless, Hct and Hb gradually augmented after toxicity (Table 3).

The IgM and serum lysozyme of the rainbow trout in T2 were significantly ($p < 0.05$) higher than in T3 (Table 4). A lower glucose level was found in fish that received garlic at a lethal dose. The glucose level of the fish significantly rose in the fish exposed to copper ($p < 0.05$). However, the glucose level did not significantly change in T2 and T3 ($p > 0.05$).

Table 1.
The relationship between the copper sulfate concentration and the mortality rate of *Oncorhynchus mykiss*

Treatment		Copper concentration (mg/l)	Mortality No.					Cumulative Mortality % (96h)
			NO.	24 h	48 h	72 h	96 h	
Without garlic	0	0	11	0	0	0	0	0
	1	0.02	11	0	0	0	1	9.09
	2	0.1	11	2	1	1	0	36.36
	3	0.3	11	4	3	0	1	72.73
	4	0.4	11	6	5	0	0	100
With garlic	0	0	11	0	0	0	0	0
	1	0.02	11	0	0	0	0	0
	2	0.1	11	1	1	1	0	27.27
	3	0.3	11	3	3	1	1	72.73
	4	0.4	11	5	5	1	0	100

Table 2.
LC1–99 of Cu (mean ± standard error) of *Oncorhynchus mykiss*

LC	Estimate							
	24 h	**24 h**	48 h	48 h	72 h	72 h	**96 h**	**96 h**
	Without garlic	With garlic	Without garlic	With garlic	Without garlic	With garlic	Without garlic	With garlic
LC1	-.082	-.019	-.036	-.022	-.062	-.036	-.094	-.032
LC10	.119	.175	.079	.104	.060	.079	.032	.075
LC20	.204	.257	.127	.158	.111	.127	.085	.120
LC30	.265	.316	.162	.196	.148	.162	.123	.153
LC40	.317	.367	.192	.229	.180	.192	.155	.181
LC50	.366	.414	.220	.260	.209	.220	.186	.207
LC60	.414	.461	.248	.291	.239	.248	.216	.233
LC70	.466	.511	.277	.323	.270	.277	.248	.260
LC80	.527	.570	.312	.362	.307	.312	.287	.293
LC90	.612	.652	.361	.415	.359	.361	.339	.338
LC95	.682	.720	.401	.459	.401	.401	.383	.375
LC99	.813	.847	.476	.542	.480	.476	.465	.445

Table 3.
Hematological changes in Oncorhynchus mykiss acute exposed to copper after 30 minutes. Different lowercase letters within a column show significant effects of the treatments ($p > 0.05$)

Hematological parameters	Treatment 1 (Without garlic and copper)	Treatment 2 (lethal dose, garlic)	Treatment 3 (lethal dose, without garlic)
RBC (106 /µl)	0.41 ± 0.18^{c}	0.58 ± 0.02^{b}	0.69 ± 0.08^{a}
WBC (103 /µl)	113.35 ± 3.85^{b}	127.17 ± 2.11^{a}	131.53 ± 2.7^{a}
Hb (g /dl)	8.15 ± 0.45^{b}	8.99 ± 0.21^{a}	9.19 ± 0.45^{a}
Hct %	8.35 ± 0.65^{b}	18.70 ± 0.65^{a}	19.13 ± 0.20^{a}
MCV %	201.00 ± 14.90^{a}	182.63 ± 5.74^{b}	179.13 ± 6.27^{C}
MCH (Pg)	246.35 ± 7.15^{a}	221.17 ± 6.01^{b}	207.35 ± 5.45^{c}
MCHC (g /dl)	194.50 ± 3.80^{a}	171.23 ± 2.61^{b}	169.35 ± 5.45^{b}
Neutrophils %	8.20 ± 0.50^{b}	9.01 ± 0.22^{a}	9.12 ± 0.31^{a}
Lymphocytes %	79.01 ± 1.12^{c}	86.66 ± 2.51^{b}	96.11 ± 3.04^{a}
Monocytes	1.19 ± 0.01	1.11 ± 0.31	1.13 ± 0.57
Eosinophils %	1.05 ± 0.51^{b}	1.38 ± 0.57^{a}	1.41 ± 0.16^{a}

Behavioral Changes

The behavioral reaction of rainbow trout fingerlings was assessed every 12 h throughout the acute poisonousness experiments. The control group indicated usual behavior during the experiment time. The behavioral alterations in *O. mykiss* exposed to different concentrations of copper sulfate (ppm level) are as follows:

Control group: There were no behavioral alterations and losses detected during the test. The hypothetical spontaneous reaction was zero.

Test groups:There were downward and vertical swimming forms and unexpected activities. The motion of the fish became enormously relaxed and they showed behavioral irregularities, such as the loss of balance and water capsizing. Finally, the fish sank to

Table 4.
Immunological indices in Oncorhynchus mykiss acute exposed to copper after 30 minutes. Different lowercase letters in a column show significant effects of the treatments ($p > 0.05$)

Hematological parameters	Treatment 1 (Without garlic and copper)	Treatment 2 (lethal dose, garlic)	Treatment 3 (lethal dose, without garlic)
Glucose (mg/dl)	109.21 ± 6.18^{b}	129.21 ± 4.18^{a}	134.21± 6.18^{a}
Total protein (g/dl)	3.35 ± 0.05^{a}	1.18 ± 0.05^{b}	1.21 ± 0.01^{b}
Lysozyme (u/ml)	32.60 ± 2.38^{a}	26.20 ± 2.38^{b}	21.60 ± 3.38^{c}
IgM (mg/ml)	16.71 ± 1.74^{a}	12.31 ± 2.07^{b}	10.01 ± 1.14^{c}

the bottom and became immobile. Variations in behavioral reactions were initiated 45 min after dosing. In contradiction of the control group, slowing motion, losing balance, and spending more time at the bottom were detected. After 1 h, the fish were continually swimming indirectly with increased operculum motion and opening mouth for oxygen. Afterwards, the dead fish were observed while their mouth and operculum were open. Nevertheless, the fish exposed to copper indicated two patterns of behavior throughout the first 24 h. Those exposed to higher concentrations showed tension followed by unpredictable swimming and arranging on the water surface instantly after the addition of the material. However, those exposed to lower concentrations remained standing, made small movements, and rest at downward on the containers. Although there were similar signs in all treatments at high concentrations of copper, the fish fed with garlic showed high resistance to toxicity at low concentrations.

Histopathology

The histological samples indicated that the lesions of the fish gill, including edema, hyperemia, hyperplasia, hemorrhage, inflammation, epithelial cells necrosis of gill, and expansion of secondary lamella, were observed in diverse cases exposed to the fatal density of copper (Figures 1 and 2). The main lesions were found on the kidney of the fish, such as the expansion of Bowman's capsule, degenerated tubules of the kidney, epithelial cells necrosis of the kidney, hyperemia, hemorrhage, and migration of inflammatory cells. No lesions were detected in the kidney of the control fish samples (Figure 3). The liver lesions in the samples comprised inflammatory cell infiltration, hepatocyte necrosis, hemorrhage, and hyperemia (Figures 4 and 5). However, the control samples showed no lesions. Nevertheless, the impacts of poisonousness were diverse amongst the studied fish.

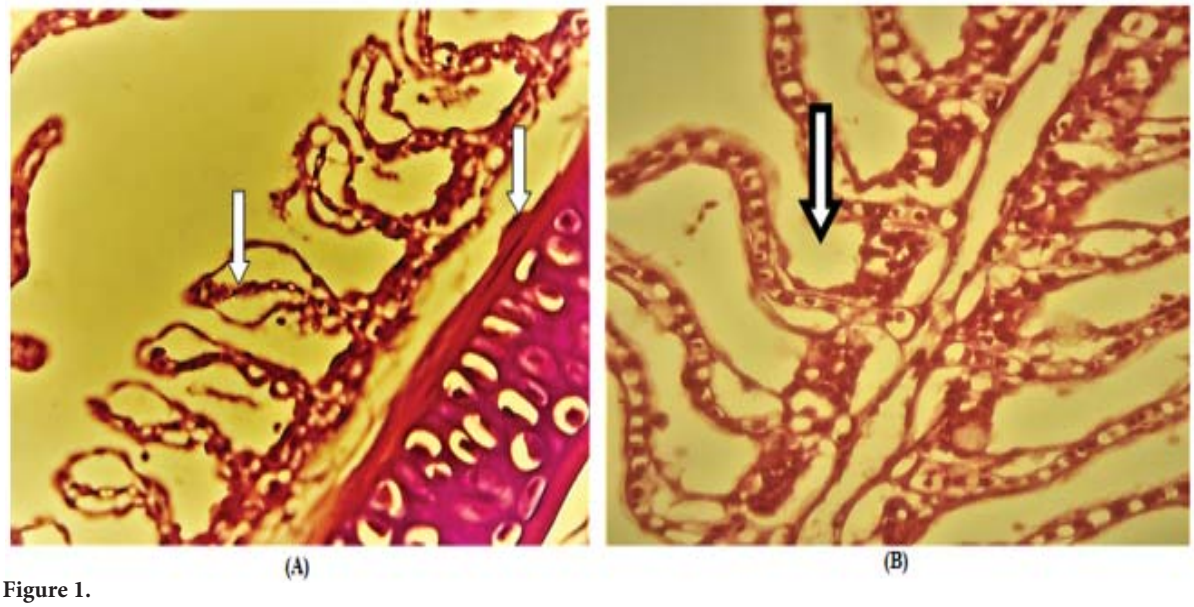


Figure 1.
Photomicrographs of gill in the fish exposed to lethal copper toxicity. Arrows show edema; A: with garlic, b: without garlic, (H & E, ×400)

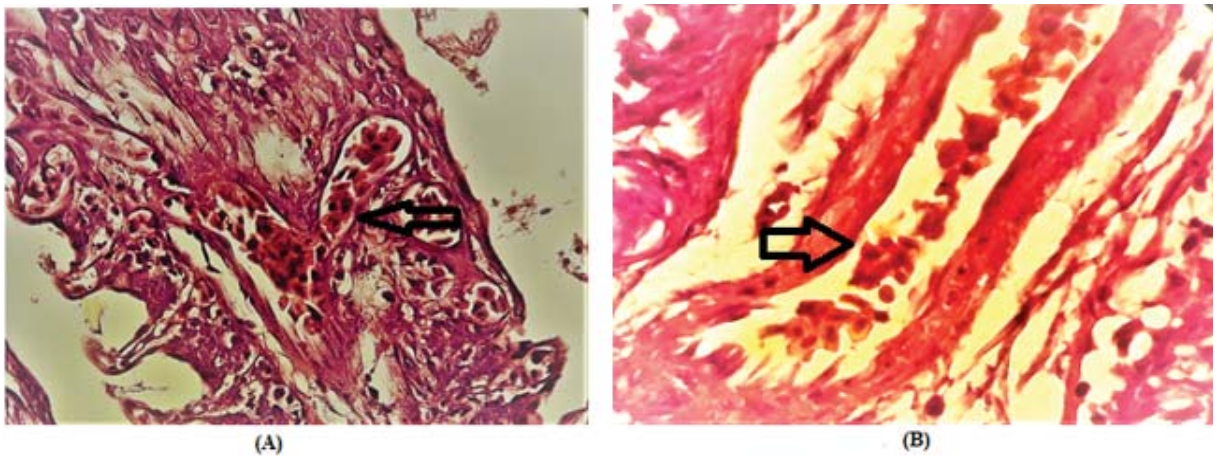


Figure 2.
Photomicrographs of gill in the fish exposed to lethal copper toxicity. Arrows show hyperemia; A: with garlic, B: without garlic, (H & E, ×400)

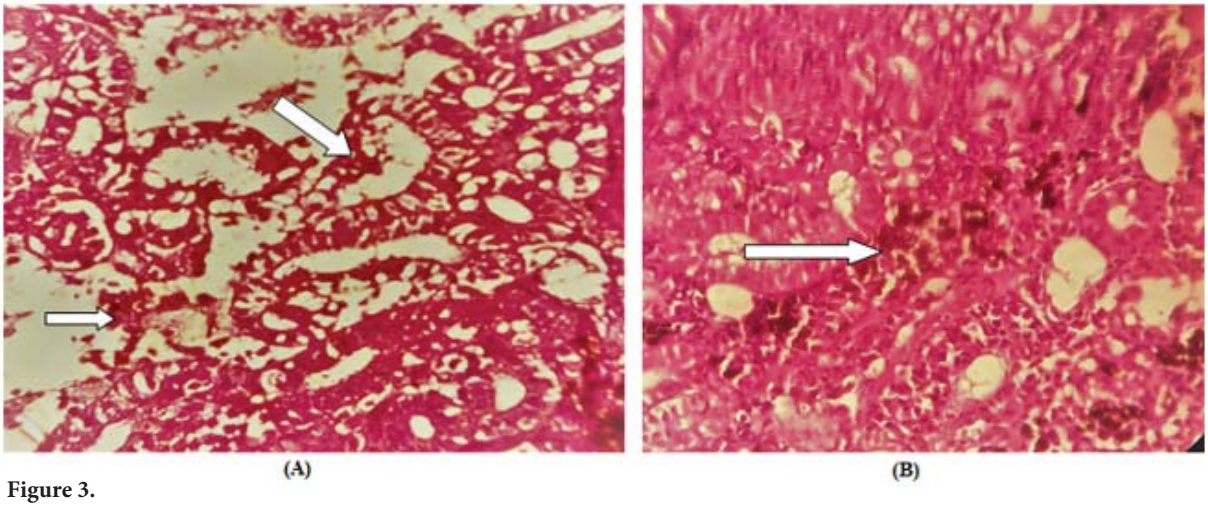


Figure 3.
Photomicrographs of the kidney in the fish exposed to lethal copper toxicity. Arrows show hemorrhage and degenerated tubules of the kidney; A: with garlic, B: without garlic, (H & E, ×400)

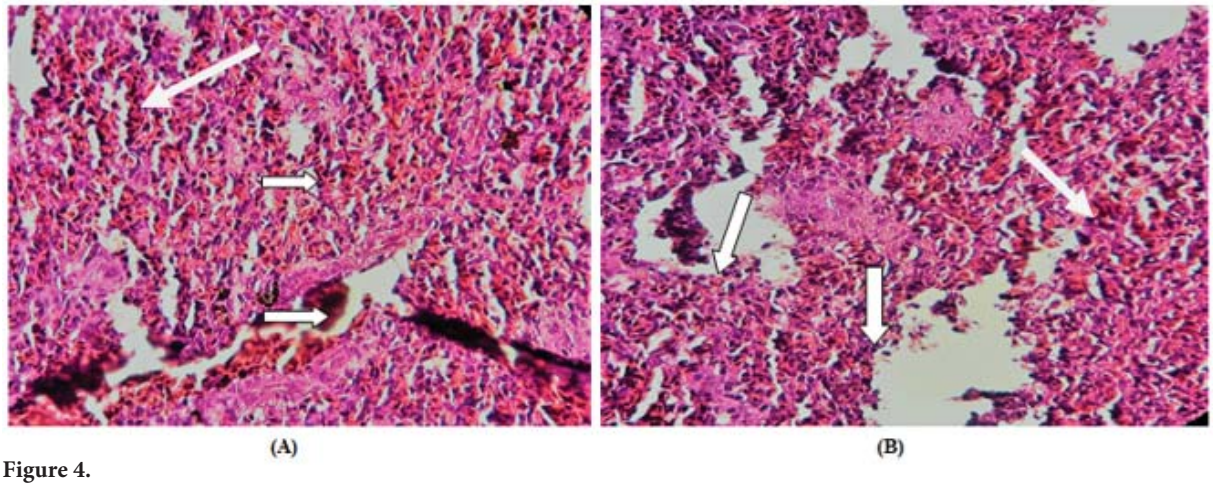
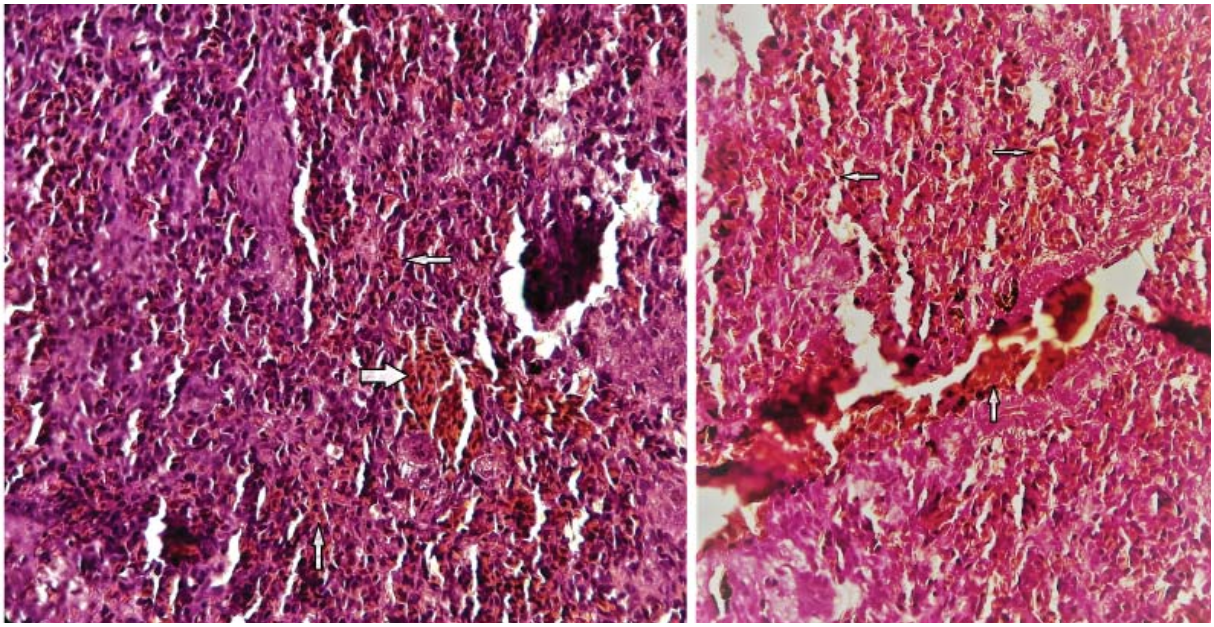


Figure 4.
Photomicrographs of the liver in the fish exposed to lethal copper toxicity. Arrows show hemorrhage, inflammatory cells infiltration, and hepatocytes necrosis; a: with garlic, b: without garlic, (H & E, ×400)



(A) (B)

Figure 5.
Photomicrographs of the liver in the fish exposed to lethal copper toxicity. Arrows show hemorrhage and hyperemia; a: with garlic, b: without garlic, (H & E, ×400)

Discussion

The current study aimed to investigate the poisonous impacts of copper on rainbow trout fish nourished with garlic at diverse period intervals of 24, 48, 72, and 96 h. Each species responds differently to material, so the significance of leading poisonousness examinations with diverse animals [9]. Nevertheless, behavioral variations in fish are a suitable biomarker for screening contamination and managing the marine environment [10]. In this research, the fish exposed to copper were detected to be highly irritable and showed demoniac swimming when handled. Their bodies were protected with thick mucus and finally deceased with open mouths. At the beginning of the bioassays and especially at higher concentrations, the fish would move to the water surface.

After 6-8 h of confusion at the upper concentrations, the behavior was more irregular amongst the rainbow trout. Fish became stiff, keeping a situation perpendicular to the downward of the tank and gulping air. However, the control fish were kept under the same conditions as the test fish in all respects except for the addition of copper toxicant. The fish acted regularly, showing none of the indications detected in the fish exposed to copper. Several authors have described the same variations in the gills of the fish exposed to diverse types of poisons.

Similar to the results obtained in this study, condensing of the lamellar epithelium (fusion) was described following the exposure of Nile tilapia (*Oreochromis niloticus L.*) to deltamethrin and glyphosate [11]. In the current study, Finney's Probit analysis presented 96-h LC50 value for *O. mykiss* exposed to copper sulfate densities of 0.186 and 0.207 mg/l for T2 and T3, respectively. Gul et al. [12] stated that 96-h LC50 for *P. reticulata*, Pallas, 1859 in hard water (235 mg CaCO3 / l, pH: 7.88, DO = 5.8 mg/l) was 30.8 and 30.9 mg/l based on Finney's Probit and Behrens-Karber's methods, respectively. Their reported values are slightly higher than our results. The dissimilarity can be attributed to changes in the water temperature, pH, and hardness. Bagdonas and Vosylienė [13] investigated Cu toxicity for rainbow trout. The 96-hr LC50 value for Cu was 0.2 mg/l. Hosseini et al. [14] reported the LC50 value of copper sulfate for rainbow trout fish in 24, 48, 72, and 96 h as to be 0.96, 4.79, 8.38, and 10 mg/l, respectively. Finlayson and Verrue [15] evaluated the toxicity of zinc, cadmium, and copper mixture for juvenile Chinook salmon. Bello-Olusoji and Adebola [16] investigated the toxicity of copper for freshwater prawn, *Caridina Africana*. The 96-h median fatal concentration for copper salts was 0.15 mg/l.

The variations in toxicity stated by different researchers may result from the changes in species, life stage, organism dimension, examination method, and water quality [9]. The fatal level varies with fish depending on species, age, and environmental factors. Inyang et al. [17] proved that hardness and water pH had a noteworthy direct relationship with the 96-h LC50 of copper in the examined fish. The highest death rate occurred throughout the early hours of exposure. However, all these remarks were more noticeable with the increasing densities of the poisons. A fish extremely vulnerable to the toxicity of a metal can be less or not vulnerable to the toxicity of another

metal at a similar concentration [18].

Salmonids are usually more susceptible to toxic metals in comparison with other fish species. Different experiments have revealed that the lethal concentration of copper is 0.1-10 mg/l for fish. We observed that the fatal level of copper was 0.4 mg/l for all the treatments. As CWQC [19] recommended, there was a safe level of LC50 96-h concentration (0.01 mg/l) for all aquatic animals. In the present study, NOEC was 0.02 mg/l in fish fed with *A. sativum*, whereas at the 0.02 mg/l concentration of copper, the mortality rate was 9.09% after 96 h in fish not fed *A. sativum*. This reveals that fish fed *A. sativum* are more resistant than others. However, given the classification of Louis et al. [20] for the degree of metal toxicity and the lethal concentration obtained in this research (0.4 mg/l), copper sulfate is very toxic (at the rate of 0.1-10 mg/l) to rainbow trout.

Hematological factors, such as complete blood cell count, are usually used for checking the health condition of farmed fish to several infections [21]. According to our results, a significant increase was observed in some hematologic indices, namely RBC, WBC, and HCT. The WBC and RBC count gradually rose after exposure. We observed that garlic could decrease WBC, MCV, and HCT values after 96 h exposure (Table 1). A decreasing effect was noted in the MCHC value in T2 and T3 after 96 h compared to the control value. Such damage to the cell organelles has been reported in various studies [22, 23]. According to KO et al. [22], hematological parameters, such as RBC count, Hct, and Hb value are sensitive indicators in the evaluation of fish metabolism under metal stress. In the present study, copper exposure induced an important rise in RBC, WBC, and Hct values in rainbow trout, which may be attributed to the augmentation in oxygen supply. The increased level of stress hormones (corticosteroids) caused a fall in leucocytes and erythrocytes in studied fish [7]. Kumar and Banerjee [24] reported that metals directly affected hematopoietic cells in the kidney and spleen and induced anemia by reducing the oxygen supply due to RBC concentration and decreased Hb. First, leucocytes initially increase to keep the phagocytosis mechanism and create antibacterial or antiviral compounds to halt the extent of the agent [25]. The attained results indicated a fascinating pattern of reaction in the hematological factors to garlic. Therefore, copper exposure induced a significant increase in WBC and RBC count in the treatments. Studies have shown that RBC and WBC counts were lower in fish fed garlic (T2). This reveals that the use of garlic could improve fish resistance. Similar results have been observed in erythrocytes and leucocytes in fish exposed to various toxicants and pathogens [7, 25]. On the other hand, the reduction in MCV detected after copper exposure can cause the shrinkage of erythrocytes due to hypoxia or microcytic anemia [25]. However, the quality and quantity of leukocytes are generally used to determine immune reactions, disease, and toxicants. Leukocytes are normally lower in healthy fish than in infected fish. As a result, they can be used as an indicator of infectious diseases similar to our study [26]. However, diminished Hb or RBC count can be an indicator of anemia [27]. Alterations in differential leukocyte count are identified as susceptible indices of poisons or dysfunction in hematological tissues or some infected illnesses [28]. Lymphocyte percentage was lower than usual lymphopenia and can be an appropriate marker of immune system shortage and xenobiotic material treatments that can also reduce the body's source of lymphocytes [16]. Furthermore, increases in monocyte and eosinophil and the reduction of lymphocytes were detected in WBCs (Table 1). According to Banaee et al. [29], utmost infections cause a type of neutrophilia. Analysis after 96 h exposure indicated that copper treatment (T3) was most efficient in RBC and WBC. The decrease in RBC and HCT levels in toxicant-treated fish can be attributed to the disturbance in erythropoiesis and the development of RBC [30]. Several researchers have described that the reduction in RBC count and HCT levels could be associated with the pressure after little exposure to metal [28, 30]. According to the results, garlic can rise antibody production and inhibit the undesirable effects of copper. During the experiment, RBC and hemoglobin rose in the garlic treatments in fatal doses in comparison with the other investigated treatments.

Histopathological variations in fish tissue can be used to identify the direct toxic impacts of compounds on target tissues because they reveal the loss instigated by the period and severity of exposure to the poisonous component and the tissue's adaptive capability [31, 32]. The gill is the first organ exposed to and influenced by toxins and pollutants. We observed significant deformations in the gill lamellae. The fusion of lamellae and the hyperemia of gill epithelium were apparent and telangiectasia was less common. Gills are in direct contact with water and react to ecological contamination and will be affected by copper. Nevertheless, in gill, at upper levels (> 0.2 mg/l), cell hyperplasia happened and the interlamellar spaces were filled. Several authors have described the same changes in the gills of fish exposed to diverse types of toxicants, such as Deltamethrin on Nile tilapia (*Oreochromis nilotica*) [33] and formalin on *Corydoras melanistius* [9]. The toxicity of copper is the reason for pathological changes in the gills, respiratory imbalance, instigating gill dysfunction, mortality, and osmoregulatory changes correlated with the fact that

the gill epithelium is the main contact surface because of having an enormous contact surface area with the external environments. Therefore, it is a target of the contaminants in the water [34]. Copper can disturb the nervous and cardiovascular systems of the fish when it is aggregated in the gills because it can adjust the transfer of salt (NaCl) into and out of the fish [35]. It can disturb the cellular structure and glucose metabolism of fish [36]. At the high concentrations of copper (> 0.3 mg/l), after 96 h of exposure, observations in the kidneys included the congestion of capillaries, focal necrosis, increases in Bowman's space, and necrosis of renal tubules. Similar results have been reported by others [9]. Various reasons have been put forth for fish mortality, including degenerated renal tubules and glomerulus, disrupted kidney function, disoriented osmoregulation caused by injuries to the gills, brain injuries causing convulsion [37], decreased oxygen transmission by blood, and increased plasma ammonia [35].

Comparing the data and figures demonstrates that at the 0.1 mg/l concentration of copper, the fish fed with garlic showed the lowest symptoms of sickness compared to others. Pathological results in the liver comprised swelling of hepatocytes, cellular deteriorations, and focal necrosis. These histopathological changes approved the toxic impact of copper. The liver is the main organ of several key metabolic pathways. Consequently, the toxic effects of compounds typically become visible in the liver. Numerous carbon-based combinations compel toxicopathic lesions in the liver of fish species. Acute toxic harms generally comprise cloudy swelling or hydropic degenerations along with the pyknosis, karyolysis, and karyorrhexis of nuclei [33].

In the liver, at lower concentrations (0.02 mg/l), as well as in gills, no important alterations were detected throughout the test. Finally, several injuries can result in fish death, including deteriorated renal tubules and glomerulus, and necrosis of hepatocytes. Toxicity disturbs liver and kidney function.

In Conclusion, Fish species were recently suggested as environmental biomarkers. Measuring metals in aquatic organisms may be a bioindicator of their impact on organisms and such information is beneficial in environmental threat evaluation. According to our findings, *A. sativum* is beneficial in the diet for improving fish resistance in fish systems.

Materials and Methods

Experimental Settings

This investigation was conducted in the Fish Research Laboratory of the Fishery Division, Gonbad Kavous University, Golestan, Iran. The rainbow trout fish was prepared from a cold-water fish farm located in Fazel Abad, Golestan province, Iran, and transported to the research laboratory in 2 h in special plastic bags with adequate oxygen. The experimental tanks were of 25-liter capacity. The weight of the fish used was 51.2 ± 3.73 g. The tanks were aerated for 24 h and dissolved oxygen was maintained at a saturation range by aeration. The test temperature (17.1°C ± 0.74°C), pH (8.09 ± 0.15), and dissolved oxygen (8.12 ± 1.02 mg/l) were measured using a portable multi-parameter Hack (Model 2000). The experimental diet was prepared by supplementing a basal formulated diet with 1% of garlic microencapsulation [38]. Fresh garlic (A. sativum) samples were bought from a local grocery store. The fish were fed a phytobiotic-enriched diet at least thrice a day for 40 days. The garlic was peeled and then powdered in the oven (ON-11E). Garlic powder was mixed with ethanol (purity of 70%) in a shaker at room temperature for 48 h. The solution was passed through a Whatman filter paper (42 µ) and placed in Rotary (HS-200S, Korea) at 75°C for 1 h to remove the alcohol. Next, the extract was placed in the oven at 38°C for 30 min. In order to encapsulate garlic extract, 30 g maltodextrin and 10 g Arabic gum were mixed with 60 g distilled water at 70°C-80°C and then homogenized (IKA T 25 digital ULTRA, Germany) for 1 h by homogenizer at 7000 g. The material was stored at 60°C inside the Ben-Marie (Memert. WNB 14, Germany) for 24 h. Coating materials and garlic extract (3:1 ratio) were mixed for 30 min. Microencapsulated garlic extract was preserved in a freeze dryer (Alpha-2 LD plus, Germany) for 24 h [38]. A commercial diet (Bezae Company, Iran) was employed as the experimental diet. The analyzed content was crude protein 44%-45%, crude fat 14%-14.5%, moisture 10%, crude fiber 2%-2.2%, absorbable phosphor 0.8%, and digestible energy 4300 kcal/kg.

The fish were not nourished 24 h previous to or throughout Cu exposure. Finally, the fish specimens were considered in three investigational groups (11 fish in separate treatment; replications with 2 fish specimens). The experiment treatments comprised a control with no Cu or garlic treatment (T1), feeding with garlic additive and Cu exposure (T2), and exposure to a lethal dose of Cu with no garlic additive (T3).

Toxicity Test

For the acute bioassay experiments, 11 fish specimens were separated for each concentration. The same number of fish served as control. Stock solutions of copper sulfate were provided by liquidation methodical grade copper sulfate (CuSO4.5H2O from Merck) in ddH2O. The added copper sulfate to each aquarium was considered after the volume of each tank was exactly specified. The control group was preserved in water without adding copper sulfate and garlic. Based on the preliminary tests and previous results (range finding test), the fish were exposed to 0, 0.02, 0.1, 0.3, and 0.4 mg/l of copper to determine LC50 for all the fish. No mortality was observed during this period. However, deceased fish were separated every 12 h and were eliminated from the aquaria immediately. The mortality rate was recorded at 24, 48, 72, and 96 h after the challenge. The behavior variations in all fish and the exposed fish to different doses of copper sulfate were assessed (e.g., breathing and general activity). The tests were performed by a stationary acute investigational technique [12] and the bioassay structure was implemented as defined in identical techniques [39].

Histopathological and Hematological Studies

Nine fish specimens were randomly selected and blood samples were taken from their caudal veins [40] in 2 ml disposable heparinized syringes. The blood samples were kept in test tubes containing EDTA (10 mM Tris–HCl, 250 mM sucrose, 100 mM sodium citrate, pH 7.6). In blood samples, WBC count, RBC count, Hct, and HB were evaluated [41]. The RBC count, Hct, and HB were analyzed immediately. After diluting with Hen-

dricks's diluting solution, the number of RBCs was counted using an optical microscope equipped with a hemocytometer (Improved Neuberger, Germany). HB concentration was measured by the Cyan-methemoglobin technique (Asan Pharm. Co., Ltd.). Hct value was analyzed by the microhematocrit centrifugation technique using a capillary tube and a microcentrifuge (Hawksley & Sons, Ltd.) [42].

To measure immune factors, blood was obtained from the caudal vein without heparin [43]. The blood samples were centrifuged at 5000 g at 4°C for 10 min and the serum samples were stored at -80oC until analysis [44].

In addition, 12 fish were randomly selected, euthanized, and dissected to collect the whole digestive tract, gill, liver, and kidney tissues [42]. Lysozyme levels were evaluated according to the Ellis method [45]. Immunoglobulin M content was estimated based on the technique explained by Saha et al. [46]. Alternative hemolytic supplement activity (ACH50) was assessed through the method explained by Sunyer and Tort [47] based on the hemolysis of rabbit red blood cells. The volume of the serum yielding 50% hemolysis (ACH50) was found and used to estimate the supplement activity of the samples (ACH50 is in units/ml). Serum glucose and total protein were measured by Pars Azmoon kits (Pars Azmoon Company, Iran) according to the manufacturer protocols. The samples were derived from the kidney, liver, and gill of the fish for histopathological evaluation. The arbitrarily deprived sections for tissue processing were fixed in 10% neutral buffered formalin [48]. Next, the samples were observed under an optical microscope for histological alterations and the histology of the control group was compared with the treated groups.

Statistical Analysis

The acute toxic influence of copper sulfate was assessed on the standard experiment species rainbow trout (O. mykiss) using Finney's [49] Probit Method (LC50 analysis). No observed effect concentration (NOEC) was considered the highest concentration that led to no death, while the lowest observed effect concentration (LOEC) was regarded as the lowest concentration that caused fish death [50]. Statistical analysis was performed by the analysis of variance (ANOVA) followed by Duncan's ($p < 0.05$) [51] Multiple Range Test (DMRT). SPSS version 16 was used for the analyses and the results are presented as mean ± SD.

Authors' Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Evaluation of supracondylar femur fractures in cats: a retrospective study

Sadık Yayla, Semih Altan, Emine Çatalkaya, Berna Ersöz-Kanay, Nahit Saylak

Department of Surgery, Faculty of Veterinary Medicine, University of Dicle, Diyarbakir-Turkey, 21280.

ABSTRACT

Femoral fractures in cats are common and are usually treated using pins and wires, lag screws, intramedullary pins, as well as interlocking pins and plates. Among femur fractures, supracondylar femur fractures have an important place, and both proximal and distal femur fractures are more difficult than diaphyseal fractures. This study, which was conducted on 55 cats with the distal femur and supracondylar fractures, aimed to retrospectively evaluate the results of three different methods used traditionally. These methods were Rush pinning, cross pinning, and locked anatomic plates. Despite the disadvantages of each of the techniques, no problem was found in the application of either of them. No complications, including pin migration or plate and screw related complications, were encountered in the postoperative period. Functional improvement was achieved in all cats. As a result, it can be said that the method to be used in supracondylar fractures in cats varies according to the surgeon's preference and the orthopedic materials in his or her inventory. However, the anatomical locking plates may be preferred due to their advantages, such as not using bandages and starting to use the limb in the early postoperative period.

Keywords

Cats, Cross pinning, Locked L plates, Rush pinning, Supracondylar femur fractures

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Abbreviations

IV: intravenous
IM: intramuscular

Corresponding author: Sadık Yaylaa
Email: sadik.yayla@dicle.edu.tr
Tel: +90(41)2241-1000

Introduction

Femoral fractures in cats are common due to traumatic causes, such as falling from height and traffic accidents and constitute 20%-26% of total fractures [1-3]. According to the Salter-Harris classification, distal femur fractures are classified as metaphyseal, physeal, or epiphyseal in cats that are not yet fully grown, whereas distal metaphyseal fractures are defined as supracondylar fractures in adult cats [1, 3, 4]. Supracondylar fracture is one of the most common types in dogs and cats. Important factors affecting the treatment of distal femur fractures can be listed as correct surgical approach, minimal dissection, preservation of soft tissue and fracture line, anatomic reduction, adequate stabilization, appropriate material selection, and effective postoperative care [1, 4-7].

In any treatment method chosen, the desired goal is functional recovery by providing rapid bone healing. Distal femoral fractures in cats are successfully treated with pin application techniques (e.g., pin and wires, Rush pins, intramedullary threaded pins, interlocking pins, and cross pin) and plate osteosynthesis techniques [1, 8-12]. The aim of this study was to retrospectively compare different pin applications, such as Rush pin and cross-pin, and locked L plate osteosynthesis in supracondylar femoral fractures, which are common in cats.

Results

In the cases investigated in the current study, fracture occurred due to falling from height in 26 cases, traffic accident in 19 cases, crash in 3 cases, and unknown reason in 7 cases. Cats with other fractures, diaphragmatic hernia, or head trauma were not included in the study. In terms of the technique used, the cases were randomly distributed, and 20 cats were treated with the first method (rush pin, Figure 1), 17 cats with the second method (cross rush pin, Figure 2), and 18 cats with the third method (locking L plate, Figures 3 and 4).

It was determined that none of the cases had adverse problems during the operation and the treatment was completed without any problems. Cats who underwent plate osteosynthesis started walking within 3 days, while the functional recovery was achieved in all cats within 3 weeks postoperatively. There was atrophy, albeit very little, since the movement was limited in the bandaged cats. However, this situation resolved spontaneously when the cat started to use the relevant extremity. No serious complications, such as pin migration or plate- and screw-related complications were encountered in the postoperative period.

Figure 1.
An example of Rush pin implementation

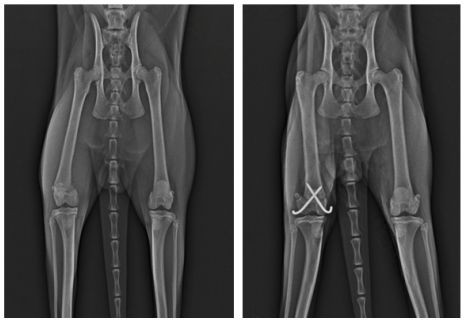

Figure 2.
An example of a cross pin application

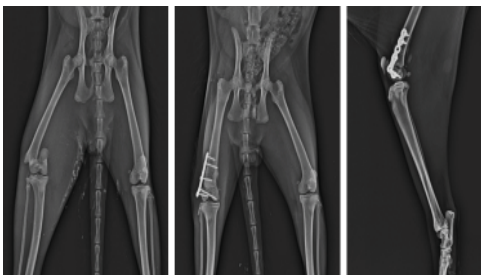

Figure 3.
A case with a locked anatomic L plate

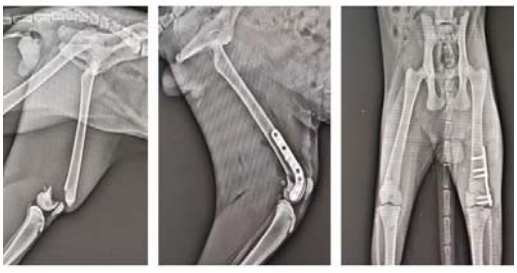

Figure 4.
A case with a locked anatomical cane plate

Discussion

Femoral fractures are common problems in cats and dogs, with supracondylar femur fractures having special clinical importance. The etiology is mostly a trauma, such as a traffic accident or falling from a height. Since cats have a lower body weight than dogs, post-operative care conditions are relatively easier.

However, the treatment options planned for supracondylar femur fractures in cats are very similar to those in dogs. Among these, cross rush pin application, cross pin technique, and anatomical plate osteosynthesis technique are widely used [1, 5, 9]. The present study aimed to retrospectively evaluate different treatment options, namely Rush pin application, cross-pin application, and locked L plate osteosynthesis for supracondylar femur fractures in cats.

Although some methods, such as intramedullary pinning or interlocking nailing techniques are widely used in femur fractures, it is known that intramedullary nails are insufficient for fixation compared to the femoral body for distal femur fractures, especially supracondylar fractures [1, 5, 9, 13-18]. Therefore, in contrast to retrograde pinning in supracondylar fractures, cross Rush pin application is preferred to the femoral medullary canal from the distal of the condyle, which is exposed by cutting the joint capsule. In addition, the fixation technique has become widespread in recent years by sending a Kirschner wire crosswise from the lateral and medial condyles to the femoral medulla. It is necessary to open the joint capsule both for rush nails and for the use of the cross pinning technique. In addition, in these two methods, it is difficult to maintain the immobility of the extremity, especially in hyperactive cats, to provide adequate immobilization in the postoperative period [1, 5]. We think that the bandage applied to prevent pin migration may cause some complications. Sometimes atrophy may develop in the extremity muscles after bandage usage. Despite these described disadvantages, Rush pin and cross pin techniques also have some advantages, including being easy to apply and being economical. The approaches to the condyles are very similar in Rush pin and cross pin. In the intraoperative process, there is almost no joint damage. However, extremity movements should be limited against pin migration in the postoperative period.

Recently, the number and variety of plaques used in veterinary orthopedics have increased with technological developments. With the widespread use of locking plates, the usage of shaped anatomical plates, such as T plate, L plate, and C plate became easier. Among these, the use of a locked L plate is extremely easy, especially for supracondylar femur fractures [4, 5, 9-11]. In addition, the use of locking plates, which allow animals to walk immediately after the operation, eliminates the need for bandages in the postoperative period. This is important to avoid bandage-related complications [9-11]. In our study, no complications related to the plate or screw were observed in the cats to which the plate was applied. Since bandages were not applied to these cats, they started to use the relevant extremity immediately after the surgery.

According to the data obtained from this study, it can be said that the Rush pin technique, cross pin technique, and locking plate are sufficient for fracture stabilization in supracondylar femur fractures in cats. Each technique has advantages as well as disadvantages. We observed in our study that bandage restricts the comfort of cats in the postoperative period rather than the success of the preferred method. As a result, the method to be used in supracondylar fractures in cats might vary based on the type of fracture, the surgeon's preference, and the available orthopedic materials in the inventory. However, the use of an anatomical locking plate may be preferred due to its advantages, including not using postoperative bandages and starting to use the limbs in the early postoperative period. Although these plates may provide more stability, they are more expensive than other methods, and the left-side and right-side plates should be available. In addition, caution should be exercised in the use of plaques in young cats before growth plates close.

Materials and Methods

In this study, 55 cats with supracondylar or distal femur fractures were selected out of the patients who presented to the Department of Surgery, Faculty of Veterinary Medicine, Dicle University, during 2019-2022 with the complaint of hind limb lameness. Cats (n = 55) included in the study were of different breeds (26 mixed breeds, 9 tabby breed cats, 5 Persian cats, 7 Van cats, 3 Ankara cats, 3 British, and 2 Siamese), different genders (32 female and 23 male), with the mean ± standard deviation age of 14.78 ± 5.80 months and mean ± standard deviation body weight of 3.18 ± 0.93 kg. Surgery was planned after the fracture was confirmed based on the physical and radiological examinations.

All operations were performed under general anesthesia. Each animal was administered 1 mg/kg IV xylazine for premedication and 15 mg/kg IV ketamine for anesthesia. Moreover, for multimodal analgesia during operation, a combination of 12 mg ketamine + 4.8 mg butorphanol + 40 µg medetomidine in 100 ml saline was given IV as 5ml/kg/h. After each animal in the study was placed in the lateral recumbency, routine preparations for the operation were made and the area was limited with sterile covers. A lateral parapatellar skin incision was made to the fracture line for an appropriate surgical approach. Subcutaneous tissues were dissected and the capsular incision was made. The fracture line was exposed by pulling the patella and patellar ligament medially. Next, the stifle joint was reached and the condyles were exposed. After this stage, three different methods were followed.

In the first method (n = 20), Rush pins sent over the side face of the medial and lateral condyles were placed in the proximal femur medulla (Figure 1). In the second method (n = 17), two pins, which were sent in a similar way to the Rush pin technique, were diagonally based on the proximal femoral cortex (Figure 2). In the third method (n=18), a locking L plate was placed craniolaterally to the femur (Figures 3 and 4). The area was routinely closed after the patella was brought to its normal position.

All animals using the first and second methods were protected with a bandage for 10 days postoperatively. A postoperative bandage was not applied in cats with plaque. Amoxicillin clavulanic acid at a dose of 12.5 mg/kg IM was injected for 5 days. In addition, 0.2 mg/kg IM meloxicam was used for 3 days. All cases were followed up clinically and radiologically with 1-week intervals postoperatively until functional recovery and callus formation

Table 1.
Thermal environmental parameters of the experimental site in the hot-dry season

Method of treatment	Breed	Gender		Bandage use	Comparison
		Male	Female		
First method (Rush pinning n=20	15 mixed	6	9	Bandage used	Bandage-related atrophy or poor circulation may be seen. Enough for good stabilization.
	2 tabby	2	0		
	2 Persian	1	1		
	1 Van	1	0		
Second method (Cross pinning) n=17	7 mixed	2	5	Bandage used	Bandage-related atrophy or poor circulation may be seen. It can be applied more easily than Rush pinning. Enough for good stabilization.
	4 tabby	3	1		
	3 Van	1	2		
	3 Ankara	1	2		
Third method (Locking L plate) n=18	4 mixed	2	2	No bandage	A more comfortable postoperative process. Enough for good stabilization. It is a great advantage to not have bandages and to start walking immediately the day after the operation. May be more expensive than other techniques and requires experience.
	3 tabby	1	2		
	3 Persian	0	3		
	3 Van	1	2		
	3 British	1	2		
	2 Siamese	1	1		

* Humerus, radius-ulna, tibia, and distal fractures were ignored in the animals included in the study. However, cats with vertebral and pelvic fractures requiring surgery were
excluded from the study.
** All of the cases had a distal fracture of the femur and there was no second fracture of the same femur.

was completed. In animals with Rush pins and cross pins, these pins were removed by the decision of either the veterinarian or the owner of the patient due to concerns that they would disrupt bone growth and cause irritation.

Authors' Contributions

S.Y., S.A., E.Ç., B.E.K. and N.S. conceived and planned the experiments. All authors took part in the operations. S.Y. wrote the manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

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Protective effect of abscisic Acid in a spinal cord injury model mediated by suppressed neuroinflammation

Maryam Rezaeezadeh Roukerd [a], Sahel Motaghi [a], Balal Sadeghi [b], Mehdi Abbasnejad [c]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
[b] Food, Hygiene and Public Health Department, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
[c] Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.

ABSTRACT

Abscisic acid (ABA) is a phytohormone with modulatory roles. The anti-inflammatory effect of this hormone has been reported on different animal tissues. Immediately after spinal cord injury (SCI), neuroinflammation causes neuropathic pain and locomotor impairments. We investigated the impacts of ABA as an anti-inflammatory substance on an acute SCI model. The weight-drop contusion injury model was applied for inducing SCI in rats. The solvent, ABA (10, 15 µg/rat, IT), and MP (30 mg/kg, IP) were administered after injury. For the evaluation of proinflammatory gene expression, a real-time polymerase chain reaction was applied for the two inflammation markers tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Moreover, the tail-flick and Basso, Beattie, Bresnahan (BBB) tests were performed to determine the effects of ABA on the neuropathic pain and locomotor function in the chronic phase of injury, respectively. Our data showed that ABA reduced the gene expression of TNF-α and IL-1β in the spinal cord of injured rats. It also increased the latency response to nociceptive thermal stimuli and improved locomotor function. Our findings showed the anti-inflammatory impacts of ABA in improving neuropathic pain and locomotor functional recovery after SCI.

Keywords

Abscisic acid, neuroinflammation, neuropathic pain, spinal cord injury

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Number of Pages: 10

Abbreviations

SCI: Spinal cord injury
CNS: Central nervous system
ABA: Abscisic acid
CSF: Cerebrospinal fluid
MP: Methylprednisolone
IL-1β: Interleukin-1β
TNF-α: Tumor necrosis factor
BBB test: Basso, Beattie, Bresnahan test

Corresponding author: *Sahel Motaghi*
Email: sahelmotaghi@uk.ac.ir
Tel: +98 9128191929

Introduction

Spinal cord injury (SCI) is a debilitating disease that leads to long-life physical impairment [1]. The last statistics in 2020 showed that approximately 54 cases per million people in the United States were affected by SCI [2]. The pathological events following SCI are divided into two broad events of primary and secondary injuries [3]. Primary injury is mechanical damage to the spinal cord and elicits a series of pathophysiological cascades that are termed secondary injuries [4]. The secondary insults exacerbate injury to the spinal cord because of leading to the destruction of axonal tracts and being one of the main barriers against functional recovery after SCI [5]. Neuroinflammation in the lesion area is the main biological event in secondary injuries [6]. Inflammation is one of the causes of neural damage after SCI [7]). Neuropathic pain is one of the other consequences of neuroinflammation, which occurs in the chronic phase of CNS injuries. Neuropathic pain is mediated by the neuroinflammatory response and follows pathological changes after injury. The role of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 is clarified by their pathological effects on neuropathic pain in the CNS [8, 9]. TNF-α has a critical role in recruiting other immune cells to participate in neurodegeneration procedures. It seems that preventing TNF-α upregulation can reduce pain and the progress of Wallerian degeneration [9]. The spinal cord has a more pronounced inflammatory response to injury than the brain, with twice as much neutrophil infiltration within 24 h, sustained macrophage infiltration, and enhanced lymphocyte infiltration [10]. This property makes the spinal cord vulnerable to secondary lesions [11]. Some studies demonstrated that the prevention of proinflammatory cytokines, including IL-1β and TNF-α, markedly improved functional recovery and induced injury apoptosis after SCI [11-13]. Therefore, inflammation limitation after SCI can be considered an important therapeutic target. Out of many chemical and natural agents with anti-inflammatory effects, MP is used widely [14].

Unfortunately, the role of steroids such as MP in acute SCI (ASCI) is unclear, and it has been correlated with an increased risk of pneumonia and hyperglycemia [15]. ABA presents in vegetables and fruits and can be obtained naturally through food. It is also generated endogenously in some human and animal tissues. Many studies have shown that ABA benefits human health. ABA has antidiabetic [16], antioxidant, and antiapoptotic [17] properties. It is also effective in curing ischemic retinopathy because of its angiogenic properties [18]. Another property of APA is its anti-inflammatory role in the CNS and other tissues [19-21]. ABA has no side effects even in high doses [22]. Therefore, we investigated the effect of ABA treatment on neuropathic pain and functional motor recovery in the chronic phase of SCI. Furthermore, molecular assessments were applied for evaluating the results of preventing inflammation in the recovery of insult.

Abbreviations-Cont'd

PPAR: Peroxisome Proliferator-Activated Receptor
IT: Intrathecal
IP: Intraperitoneal
DMSO: Dimethyl sulfoxide

Results

Effects of abscisic acid on TNF-α and IL-1β gene expression

As shown in Figure 1, in comparison with the control group, TNF-α was significantly upregulated in SCI and SCI + solvent injected groups ($p < 0.001$). Compared to SCI and SCI + solvent injected groups, the expression of TNF-α significantly reduced in the ABA and MP-treated groups ($p < 0.001$) (Figure 1A). IL-1β expression was higher in the SCI, SCI + solvent ($p < 0.001$), and SCI + ABA (10 µg/rat) groups ($p < 0.01$) in comparison with the control group. The expression of IL-1β significantly decreased in the SCI + ABA (10 µg/rat) ($p < 0.01$), SCI + ABA (15 µg/rat), and MP-treated groups compared to the SCI and SCI + solvent groups ($p < 0.001$). All melting curves obtained from PCR light cycler Roche are shown in Figure 2 (A, B, C).

Tail-flick test

As shown in Figure 3, the tail flick nociceptive threshold was significantly lower in the SCI and all SCI-treated groups when compared with the control group ($p < 0.001$). This parameter showed a significant increase from day 1 to 30 in the SCI + ABA (10, 15 µg/rat) and SCI+MP-treated groups in comparison with both SCI and SCI + solvent groups ($p < 0.01$ and $p < 0.001$). The thermal threshold was significantly higher in the SCI + MP-treated group in comparison with SCI + ABA (10 µg/rat) injected rats after 20 days ($p < 0.001$). Except on day 5 ($p < 0.01$), there was no significant difference between the SCI + MP and SCI + ABA (15 g/rat) groups. The tail flick nociceptive threshold showed a significant rise from day 5 to 30 in SCI + ABA (15 µg/rat) treated animals in comparison with SCI + ABA (10 µg/rat)-treated group ($p < 0.001$ and $p < 0.01$).

BBB test

Locomotor functional recovery after treatment

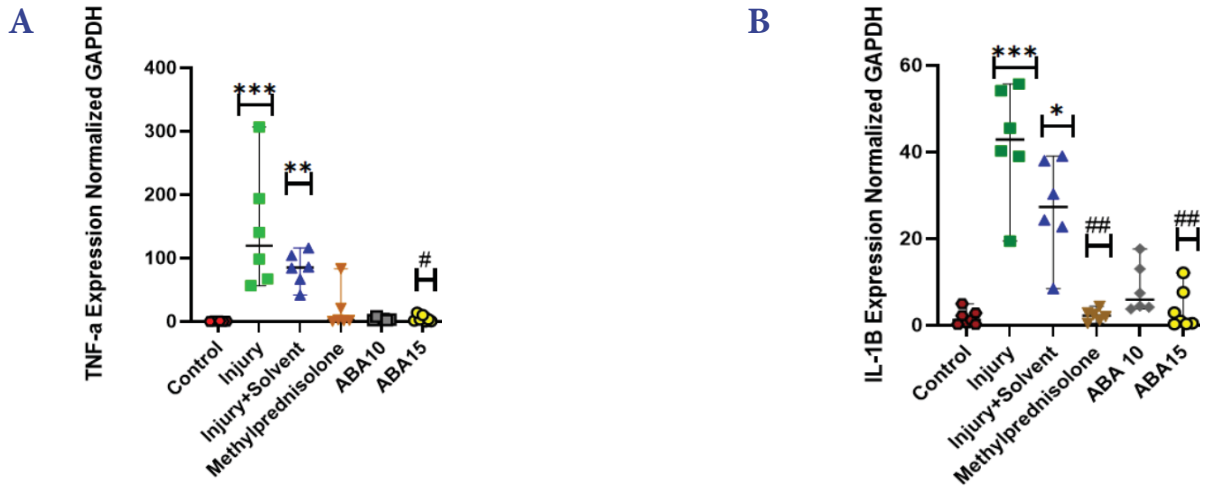

Figure 1.
The effects of abscisic acid (ABA) on gene expression of the proinflammatory markers TNF-α (A) and IL-1β (B) in the acute phase of SCI. Reported *p* values on graphs represent the statistical testing with ANOVA and Tukey's post hoc test. **: $p < 0.01$ and ***: $p < 0.001$ indicate the significant differences in comparison with the control group. ##: $p < 0.01$ and ###: $p < 0.001$ show the significant differences versus SCI and SCI with solvent injected groups.

by ABA was assessed with the BBB test. Our results showed a remarkable rise in BBB scores in the control group compared to all SCI-induced groups ($p < 0.001$). The BBB scores were significantly higher in the SCI+ABA (15 µg/rat)-treated rats during days 3-30 in comparison with the SCI and SC + solvent injected groups ($p < 0.01$ and $p < 0.001$). As presented in Figure 4, the same effect was seen in SCI + ABA (10 µg/rat)-treated rats on day 3 and from day 13 to 30 ($p < 0.05$, $p < 0.01$, and $p < 0.001$). The BBB scores in the SCI + MP-treated group showed a significant increase from day 11 to 30 ($p < 0.01$ and $p < 0.001$).

There was a significant difference between SCI + ABA (15 µg/rat) and SCI + MP-treated groups from day 7 to 9 ($p < 0.05$). A significant difference was found between SCI + ABA (10 µg/rat) and SCI + MP-treated groups on day 19 and during days 25-30 ($p < 0.05$ and $p < 0.01$). The BBB scores showed a significant increase from day 7 to 9 ($p < 0.01$), and on day 17 ($p < 0.001$) in SCI + ABA (15 µg/rat)-treated group in comparison with SCI + ABA (10 µg/rat)-treated group (Figure 4).

Discussion

The results of the present study indicated that the IT administration of ABA immediately after SCI inhibited proinflammatory cytokines TNF-α and IL-1β and subsequently improved neuropathic pain and locomotor function.

The damage after SCI triggers inflammatory responses with enhanced TNF-α, Il-1β, and other proinflammatory marker levels [23]. This process started by the blood-brain barrier damage and neural insult causes the early expression of cytokines in the injured spinal cord models in humans and animals. Just 30 min after SCI, both TNF-α and IL-1β levels raised and remained at the peak levels for 6 h. Different cells in the CNS tissues, such as astrocytes and microglia cells, immediately express these inflammatory mediators [24]. The molecular results of this study confirm the anti-inflammatory role of ABA. In line with this study, other investigations showed the anti-inflammatory effect of ABA in the CNS and other tissues [25-27]. There is an interaction between the immune system and the sensory nervous system in the generation of neuropathic pain. In other words, neuropathic pain is the result of somatosensory lesions that induce extreme inflammatory responses [28]. The results of the tail flick test in the current study indicated that the administration of ABA had antinociceptive effects and rose the latency response to thermal stimuli. Moreover, the BBB test showed an improvement in locomotor function following ABA administration. Our findings are in line with those of Mollashahi et al. (2018) who discovered that ABA had antinociceptive results in animal models for nociceptive tests, including hot plate, tail flick, and formalin tests. Furthermore, ABA was shown to impose its antinociception effects via the PPAR β/δ and opioid receptors [29]. Guri et al. demonstrated that human ABA-related genes are anchored to PPARγ with four homologs. In obese mice, ABA reduced the inflammation activated by microglia and white fat tissue-infiltrated macrophages. Nuclear receptors of PPARγ were activated by ABA in pre-adipocyte cell culture [25, 30].

The PPARs, which are defined as nuclear hormone receptors, are expressed in different sites of the CNS

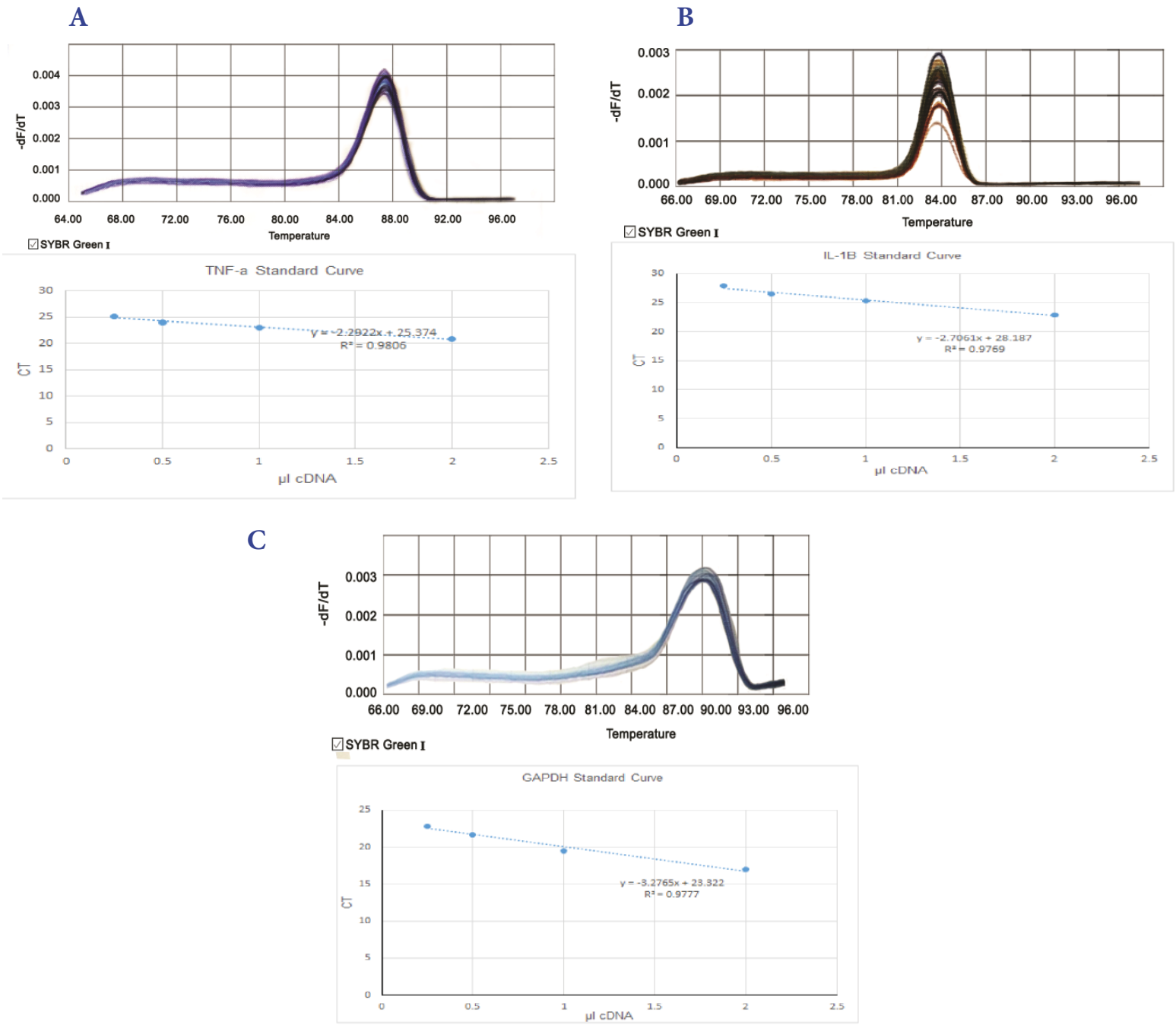

Figure 2.
The melting and standard curves.
A: TNF-α melting curve and standard curve. B: IL-1β melting curve and standard curve.
C: GAPDH melting curve and standard curve.

related to pain modulation. These areas are composed of the thoracic and lumbar spinal cord, rostral ventromedial medulla, amygdala, and periaqueductal grey [31, 32]. All three isotypes of PPARs, β/δ, and γ, can modulate the inflammatory response and nociceptive reactions after SCI [33]. The result indicates the neuroprotective effects of PPAR agonists in the model of SCI, although their specific roles are not entirely understood based on their isotype [33]. The results of some studies indicated that the neurogenic inflammation mechanism possibly is modulated by PPARs agonists by preventing substance P and histamine release from dorsal root ganglion. Moreover, the role of PPARs in pain modulation confirmed by molecular and pharmacological investigations [34], and its peripheral anti-inflammation effects have been reported [35]. The agonists of PPAR-γ ameliorate functional motor recovery via reducing gliosis and apoptosis, as well as preventing inflammatory mediators in the rat model of SCI approved by the FDA [36-38]. It is sug-

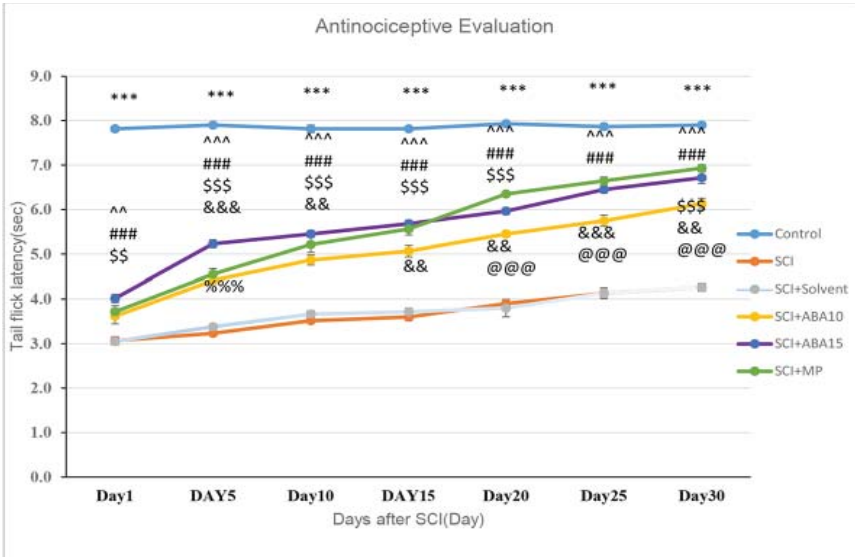

Figure 3.
The assessment of antinociceptive effect of abscisic acid (10, 15µg/rat) and methylprednisolone in the tail flick test during 30 days. Values are shown with mean ± S.E.M (n = 6 rats/group). ***$p < 0.001$ shows a significant difference in comparison with all SCI-induced groups. ### $p < 0.001$ shows the significant difference between SCI+ABA (15µg/rat) group and SCI and SC+ solvent injected groups. $$$p < 0.01$ and $$$ $p < 0.001$show significant differences between SCI+MP and SCI and SC+ solvent injected groups. ^^ $p < 0.01$ and ^^^$p < 0.001$show significant differences between SCI+ABA (10µg/rat) group and both SCI and SC+ solvent injected groups. %%%$p < 0.001$shows the significant difference compared to SCI+ABA (15µg/rat) and SCI+MP group. @@@$p < 0.001$shows the significant difference between the SCI+MP group and SCI+ABA (10µg/rat) group. && $p < 0.01$ and &&&$p < 0.001$show the significant difference in comparison between SCI+ABA (15µg/rat) and SCI+ABA group (10µg/rat) group. ABA10: abscisic acid-treated (10µg/rat), ABA15: abscisic acid-treated (15µg/rat), MP: methylprednisolone.

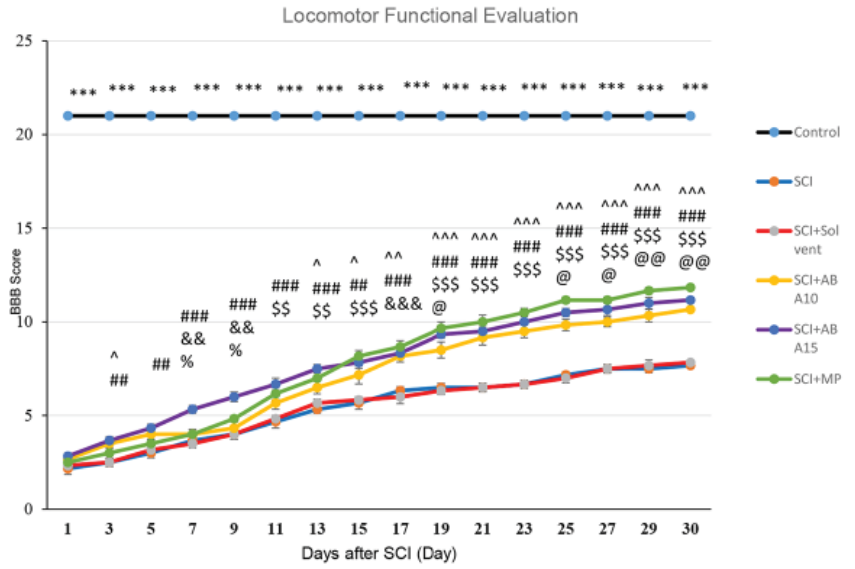

Figure 4.
The assessment of abscisic acid (10, 15µg/rat) and methylprednisolone effects on locomotor function via the BBB test during 30 days. Values are shown with mean ± S.E.M (n = 6 rats/group). ***$p < 0.001$ shows a significant difference compared to all SCI-induced groups. ## $p < 0.01$ and ### $p < 0.001$ show the significant difference between SCI+ABA (15µg/rat) group and both SCI and SCI+ solvent injected groups. ^$p < 0.05$, ^^ $p < 0.01$, and ^^^ $p < 0.001$ show the significant differences when comparing the SCI+ABA (10µg/rat) group with both SCI and SCI+ solvent injected groups. $$ $p < 0.01$ and $$$ $p < 0.001$ show the significant difference in comparing the SCI+MP group with SCI and SC+ solvent injected group. %$p < 0.05$ shows a significant difference when comparing SCI+ABA (15µg/rat) group with the SCI+MP group. @$p < 0.05$ and @@$p < 0.01$ indicate the significant differences between ABA+SCI (10µg/rat) and the SCI+MP groups. && $p < 0.01$ and &&& $p < 0.001$ show significant differences between ABA+SCI (15µg/rat) and ABA+SCI (10µg/rat) groups. ABA10: abscisic acid-treated (10µg/rat), ABA15: abscisic acid-treated (15µg/rat), MP: methylprednisolone.

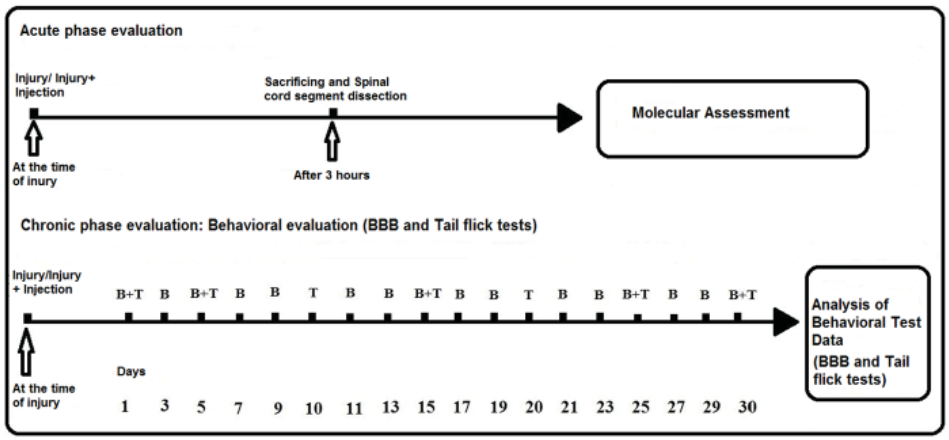

Figure 5.
The procedure of molecular and behavioral experiments.

gested that PPARγ agonist has anti-inflammatory potential and can cooperate in neuroprotective actions in CNS injuries [39, 40]. ABA structurally resembles thiazolidinediones which is the PPAR-γ agonist and may control neuroinflammation by decreasing the TNF-α level [25, 41].

Recently, it was shown that the IT administration of ABA enhanced the nociceptive threshold in the tail flick and hot plate tests. Indeed, the pharmacological suppression of protein kinase A prevented these effects [42]. These studies showed that ABA imposed its beneficial effects on reducing neuropathic pain and improving recovery after SCI via PPAR and PKA signaling pathways.

Overall, the present study indicated that ABA causes a significant reduction in the expression of proinflammatory genes TNF-α and IL-1β in the spinal cord of injured rats. Behavioral data in line with molecular findings demonstrated that ABA increases the latency response to nociceptive thermal stimuli and improves locomotor function. The findings of this research confirm the potential role of ABA as an anti-inflammatory and antinociceptive after SCI. However, more detailed studies are required.

Materials and Methods

Animals

Male Wistar rats (n = 108, W = 220-250 gr) were procured from the animal house of Shahid Bahonar University of Kerman. Animals had ad libitum access to food and water. They were kept under a 12 h light/dark cycle at 22°C ± 2°C temperature and were randomly divided into two groups for molecular and behavioral assessments. All tests were approved by the Ethics Committee of Shahid Bahonar University of Kerman (Approval No. IR.KMU. REC. 1399. 096), which follows the ARRIVE guidelines [43]. Moreover, standard ethical guidelines were considered for assessing pain in animals [44].

Medications

The (±)-cis trans-ABA was acquired from Sigma-Aldrich Co. (USA). It was diluted with artificial CSF (aCSF) after dissolving in DMSO. ABA was injected into the spine after inducing SCI by two different dosages: ABA10 (10 µg/rat, IT), and ABA15 (15 µg/rat, IT). The solvent was also administered IT (aCSF + DMSO at the ratio of 2:1 v/v). MP sodium succinate (30 mg/kg, IP) was procured from Exir Pharmaceutical Co. (Iran).

Experimental design

Rats were randomly divided into six experimental groups (n = 6). The control group did not receive any surgery or treatment and in the SCI group, just SCI was induced without any treatment. The SCI + solvent-treated group received ABA vehicle (aCSF + DMSO) and SCI + ABA-treated groups received different doses of ABA (10 or 15 µg/rat) administrated IT and the SCI + MP-treated group was administered 30 mg/kg of MP IP. All IT injection volumes were 5 µl, and MP injected volume was 0.5 ml. Figure 5 shows the procedure of this study.

Spinal cord injury induction

Ketamine and xylazine (60 and 10 mg/kg IP, respectively) were applied to anesthetize the animals. Next, the rats were fixed in stereotaxic apparatus during the surgery. After shaving the skin and completing antiseptic procedures with 7.5% povidone-iodine, fascia and paravertebral muscles were gently dissected to expose the lamina. Dorsal laminectomy was performed without insulting the dura matter at T9-T10 to expose the spinal cord. In order to induce SCI, we used the weight-drop contusion injury model [45]. Briefly, it was performed by dropping 10 gr weight from a height of 25 mm above the exposed spinal cord [46]. Then injections were completed according to the study design. In the end, the muscle and fascia layers were sutured with absorbable sutures, and animals were transferred to a cage with a circulating heating pad for recovery.

The animals were anesthetized and sacrificed 3 h after SCI and injections. For the molecular study, intracardial perfusion was performed by phosphate buffer (pH = 7.35) and after tissue sampling, the specimens were stored in a -80°C freezer. For evaluating locomotor functions and pain response in the chronic phase, all SCI-induced groups received gentamicin for up to 5 days (12 mg/kg/day, IM) after the operation to diminish the infection rate. In addition, the rats received bladder massage three times a day to evacuate the bladder until the micturition reflex was re-established.

Tail flick test

The tail flick analgesia test estimates the analgesic properties of pharmacological substances at spinal and supraspinal levels. The tests began on the first day after the injury and continued every day until day 30. In this apparatus, the central analgesic effect of ABA was determined via the radiant heat algesimeter (Hugo Sachs Electronic, Germany). The last third of the tail was placed on a heat source to determine the analgesic reaction time (delay to tail withdrawal). Baseline threshold and deviation from the baseline due to treatments were recorded. Finally, the mean value of three measurements was applied for analysis. We considered 10 sec as the cut-off time to prevent the irritation of the animal's tail.

BBB tests

To assess the effect of the acute administration of ABA on functional recovery after SCI, we recorded the behavioral BBB test, which monitors each rat's movement in an open-field area for 5 min. The range of BBB score is from 0 (without any movement in a hind limb) to 21 (normal motion with the same interval steps), defined in three stages early, intermediate, and late.

In the early stage, scores are 0-7, defined as no hindlimb movement or isolation in motion in hindlimb joints. The intermediate stage scores are 8-13, implying proportion movement between the hindlimb and forelimb without solidarity in steps interval. The last stage score of 14-21 indicates coordination between the forelimb and hindlimb, return of toe and tail position, and trunk constancy during stepping. The test was performed a day after injury and continued every 2 days until 30 days after injury.

Molecular assay

Tissue isolation, RNA extraction, and reverse transcription

Total RNA from all spinal cord tissues of all groups was extracted with the Wizbio Reagent Master Kit protocol (Wizbio, South Korea), and the NanoDrop spectrophotometer (Thermo Scientific, USA) was used to verify the RNA isolation method and

Table 1.
Primer sequences, RT-PCR fragment lengths, and NCBI accession numbers.

Primer name	Primer sequence	PCR amplicon	NCBI accession number	Pre-denaturation Temperature/ time (s)	Denaturation Temperature/ time (s)	Annealing Temperature/ time (s)	Extension Temperature/ time (s)	cycles
GAPDH	F: GTCTTCACCACCACGGAGAAGGC R: ATGCCAGTGAGCTTCCCGTTCAGC	392	NM_017008.4	95/300	95/20	60/30	72/20	40
TNF-α	F: ACCAGCAGATGGGCTGTACCTTAT R: ATGAAATGGCAAATCGGCTGACGG	107	NM_012675.3	95/300	95/20	60/30	72/20	40
IL-1β	F: AAGACACGGGTTCCATGGTGAAGT R: TGGTACATCAGCACCTCTCAAGCA	97	NM_031512.2	95/300	95/20	60/30	72/20	40

evaluate the concentration of RNA. We applied 1 ml Wizol Reagent for homogenizing 50 mg of spinal cord tissue. Afterwards, 200 µl of chloroform was added to each tube for RNA isolation. The washing stage was completed with 500 ml of isopropanol and 75% ethanol. The tubes were centrifuged after adding the chemical solution for isolating RNA from tissue samples and ultimately, a proper volume of RNase-free water was added to each tube. A volume of 10 µl of RT master mix which contained MMLV RTase (Wizbio, South Korea) and 1 µl oligo (dT) were added to 1 µg RNA of each template. Next, each sample volume was increased to 20 µl with RNase-free water.

Real-time PCR

The assessments were improved by applying Bio-Rad PCR iQ5 Thermal Cyclers (Bio-Rad, Richmond, CA, USA) to synthesize the first strand of cDNA. Next, 1 µl forward and 1 µl reverse primers and qPCR master mix were added and prepared for PCR with 10 µl SYBR green reporter dye. The Real Q Plus 2X master mix (Bio FACT, South Korea) was used in the PCR reactions and the final volume reaction in each tube was 20 µl. Samples were performed in triplicates on Roche PCR light cycler (Roche Life Science, Germany), and its software version 1.1 was utilized for analyzing the gene expression data. The fluorescence melting curves were generated to screen the primer dimers. For predicting all the sizes of primer, the PCR products were run on the electrophoresis 1.5% agarose gel (Sigma) with DNA loading dye (Smobio, Taiwan) and then using Ingenius 3 Gel Documentation (Syngene Bio-Imaging, UK) for visualization. Samples were normalized with the housekeeping gene GAPDH, as well as TNF-α and IL-1β genes as the proinflammatory markers (47). We evaluated PCR efficiency and linearity using four points of diluting and over twofold concentration of cDNA which obtained 0.9769, 0.9806, and 0.9777 for IL-1β, TNF-α, and GAPDH, respectively (Figures 2A, 2B, 2C). All the information on the primers, including their sequences, size of PCR product, NCBI accession numbers, and PCR stages based on temperature and time(s) are mentioned in Table 1 (48). The PCR efficiency for the primers was not 1. As a result, we applied 1.96^-ΔΔCT for TNF-α and 1.95^- ΔΔCT to determine the relative gene expression ratio.

Statistical analysis

Molecular and behavioral datasets were analyzed by the SPSS software. The one-way analysis of variance with Tukey's posthoc test was applied to assess the differences between the study groups. The mean ± SEM was presented for all data, and values of $p < 0.05$ were considered statistically significant.

Authors' Conributions

All authors conceived and planned the experiments. Maryam Rezaeezadeh Roukerd carried out the experiments and analyzed data set. Mehdi Abbasnejad contributed to the interpretation of the results. Sahel Motaghi took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest. This work was supported by Shahid Bahonar University of Kerman under Grant number 96/5/2736.

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Clinical and mycological features of dermatophytosis in domestic cats at Ferdowsi University of Mashhad Veterinary Teaching Hospital

Zohreh Ghiasi Tarzi[a], Samaneh Eidi[b], Javad Khoshnegah[c]

[a] Graduated from Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
[b] Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
[c] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

ABSTRACT

The aim of this study was to investigate the clinical manifestations and mycological features of feline dermatophytosis in a population of cats with suspected lesions of dermatophytosis at Ferdowsi University of Mashhad Veterinary Teaching Hospital (northeast of Iran). Skin, hair, and nail samples from 122 clinically suspected cases of feline dermatophytosis were screened by direct microscopic examination. Of the 122 samples examined, 91 (74.59 %) were positive for fungal elements by direct microscopic examination, and 66 (54 %) were culture-positive for *Microsporum canis*. *M. canis* was the only dermatophyte species isolated from the cats. In the studied population, young cats (< 7 months) showed a statistically higher rate of *M. canis* infection than older animals ($p < 0.001$). The occurrence of dermatophytosis did not correlate with gender or the type of cats' hair. The isolation rate of dermatophytes was relatively high in winter. However, the association between season and the dermatophyte inflation rate was not significant. The diagnostic value of microscopic examination proved to be high compared to fungal cultures as 64 of 66 samples that had positive *M. canis* culture were positive at microscopic examination. The agreement between culture and microscopic examination was moderate ($k = 0.49$). The information acquired in this study provides helpful insights into dermatophytoses in northern Iran highlighting the most important clinical and mycological features of skin mycoses in pet cats.

Keywords

Dermatophyte, Dermatophytosis, Microsporum, Microsporum canis, Cat, Iran

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Abbreviations

M. canis: Microsporum canis
KOH: Potassium hydroxide
DMSO: Dimethyl sulfoxide
SCC: Sabouraud agar with cycloheximide and chloramphenicol
$\chi 2$: Chi-square

Corresponding author: *Javad Khoshnegah*
Email: *khoshnegah@um.ac.ir*
Tel: *+98 (511) 3880-5612*

Dermatophytosis is the most common fungal infection of cats worldwide and one of the most important infectious skin diseases in this species [1]. The prevalence of dermatophyte isolation, especially that of *M. canis*, varied among the populations depending on the geographic region, whether or not the cat was a stray or pet cat, and the presence or absence of skin disease at the time of sampling [1]. In Mashhad (northeast of Iran), a very low prevalence of dermatophytosis has been reported in dogs (1/218 dogs with dermatologic manifestations) and a very high prevalence in cattle (490/684 cattle with dermatologic manifestations) based on culture results [2, 3]. According to a study on the fungal agents of animals, cats (36.3%) were the most prevalent infected animals, followed by camels (13.4%), dogs (12.8%), horses (12.5%), cows (12.3%) [4]. In Turkey (located in the west of Iran), the isolation rates of dermatophyte species from dogs and cats were 18.7% and 20.1%, respectively [5]. In other countries, a wide range of prevalence of feline dermatophytosis from 4% to 91% has been reported [6-13].

Feline dermatophytosis is most often characterized principally by one or more irregular or annular areas of alopecia with or without scales [14, 15]. However, there are some other clinical presentations of feline dermatophytosis, such as inflammatory areas of folliculitis characterized by alopecia, erythema, scale, crust, follicular papules, military dermatitis, chin folliculitis, dermatitis of the dorsal tail and onychomycosis [16, 17]. In general, the nature of the dermatophyte cannot be determined from the clinical presentation [15, 18].

Various risk factors have been described for feline dermatophytosis. Some authors suggest that the highest incidence of dermatophytosis might be found in kittens, immunocompromised animals, and long-haired animals (e.g., Persians) [5-7, 15, 19]. The current study was designed to assess the clinical manifestations and mycological features of feline dermatophytosis in a population of Iranian domestic cats with suspected lesions of dermatophytosis presented at a veterinary teaching hospital.

Location of the study: Mashhad is a large city located in the northeast of Iran close to the borders of Afghanistan and Turkmenistan. It is located in the valley of the Kashaf River between the two mountain ranges of Binalood and Hezar-Masjed. The city's climate is semi-arid with cold winters and moderate summers. It has a resident population of approximately 3,400,000 [20].

Ethical Considerations: This research proposal has received ethical approval from Ferdowsi University of Mashhad Research Office. In addition, the National Research Council's guide for the care and use of animals was followed.

Study population and sample size: During the study period (September 2017 to November 2018), animals with skin lesions, such as alopecia, peripheral scaling, erythema, crust, popular lesions, military dermatitis, and onychomycosis, classified as suspected cases of dermatophytosis, were sampled [15]. Convenience sampling was used in the selection of animals. Overall, in the present study, 122 cats with suspected lesions of dermatophytosis were examined.

Sampling and examination procedures: All cats with suspected skin lesions of dermatophytosis were closely examined, including observation and palpation of the skin for any kind of primary and/or secondary skin lesions. The anatomic location(s) of the skin lesions were recorded.

Hair samples were collected based on the clinical signs using Wood's lamp examination. The method of hair sampling was chosen according to the clinical signs and was either by the toothbrush technique when lesions were generalized or by hair pluck of the margins of localized lesions [21, 22]. The samples were sent to the Mycology Laboratory of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. None of the sampled animals was treated with an antifungal agent.

Data collection: Age, gender, and hair type of sampled animals were recorded. Cats were classified into four groups based on their age [23] as younger than 7 months (≤ 7 months), 7 months to 2 years (7 months-2 years), 2-6 years (≥ 2-6 years), and older than 6 years (≥ 6 years). To evaluate seasonal effects, the samples were categorized according to the sampling periods into spring, summer, autumn, and winter groups.

Direct microscopic examination: All samples were examined for fungal elements under a light microscope at ×40 magnification using 20% KOH / DMSO (Merck Co., Darmstadt, Germany).

Culture and identification: All samples were inoculated onto Mycosel agar (Merck Co., Darmstadt, Germany). The plates were incubated at 27°C and examined daily for four weeks. Dermatophyte isolates were identified by colony morphology and microscopic examination with lactophenol cotton blue preparation.

Statistical analysis: The chi-square ($\chi 2$) test was used to assess statistical differences between the groups. Comparisons of prevalence between male versus female and long-haired cats versus short-haired cats were made using the $\chi 2$ test for two inde-

pendent proportions. Comparisons of three or more prevalences (four age categories and four seasons) were made using the $\chi 2$ test for multiple independent proportions. Agreement between the results of the two methods used to examine the animals was evaluated by Kappa statistics. All statistical analyses were performed with the SPSS software for Windows (version 26) and $p < 0.05$ was considered statistically significant.

Results: Of the 122 examined samples, 90 (73.77%) were positive for fungal elements by direct microscopic examination and 66 (54%) were also culture-positive. Three (2.45%) samples considered negative upon direct microscopic examination yielded positive cultures for dermatophytes. Agreement between the tests was moderate as shown by the kappa value of $k = 0.49$ between direct microscopy and fungal culture ($p < 0.05$). According to the culture results, *M. canis* (54%) was the only dermatophyte species isolated from the cats (Fig. 1).

In the present study, the lesions of dermatophytosis mostly appeared as one or more irregular or annular areas of alopecia with or without scales, with the trunk (27/66), ears (24/66) (Fig. 1a), and face (18/66) (Fig. 1b and 1c) affected most commonly according to culture results (Table 1). Overall, most of the infected cats presented the generalized distribution pattern of the disease.

The infection had no statistically significant association with the gender or hair type of the cats. According to our model, the only risk factor found to be significantly associated with infection was age. Cats younger than 7 months of age showed a statistically higher prevalence of infection than older animals ($p < 0.001$). The isolation rate of dermatophytes was relatively high in the winter. However, no statistically significant difference was detected in the seasonal distribution of feline dermatophytoses. The variables are reported in Table 2.

According to the results of the present study, among 122 examined samples, 90 (73.77%) were positive for fungal elements by direct microscopic examination and 66 (54%) were culture-positive. While 3 (2.45%) negative samples at direct microscopic examination had positive culture, 26 (21.31%) positive samples at direct microscopy had negative culture. Agreement between the examination methods was moderate as shown by the kappa value of $k = 0.49$ between direct microscopy and fungal culture. Thus, it can be concluded that direct microscopic examination can be helpful in the diagnosis of dermatophytoses as previously mentioned by other researchers [5]. However, we suggest that veterinary practitioners wait for the culture results to initiate sufficient treatment. Sparkes et al. (1993) emphasized that direct microscopy had positive predictive values

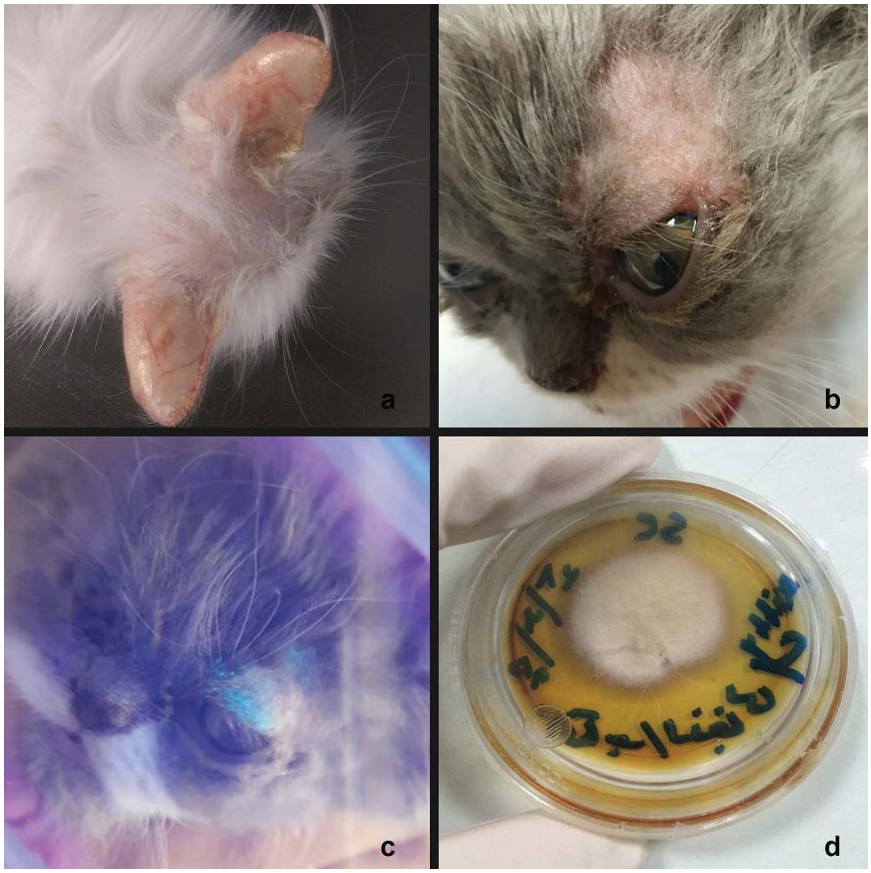

Figure 1.
Scaling and alopecia of both ears of a 6-month-old Persian cat with generalized dermatophytosis (a). Focal lesion of dermatophytosis on an otherwise healthy cat. Note the scaling, erythematous and crusting lesions in addition to alopecia in this 50-days-old Persian cat (b). The extent of lesions is highlighted by Wood's lamp (c). The culture result of the same cat is positive for *M. canis* (d).

Table 1.
Anatomical distribution of skin lesions in positive cats according to direct microscopic evaluation and culture positive results

Anatomical location	DM	Culture
Head	8	6
Face	31	18
Ears	25	24
Cervial	20	19
Trunk	32	27
Forelimbs	9	8
Hindlimbs	5	3
Onycho	4	3
Tail	11	8

of 93% and negative predictive values of 93% in determining the presence of dermatophytosis.

They stated that cultural examination alone was not enough for dermatophytosis diagnosis because of the occurrence of false positive and false negative results [7].

According to the results of the present study, only 26 negative culture samples were reported out of 122 samples, which seems to be an acceptable success. This difference in direct microscopic examination and culture results may be attributed to the sampling conditions and sample volume as well as the sensitivity of the strain to the culture medium used (SCC). Because of the existing conditions, re-sampling and using other culture media (i.e., culture media enriched with vitamins and amino acids) was not possible in the current study. It should also be noted that if a sufficient sample size was available, in cases where the direct test was positive and the culture was negative (the direct test was typically positive), sometimes the culture was repeated up to 5 times but the dermatophyte did not grow.

Our findings showed that 66 (54%) of sampled cats referred to our veterinary teaching hospital were culture-positive of which 100% of isolated dermatophytes were *M. canis*. The results cannot be generalized to the general population and also should be carefully interpreted. Some of the previous studies indicated a high prevalence of feline dermatophytosis (e.g., more than 90% as reported by Lewis et al., 1991 and Polak et al., 2014) [6, 12] and similar to the present study, *M. canis* was the most commonly isolated species in the cats with a suspected lesion of dermatophytosis. However, in Iran, two other investigations reported much lower prevalences of 25.76% and 54.8% [4, 13].

Table 2.
Prevalence of *M. Canis* in cats in relation to different variables

Variables		No. animal tested/ No. positive animal (%)
Gender		
Female		**64 / 29 (45.3%)**
	intact	50 / 21
	neutred	3 / 0
	undetermined	11 / 8
Male		**51 / 30 (58.5%)**
	intact	31 / 17
	neutred	4 / 0
	undetermined	16 / 13
Undetermined		7 / (5.73%)
Age		
	≤ 7 mos	38 / 27 (71%)
	7 mos-2 yrs	46 / 13 (28.3%)
	≥ 2 yrs-6 yrs	8 / 1 (12.5%)
	≥ 6 yrs	3 / 1 (33.3%)
	undetermined	27 / 23 (85.2%)
Hair type		
	Long hair	95 / 50 (52.63%)
	Short Hair	27 / 16 (59.2%)
Season		
	Spring	27 / 12 (44.4%)
	Summer	21 / 13 (61.9%)
	Autumn	65 / 33 (50.8%)
	Winter	9 / 8 (88.8%)

In addition, according to a recent study by Eidi et al. (2022), *M. canis* was the only species found in dogs and cats [24]. Similar to the present study, others have found that among 14 of the 38 (36.8%) culture-positive feline specimens from Brazil, 100% of isolates were *M. canis* [10]. Moreover, recently, researchers reported the isolation of *M. canis* from 60/76 lesional cats [12]. Interestingly, they emphasized that "half of the cultured dermatophytes were believed to be truly infected and half were believed to be fomite carriers". Cats are the principal reservoir for *M. canis* and the

high rate of *M. canis* isolation in cats was not an unexpected result.

Authors reported different rates of *M. canis* isolation and other dermatophytes from asymptomatic cats with a much lower rate than the symptomatic cases [25]. As a result, it can be concluded that there is a high chance of isolating *M. canis* from symptomatic cats in contrast to asymptomatic cats.

Similar to Lewis et al. (1991), one of the findings of the present research is that most of the infected cats exhibited the generalized distribution pattern of dermatophytosis involving more than one region. Sites of predilection include the trunk, ears, neck, and face. It must, however, be borne in mind that in contrast to previous studies [8, 12], the present investigation revealed a more detailed picture of the suspected lesions of dermatophytosis leading to a more accurate diagnosis of clinical dermatophytosis.

According to the present study, cats younger than 7 months of age presented a significantly higher rate of infection than older animals. Others reported similar findings concerning the age of animals [5, 9-11]. The higher susceptibility of young cats with skin lesions suspicious of dermatophytoses might be due to their immunological immaturity, the deficiency of fungistatic sebum or linoleic acid, biochemical exchange on the skin, and the physiological status of the animals [5, 6, 9, 26, 27].

We observed no statistically significant difference between male and female cats and between intact or neutered cats. Similarly, some previous studies did not find any correlation between gender and the occurrence of dermatophytosis in cats [5, 28]. In contrast, some other authors reported a higher prevalence of dermatophytosis in male or female cats; for example, Boyanowski et al. (2000) reported that neutered male cats had a 12-fold higher risk of having dermatophytosis compared to intact male cats [29]. Furthermore, Natale et al. (2007) detected a significantly higher prevalence in female cats, which might be due to the disproportion between male and female cats examined [30].

The results of the present study revealed no significant differences in the prevalence of dermatophytosis between long-haired and short-haired cats. Others found a higher percentage of *M. canis* in healthy long-haired or Persian cats. However, they did not perform a statistical analysis to check the significance of their results [10, 31]. In addition, Mancianti et al. (2002) examined symptomatic animals and found a higher percentage in long-haired cats [9]. There is indirect evidence that long-haired cats (e.g., Persian cats) are predisposed to dermatophytosis. This breed is commonly over-represented in the general cat population and also in treatment studies, supporting the observation that Persian cats are predisposed to dermatophytosis [32]. Moriello et al. (1991) provided an explanation for the more common isolation of *M. canis* in long-haired breeds. In their study on cats with and without dermatophytosis, they did not find any significant difference in hair length for the isolation of *M. canis*. They thought it is possible that *M. canis* is more difficult to be removed from long-haired cats [32].

Data on the seasonality of dermatophytoses in the literature are controversial and according to several authors, there is no correlation between the season and the occurrence of dermatophytoses [7-9, 11]. In the current research, the isolation rate of dermatophytes was relatively high in the winter. However, no statistically significant difference was identified in the seasonal distribution of feline dermatophytoses. The climate of our study region (Mashhad) is semi-arid with low humidity, cold winters, and moderate summers. Some authors suggest that the highest prevalence of dermatophytosis is found in the cold seasons. A 15-year study in Italy on 7650 suspect pet cats showed a significantly higher recovery rate for *M. canis* in the fall and winter than in summer and spring [9]. They reported a higher prevalence, but the significance is unclear.

In conclusion, further research with a larger survey is needed to get a better estimation of the prevalence and to identify the possible risk factors of dermatophytosis in cats. The information acquired in this study has provided helpful insights into dermatophytoses in northern Iran highlighting the most important clinical and mycological features of skin mycoses in pet cats.

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Conflicts of Interest

Authors declare that they have no conflicts of interest.

Authors' Contributions

JK conceived and planned the study. ZGT and JK carried out the samplings and contributed to sample preparation. The procedures of mycology have been conducted by SE. JK contributed to the interpretation of the results and took the lead in writing the manuscript.

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Abstract in Persian

آنالیز سریالی گازهای خون طی احیای سگ های هیپوولمیک با مایعات

رضا آویزه[1]، رضا آذرگون[2]، علیرضا غدیری[1]، مهدی پورمهدی بروجنی[3]، محمد راضی جلالی[1]، هادی ایمانی راستابی[1]

۱گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران.
۲ گروه بیماری های درونی و کلینیکال پاتولوژی، دانشکده دامپزشکی دانشگاه ارومیه، ارومیه، ایران.
۳ گروه بهداشت مواد غذایی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران.

چکیده

بروز اختلالات همودینامیک در بیماران هیپوولمیک مستند گشته، بنابراین مدیریت زودهنگام هیپوولمی برای کسب نتایج مطلوب ضروری است. گازهای خون که طی نوسانات همودینامیک به سرعت تغییر می یابند، می توانند به عنوان یک رهیافت تشخیصی برای نظارت بر بیماران اورژانسی استفاده شوند. اهداف این مطالعه بررسی اثرات مایع درمانی با محلول های رینگر لاکتات (LR) یا هیدروکسی اتیل استارچ (HES) بر گازهای خون سیاهرگی و سرخرگی و تفاوت بین پارامترهای گاز خون سیاهرگی و سرخرگی به منظور امکان آنالیز گازهای خون سیاهرگی به عنوان جایگزینی برای آنالیز گازهای خون سرخرگی طی احیای سگ های هیپوولمیک بود. HCO_3-، PCO_2، PO_2، pH و BE سرخرگی و سیاهرگی در پایان هر یک از مراحل مطالعه به شرح زیر آنالیز شدند: ۱) ایجاد بیهوشی، ۲) خون گیری تا فشار متوسط سرخرگی ۴۰-۵۰ میلی متر جیوه، ۳) حفظ سگ ها در وضعیت هیپوولمیک، ۴) احیا با LR (گروه A) یا HES(گروه B) در چهار مرحله و ۵) یک ساعت پس از مرحله نهایی احیاء. هیپوولمی باعث کاهش پارامترهای مورد مطالعه شد، به جز PCO_2 سیاهرگی که افزایش معنی داری نشان داد ($p < 0.05$). احیاء با مایع درمانی پارامترهای مورد مطالعه را به مقادیر کنترل برگرداند، اگرچه PCO_2 سیاهرگی در گروه A و HCO_3- در گروه B تفاوت معنی داری را نسبت به مقادیر کنترل نشان دادند ($p < 0.05$). ما دریافتیم که HCO_3^- ، pH و pCO_2 سیاهرگی را می توان به عنوان جایگزین های کمتر تهاجمی و ایمن تر برای پارامترهای سرخرگی مشابه طی پایش احیاء با مایع درمانی در سگ های هیپوولمیک استفاده کرد.

واژگان کلیدی

گاز خون سرخرگی، گاز خون سیاهرگی، هیدروکسی اتیل استارچ، رینگر لاکتات

* نویسنده مسئول: رضا آذرگون
R.azargoun@urmia.ac.ir

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Abstract in Persian

اثرات حفاظتی و ایمنی عصاره سیر (Allium sativum) بر ماهی قزل آلای رنگین کمان (Oncorhynchus mykiss) در مواجهه با سمیت مس

محمد فرهنگی*، حسین آدینه، محمد هرسیج

گروه شیلات، دانشکده کشاورزی و منابع طبیعی، دانشگاه گنبدکاووس، گنبد،گلستان، ایران.

چکیده

در مطالعه حاضر اثرات حفاظتی و ایمنی عصاره سیر در ماهی قزل آلای رنگین کمان مواجهه شده با سمیت مس مورد ارزیابی قرار گرفت. ۵۵ ماهی قزل آلای رنگین کمان با میانگین وزنی ۳/۷۳ ± ۵۱/۲۰ در معرض غلظت های از مس (۰/۰۲، ۰/۱، ۰/۳ و ۰/۴ میلی گرم در لیتر) قرار گرفتند. یک گروه به عنوان شاهد در نظر گرفته شد. تحت شرایط ثابت (درجه حرارت و پی اچ، روش آب ساکن) غلظت کشنده مس برابر ۴/۰ میلی گرم در لیتر به دست آمد. تیمارهای آزمایشی شامل: گروه شاهد بدون هیچ غلظتی از مس و عصاره سیر(T1)، تیمار حاوی عصاره سیر و غلظت کشنده مس (T2) و تیمار حاوی غلظت کشنده مس بدون عصاره سیر (T3) بود. نتایج حاصل از آزمایش بیانگر اختلاف معنی داری بین تیمارها بود، زمانی که غلظت مس در تیمارها افزایش یافت. در هر صورت در غلظت های بالای مس علایم ظاهری مسمومیت مشاهده گردید. براساس نتایج، اختلاف معنی داری بین فاکتورهای هماتوکریت، مقادیر MCV و MCHC در بین تیمارها مشاهده شد ($p < 0.05$). مطالعه آسیب شناسی بافتی نشان داد، ضایعات اصلی شامل هایپرپلازی رشته ها آبششی، اتساع کپسول بومن، و نکروز سلولهای پوششی در تمام ماهیان بود. در هر صورت نتایج ثابت کرد، استفاده از عصاره سیر در جیره غذایی می تواند جهت افزایش نرخ مقاومت ماهی در مواجهه با سمیت مس سودمند است.

واژگان کلیدی

مس، آسیب شناسی بافتی، قزل آلای رنگین کمان، مقاومت

* نویسنده مسئول: محمد فرهنگی
s.farhangi@yahoo.com

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Abstract in Persian

بررسی اثرات حفاظتی آبسزیک اسید در مدل آسیب نخاعی با واسطه مهار التهاب عصبی

مریم رضایی زاده روکرد[۱]، ساحل متقی*[۱]، بلال صادقی[۲]، مهدی عباس نژاد[۳]

۱گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان،کرمان، ایران.
۲گروه بهداشت مواد غذایی و بهداشت عمومی، دانشکده دامپزشکی، دانشگاه شهید باهنرکرمان،کرمان، ایران.
۳گروه زیست شناسی، دانشکده علوم، دانشگاه شهید باهنر کرمان، کرمان، ایران.

چکیده

آبسزیک اسید هورمون گیاهی با نقش تعدیلی در گیاهان است و هم چنین اثرات ضد التهابی آن در بافت های مختلف حیوانی گزارش شده است. پس از بروز آسیب نخاعی، التهاب عصبی ایجاد می گردد که سبب درد نوروپاتی و نقص حرکتی می گردد. هدف مطالعه حاضر بررسی اثر آبسزیک اسید به عنوان ماده ای با خاصیت ضد التهابی در مدل حاد آسیب نخاعی است و بررسی نقش ضد التهابی آن در کنترل درد نوروپاتیک و بهبود عملکرد حرکتی در مدل ضایعه نخاعی در موش صحرایی است. جهت القا آسیب نخاعی از مدل پرتاب وزنه استفاده شد و تزریق حلال، آبسزیک اسید (15µg/rat, IT ,10) و متیل پردنیزولون (mg/kg, IP 30) بلافاصله پس از ایجاد آسیب انجام شد. برای بررسی بیان ژن فاکتورهای پیش التهابی TNF-α و IL-1β در فاز حاد، از تکنیک real-time PCR استفاده گردید و جهت بررسی اثرات آبسزیک اسید بر درد نوروپاتیک و عملکرد لوکوموتور در فاز مزمن ضایعه، از تست های Tail flick و BBB (Basso, Beattie, Bresnahan) استفاده شد. نتایج نشان داد که آبسزیک اسید بیان ژن های پیش التهابی TNF-α و IL-1β در مدل آسیب نخاعی را کاهش داد و سبب تاخیر در پاسخ به محرک گرمایی گیرنده نوسیسپتیو گردید. هم چنین نتایج بهبود درعملکرد لوکوموتور پس از تیمار آبسزیک اسید را نشان داد. یافته های ما نشان داد آبسزیک اسید دارای اثر ضد التهابی بالقوه و متعاقب آن، بهبود درد نوروپاتیک و بهبود عملکرد حرکتی پس از آسیب نخاعی در موش صحرایی است.

واژگان کلیدی

آبسزیک اسید، التهاب عصبی، درد نوروپاتی، ضایعه نخاعی

* نویسنده مسئول: ساحل متقی
sahelmotaghi@uk.ac.ir

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Abstract in Persian

جنبه های درمانگاهی و قارچ شناختی درماتوفیتوز در گربه های اهلی ویزیت شده در بیمارستان دامپزشکی دانشگاه فردوسی مشهد، ایران

زهره قیاسی طرزی[1]، سمانه عیدی[2]، جواد خوش نگاه[3]*

۱دانش آموخته دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران.
۲ گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران.
۳ گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه فردوسی مشهد، مشهد، ایران.

چکیده

هدف مطالعه حاضر، شناخت جنبه‌های بالینی و قارچ شناختی درماتوفیتوز گربه سانان در یک جمعیت از گربه‌های اهلی دارای جراحات جلدی مشکوک به درماتوفیتوز بود که دربیمارستان دامپزشکی دانشگاه فردوسی مشهد (ایران) ویزیت شدند. نمونه‌های تراشه جلدی، مو و ناخن، گرفته شده از تعداد ۱۲۲ قلاده گربه دارای علایم درمانگاهی مشکوک به درماتوفیتوز، به روش میکروسکوپی مستقیم مشاهده شدند. سپس تمام نمونه‌ها در محیط کشت مایکوزیل آگار تلقیح شدند. از مجموع از ۱۲۲ نمونه، ۹۱ نمونه (۷۴/۵۹ درصد) در ارزیابی میکروسکوپی مستقیم و ۶۶ نمونه (۵۴ درصد) در کشت، مثبت بودند. تنها گونه درماتوفیتی جدا شده، میکروسپورم کنیس بود. واکاوی آماریِ داده های مطالعه حاضر نشان داد، سن گربه‌ها ارتباط معنی داری با رخداد عفونت نداشت؛ مقدار آلودگی میکروسپوروم کنیس در گربه‌های جوان (کم‌تر از ۷ ماه) بیش از گربه‌های با سن بالاتر بود ($p < 0.001$). همچنین،ارتباط معنی دار بین جنس گربه ها و نوع پوشش مویی آنها با رخداد درماتوفیتوز وجود نداشت. میزان جداسازی درماتوفیت‌ها در فصل زمستان بیشتر بود اما ارتباط معنی دار بین میزان جداسازی و شیوع درماتوفیتوز وجود نداشت. براساس نتایج مطالعه حاضر ارزش تشخیصی روش میکروسکوپی مستقیم همسانی زیادی با روش کشت دارد به نحوی که از مجموع ۶۶ نمونه مثبت کشت قارچ، ۶۴ مورد در روش میکروسکوپی مستقیم مثبت بودند و توافق نسبی بین این دو روش آزمایشگاهی مشاهده شد ($k = 0.49$). یافته‌های حاصل از مطالعه‌ی حاضر می‌توانند در درک بهتر وضعیت درماتوفیتوزها کمک شایانی کنند و مهم‌ترین جنبه های بالینی و قارچ شناختی مایکوزهای جلدی گربه های خانگی را مشخص نمایند.

واژگان کلیدی

درماتوفیت، درماتوفیتوز، میکروسپورم، میکروسپورم کنیس، گربه، ایران

* نویسنده مسئول: جواد خوش نگاه
khoshnegah@um.ac.ir

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Gene names: The standard gene names, as provided by HGNC (HUGO Gene Nomenclature Committee) should be used. Gene names must be italicized. If the case of mammalian species and if gene names refer to rodent species, they must be upper case; if they refer to non-rodent species they must be written in capitals. If they refer to other species, they must written lower case. Protein names are written in capitals and are not italicized. As an example:

Mouse beta actin gene: *Actb*
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Quantitative PCR: If the quantitative PCR method has been used, the related section in Materials and Methods must be written following the reference:

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The following information must be provided in the section:

Protocol for DNA/RNA extraction, including quantification and determination of purity;

Reverse transcription (if used): amount of RNA, concentration of all reagents: primers concentration (either random primers or oligonucleotides), reverse transcriptase and master mix components;

qPCR: sequence of forward and reverse primers, probes, amplicon size, accession number of Genebank; thermocycler parameters (i.e. denaturation, annealing and extension steps, number of cycles, melting curves); validation of PCR products; non-template controls for reverse transcription and qPCR should be included in all reactions; and

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An unhealthy diet, obesity and physical inactivity play a role in the onset of type 2 diabetes, but it has been shown that increased physical activity substantially reduces the risk [1], and participation in regular physical activity is one of the major recommendation of the evidence based guidelines for the primary prevention of diseases [2]. According to the 2004-05 National Health Survey, more than half a million Australians (3.5% of the population) have diabetes mellitus which had been medically diagnosed and most of these people have the Type 2 condition [3]. Gestational diabetes is also on the increase, rising steadily between 2000-01 and 2005-06 [4]. Approximately two thirds of those with diabetes have been prescribed medication [3], but it is of concern that a recent review of the literature found that many people do not take their medication as prescribed [5]. Many patients also self monitor the disease by measuring their blood glucose levels with a glucose meter but Song and Lipman [6] have concerns about how well this is managed.

References for the above example:

1. Hull J, Forton J, Thompson A. Paediatric respiratory medicine. Oxford: Oxford University Press; 2015.

2. Eckerman AK, Dowd T, Chong E, Nixon L, Gray R, Johnson S. Binan Goonj: bridging cultures in Aboriginal health. 3rd ed. Chatswood, NSW: Elsevier Australia; 2010.

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Tables

Please submit tables as individual files and editable text and not as images. Place all table notes below the table body. Each table should have a title which is followed by explanation of results shown in the table. Use of vertical rules must be avoided. Tables should be self-explanatory, and clearly arranged. Tables should provide easier understanding and not duplicate information already included in the text or figures. Each table should be typewritten with double spacing on a separate file and numbered in order of citation in the text with Arabic numerals. Each table should have a concise heading that makes it comprehensible without reference to the text of the article. Explain any non-standard abbreviations in a footnote to the table.

Figures

Figures must be submitted in individual files (format: TIFF, Dimensions: Width: 789 – 2250 pixels

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For the use of bar diagrams the following publication should be consulted:

Weissgerber TL, Milic NM, Winham SJ, Garovic VD. Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol. 2015; 13(4):e1002128.

The bar diagrams should be provided in color and in a well-designed and professional format. Please do not use different shades of gray. The axes of diagrams should have titles and units. Also, the source file of the image (Excel etc.) should be provided for typesetting.

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Use of Italics

Gene symbols, Latin terms (i.e. *in vivo, in vitro, ex vivo, in utero, in situ*, and etc.) and species scientific names (using the binomial nomenclature), should be typed in italics, while the first letter of the genus name must be capitalized (i.e. *Homo sapiens*).

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1. TITLE is clear and adequate
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3. INTRODUCTION well-structured and provides a rationale for the experiments described.
4. MATERIALS AND METHODS are sufficiently explained and is detailed enough to be reproduced.
5. RESULTS are clearly presented and supported by figures and tables.
6. DISCUSSION properly interprets the results and places the results into a larger research context, and contains all important references.
7. Conclusions are logically derived from the data presented.
8. English Language/style/grammar is clear, correct, and unambiguous.
9. Figures and tables are of good quality and well-designed and clearly illustrate the results of the study.
10. References are appropriate.
11. Regarding this article are you concerned about any issues relating to author misconduct such as plagiarism and unethical behavior.
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Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Azadi Square, Mashhad, IRAN
P.O. Box: 1793; Postal Code: 9177948974

Tel: +98 51 3880 3742
Fax: +98 51 3876 3852

Web: ijvst.um.ac.ir
Email: ijvst@um.ac.ir