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DERMAL TOXIC EFFECT OF ALUM STONE (Shibbe stone): TRADITIONALLY USED IN MOROCCO

Ouiam Dahmane1*, Mohamed Lemine Cheikh Brahim Ahmed2, Jorg Heukelbach3, Yahia Cherrah1 and Mina Ait El Cadi1

1Faculty of Medicine and Pharmacy, Mohammed V University, 10100 Rabat, Morocco

2 university of Nouakchott Al Aasriya (UNA), Nouakchoot Mauritania

3School of Medicine, Federal University of Ceará, Fortaleza, Brazil

*Corresponding author: Dahmane.ouiam5@gmail.com

Abstract

Alum stone is one of the most significant salts used in Moroccan traditional medicine to cure a wide range of ailments including skin problems. The present research aimed to assess the acute and subacute dermal toxicity of alum after a single and repeated doses exposure. 70 rats were divided into 7 groups (n=10 per group; 5 females and 5males) for both study. On the first day of acute experiment, Alum at single doses of 500, 1000, 2000, and 5000mg/kg were applied to the rats on the shaved area of the dorsal skin. The experimental period was determined at 14 days. In subacute study, once per day for 4 weeks, Alum was topically applied to the rats at dose of 1000 mg/kg. The weights of rats were measured over the period tests and clinical symptoms were noted on a daily basis.

No mortality or abnormal changes on body weight, general behavior and organ systems were recorded for both acute and subacute study. After exposure to repeated doses, significant changes were noted in the levels of AST and ALT and hematologic parameters showed variations in the levels of white blood cells, lymphocytes, monocytes, neutrophils and platelet count of treated rats compared to the control. The histological results revealed no deleterious effect on the skin, liver and/or kidney sections. To conclude, these findings suggest that prolonger dermal application of alum can induce serious dysfunction on biochemical and hematological parameters of rats.

Keywords: Alum stone, Aluminum, hepatotoxicity, nephrotoxicity, dermal toxicity, Morocco.

Introduction

Alum stone is a colorless natural mineral stone that is commonly used as a folk medicine for water purification and as an antisepsis in food preparation procedures such as pickling and fermentation [1]. Alum can also help to stop bleeding from small nicks and cuts and is used as a natural deodorant [2] .

Alum stone is very commonly used in the Arab region, especially in the Maghreb countries [3]. In traditional Moroccan medicine, alum stone (containing aluminum) is used as potent topical antibacterial. It is used mainly in cosmetology as a deodorant to fight bad odors, to reduce sweats and also as an aftershave to calm burns and stop bleeding caused by the blade [4].

Natural substances have multiform uses; they enter in the manufacture of drugs and cosmetics as an active ingredient and/or as additives in food products. More than 50% of drugs were approved by FDA as natural products or derived from natural products [5]. Recently, humans have returned to using natural substances to avoid any risk of toxicity or disease due to the use of synthetic or processed substances. Nevertheless, excessive and inadequate use of these substances can cause adverse effects on the human beings' health in the same way as some synthetic substances.

Since antiquity, the topical use of alum is considered safe. Over the few last years, several reports demonstrate severe symptoms and even fatalities caused by Alum poisoning, after intentional oral ingestion or intravenous application of high doses. In the review of the literature, medical reports document a patient suffering from severe renal failure treated with alum (aluminum ammonium sulfate or aluminum potassium sulfate); resulted in aluminum toxicity such as myoclonic encephalopathy accelerated joint destructions and amyloidosis after long-term hemodialysis [6]. Following intravenous injection of 1% Alum and administration of Aliminum-containing antacids, a teenager with acute lymchoblastic leukemia developed altered mental status, speech problem, coarse tremor, and aberrant EEG results. Her serum aluminum levels were mildly elevated (14-22 pg/L, normal MI pg/ L), and bone marrow biopsy specimens demonstrated aluminum deposition on special staining (Krueger's method). After a nine-week treatment of intravenous deferoxamine, all anomalies disappeared [7]. In another report, an adult woman died after ingesting 5g of alum powder dissolved in water, which caused a large anion gap metabolic acidosis, multiple organ failure including ARDS, and refractory shock [8]. After ingesting alum as a folk medicine for a prolonged fever, a young miner developed acute fulminant hepatic failure with neurotoxicity[9].

Before using a natural extract, a fraction, or a chemical on humans, it is necessary to test its toxicity in an animal model. Natural products may also reveal human toxicity and their safety has to be assessed under standardized conditions. To our knowledge, no systematic investigation has been conducted on the safety profile evaluation of topical application of alum stone as practiced in traditional Moroccan medicine. Thus, the present research aims at elucidating hepato-nephrotoxicity and skin toxicity of alum stone used in Morocco, using animal models.

Materials and methods:

Preparation of test substance:

The alum stone used in this study was purchased from an herbalist in Rabat City, Morocco. The sample was crushed using a mortar then sieved to obtain fine powder. Before each dose application, alum is diluted in 1ml of distilled water and applied to the dorsal area of animals. Control groups receives 1 milliliter of distilled water.

Animal and housing conditions:

Seventy female and male Wister rats weighting between (160-450g) and aged 3-4 weeks were used in this study. All animal were obtained from the animal colony of Faculty of Medicine and Pharmacy of Mohammed-V Souissi University (Rabat, Morocco).

The animals were kept at a temperature of 22°C–24°C and were exposed to a 12-hour light/dark cycle, kept well-disinfected polypropylene cages (females separated from males) and had access to ordinary commercial food and water throughout the studies. To reduce non-specific stress, rats were acclimatized to laboratory conditions for 5 days prior to the tests.

Skin preparation:

24 hours before testing, the animals were marked, distributed in individual cages and the dorsal area of the trunk of each animal was gently shaved by disinfected razors. The area of application of the

substance was about 10% of the total body surface of the animals.

Experimental and animal protocols were carried in agreement with the guidelines for the care and use of Laboratory of Pharmacology of the Faculty of Medicine and Pharmacy, University -Rabat. Morocco.

Study design

Acute toxicity

Acute dermal toxicity was evaluated by dermal application of a single dose of alum to female and male rats. The test was carried out in accordance with Organization for Economic Cooperation and Development (OECD) guideline 404 [10]. In total, 50 rats were randomly assigned to control group (5 males and 5 females) and treatment groups divided into four experimental groups of 10 rats each. Control group, received distilled water. The treated groups were given a single dose of 500, 1000, 2000, and 5000 mg/kg body weight of alum stone, respectively. For the 24 hour exposure period, the substance was placed to the rats' prepared region and maintained using a porous gauze dressing and a non-irritant plaster. After 24 hours, the residues were removed using distilled water. The weight of each rat was measured over the period of the 14-day test, and clinical symptoms were noted on a daily basis. The animals were sacrificed and exhumed at the end of the research to document any gross pathological abnormalities.

Repeated dose dermal toxicity - 21/28 day

The animals were divided in two groups at random (n=10 each group, 5 males and 5 females). For 4 weeks dose, 1000mg/kg body weight of alum topically applied on the shaved area of rats and maintained with a porous gas dressing for 6 hours per day and 5 days per week. Control group received distillate water on a daily basis for 28 days. Body weights of rats were measured five times during the experiment: on the first day, 7th, 14th, 21st and 28th day. To record any symptoms of clinical toxicity and/or death, regular observations were performed immediately after the substance was applied, up to 4 hours after the administration, and throughout the test period. The cutaneous toxicity test method was used in accordance with the 410 guideline limit test [11].

For hematological analysis, blood samples were obtained through cardiac puncture at the end of the experiment and stored in ethylene diamine tetra acetic acid (EDTA).

For biochemical investigations, blood samples were collected in dry tubes and then centrifuged at 4000 rpm at 5°C for 10 min. All biochemical and hematological parameters analysis were carried out in Ibn Sina hospital laboratories (Rabat, Morocco).

The liver, kidneys, and skin of animals were isolated, rinsed with physiological water, cleansed, and placed on absorbent papers until being weighed (absolute organ weight in grams). After weighting removed organs (liver and kidney) and skin isolations, they were immediately fixed in 10% formaldehyde for a period of 24hrs, then, deposited in plastic cassettes to effect the inclusion, which consists of infiltrating and coating the tissues to be examined with paraffin and then cut using a microtome. The sections obtained were spread and glued on glass slides, which will be subsequently passed through a successive coloring step and mounted using the Eukitt placed between the blade and the slide. Microscopic sections of vital organs were readied, and the slides were done and observed in anatomy-pathological laboratory, Ibn Sina hospital (Rabat, Morocco).

Statistical analysis

All data measurements of this current experience (body weight, organ weight, haematology, and serum biochemistry) were represented as mean ±SD and statistically evaluated by One-way ANOVA test, following by *Bonferroni* comparisons, using SPSS version 20.

The probability of significant differences in the mean values between groups was determined using the t-Student test. Females and males data were recorded individually, and the differences were considered significant at $P< 0.05$.

Results

Acute toxicity

Body weights of treated rats of both sexes did not show any significant change compared to the controls [**Fig.1 and Fig.2**]. Also, the observation of the skin, eyes, mucous membranes, fur, color of urine and stool, and the respiratory, circulatory and nervous systems exhibited no significant changes in any animals. No mortality was recorded over study. After the sacrifice of the rats, macroscopic examination was performed on all vital organs of

the rats, and no gross pathological findings were recorded.

Repeated dose dermal toxicity - 21/28 days

During the 28-day repeated dermal trial, no mortality or signs of toxicity were observed at the dose of 1000mg/kg body weight. As compared to the control group, treated rats showed a normal gain in body weight throughout the four weeks [**Fig.3**]. Furthermore, no changes were observed in the skin and hair, eyes and mucous membranes, respiratory and circulatory systems, or central and autonomic nervous systems of any of the treated animals. A full physical examination and weighing of animal organs, including the liver and kidneys, revealed no adverse effects or significant variations in absolute weight among the groups [**Tab.1**].

In the biochemical analysis, there were no significant differences in triglycerides, cholesterol, urea and concentrations of mineral ions (Na+ and K+) and total protein levels in the treated rats as compared to control group. For the liver and kidneys functions parameters (ALT, AST), there was a clear variance. The obtained results for AST levels in female rats were high and the ALT levels were low for males compared to the controls [**Tab. 2**].

As shown in [**tab.3**], the dermal treatment did not induce any statistical significant change for most hematological parameters (red blood cell counts, mean corpuscular volumes, mean corpuscular hemoglobin concentrations, hematocrit, hemoglobin, mean corpuscular hemoglobin), with the exception of marginal variation in white blood cell counts, lymphocytes, monocytes, neutrophils and platelet count in the female rats.

In the histological examinations, no microscopic pathological changes were reported in liver and kidneys of either treated rats or controls. However, the histological examination of skin revealed injury in the skin of two females and one male. The histological sections of dermal tissue confirmed dermal necrosis in treated male and acute exanthema in two female rats.

Discussion

This present dermal toxicity study aimed to demonstrate safety profile of alum stone using animal models. During the 14-day observation period, the acute dermal toxicity test did not cause any significant changes in body weights, food and water consumption, skin appearance, or vital organs of treated rats at doses of 500, 1000, 2000, and 5000 mg/kg body weight of alum, as compared to control group. Accordingly, this product can be classified in category 5, under the Globally Harmonized System of Classification and Labeling (GHS), with an LD50 superior than 5000 mg/kg by the dermal route.

In the sub-acute dermal toxicity test, topical application of alum at a dose of 1000mg/kg did not affected food or water consumption, body weights, color of the eyes or mucous membranes of all treated animals. This finding suggests that alum at 1000 mg/kg body weight did not interfere with the normal metabolism of the animals.

Liver and kidneys are the principal organs exposed to toxicants as their main function involves filtering toxins out of the blood into faeces and urine. Furthermore, the hypertrophy of these organs is a first-hand indication of toxicity of chemical or biological substance [12]. Relative weight and macroscopic observations of the selected organs were normal in all treated groups at dose of 1000mg/kg body weight. Similar findings were reported by Osuala et al, who demonstrated that oral administration of alum did not produce any deleterious effects in the internal organs of mice [1].

Biochemistry tests are considered a key for indicating the level of functioning of the liver and kidneys, and they measure levels of fats and sugar circulating in the body [13]. High levels of ALT, AST are reported in liver diseases or hepatotoxicity. The results of this experiment indicated no significant changes in the level of most serum biochemistry parameters in treated animals; except for the slight rise of the AST level in the treated females compared to no treatment group. Also, the ALT level in male treated group was lower compared to control group. Similar to our findings, subchronic study showed significant rise in the enzymatic activities of ALP, AST, and ALT in the rats after exposure to Al (as $AlCl_3$) [14]. Therefore, the variation of ALT and AST levels in our experience could be associated with aluminum liver damage.

The dermal application of alum had no impact on hematological parameters, with the exception of platelet concentration levels in the treated female, which showed a decrease compared to the control, which might indicate systemic toxicity. In contrast

to our results, intravenously, alum has proved its effect in platelet aggregation [15]. Result of this study, suggests that application of alum by the dermal route may influence the platelet aggregation.

Clinical tests indicated a reduction in Neutrophil, Lymphocyte, and Monocyte levels in the treated female rats, which could be related to the perturbation of blood cell production. Similar to our hematological findings, the effects of subchronic exposure to Al in rats showed a decrease in WBC and RDC levels that may represent early events in Al induced haematopoetic organ injuries. In the light of experience, the absorption of alum through the dermal application, could lead to the drop in WBC levels.

According to previous studies, skin damage can be triggered by the use of some drugs administered through systemic route (oral, intravenous or subcutaneous) [16]. Nevertheless, dermal application of alum could be the cause of these lesions as well, at a dose of 1000 mg/kg body weight. These findings obtained in the histological evaluation of the skin indicate the need for a supplementary repeated long-term study with different concentration of alum stone in order to approve its responsibility for the lesions obtained on the three animals.

Alum stone is one of the alternatives used as an antiperspirant to replace chemicals products, it is applied frequently and several times a day. Also, one of the chemical compositions of alum stone is the aluminum ions. However, Aluminum has been shown to block the sweat ducts to prevent the release of sweat, which can cause the formation of cysts in the breast [17]. Also, scientists have studied responsibility for aluminum in the development of breast cancer in women using antiperspirants containing aluminum salts on a daily basis [18]. Based on our findings, dermal absorption of aluminum salts must be carried out in order to evaluate the transcutaneous passage of aluminum containing in alum stone.

Conclusion:

In the light of these findings, we may conclude that dermal application of 1000mg/kg of alum, in vivo, had no effect on body weights or mortality during the assay. However, further researches are needed to determine the hepato and nephrotoxic potential of alum at high doses. In addition, the injury of the skin obtained in three animals treated supposed that alum stone is able to cause the dermal lesions at high doses. These findings obtained indicate the need for a supplementary repeated long-term study with different concentration of alum stone.

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Conflict of Interest statement:

The authors declare that there are no conflicts of interest.

I, the Corresponding Author, declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

I can confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

Ethical considerations

Experimental and animal protocols were carried in agreement with the guidelines for the care and use of Laboratory of Pharmacology of the Faculty of Medicine and Pharmacy, University -Rabat. Morocco.

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Figure 1. Effect of dermal application of alum stone on body weight of male rats compared to control group (distilled water) . Values are presented as mean ± S.E.M (n= 5 for each group). *P values ≤ 0.5 were considered significant using one way ANOVA followed by Bonferroni comparison tests.

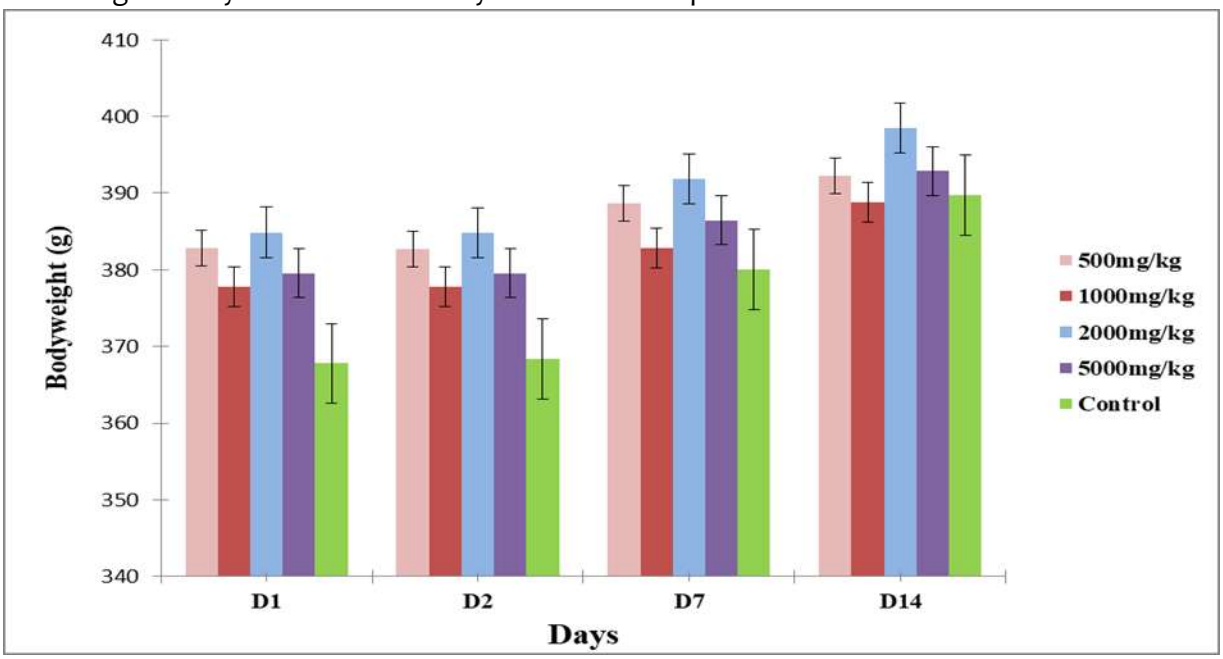

Figure 2. Effect of dermal application of alum stone on body weight of female rats compared to control group (distilled water). Values are presented as mean ± S.E.M (n= 5 for each group). **P* values ≤ 0.5 were considered significant using one way ANOVA followed by *Bonferroni* comparison tests.

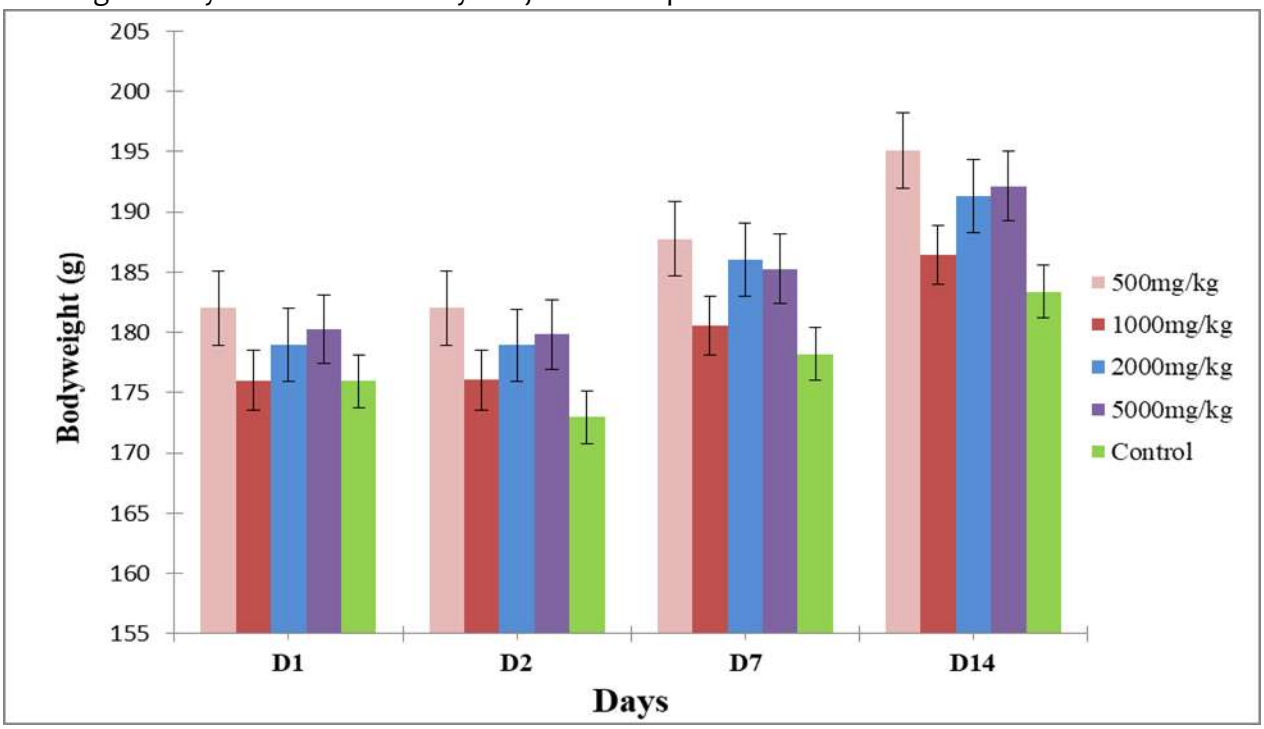

Figure 3. Effect of dermal application of alum stone on body weight of female and male rats compared to control groups. Values are presented as mean ± S.E.M (n= 5 for each group).*P values ≤ 0.5 were considered significant using one way ANOVA followed by *Bonferroni* comparison tests.

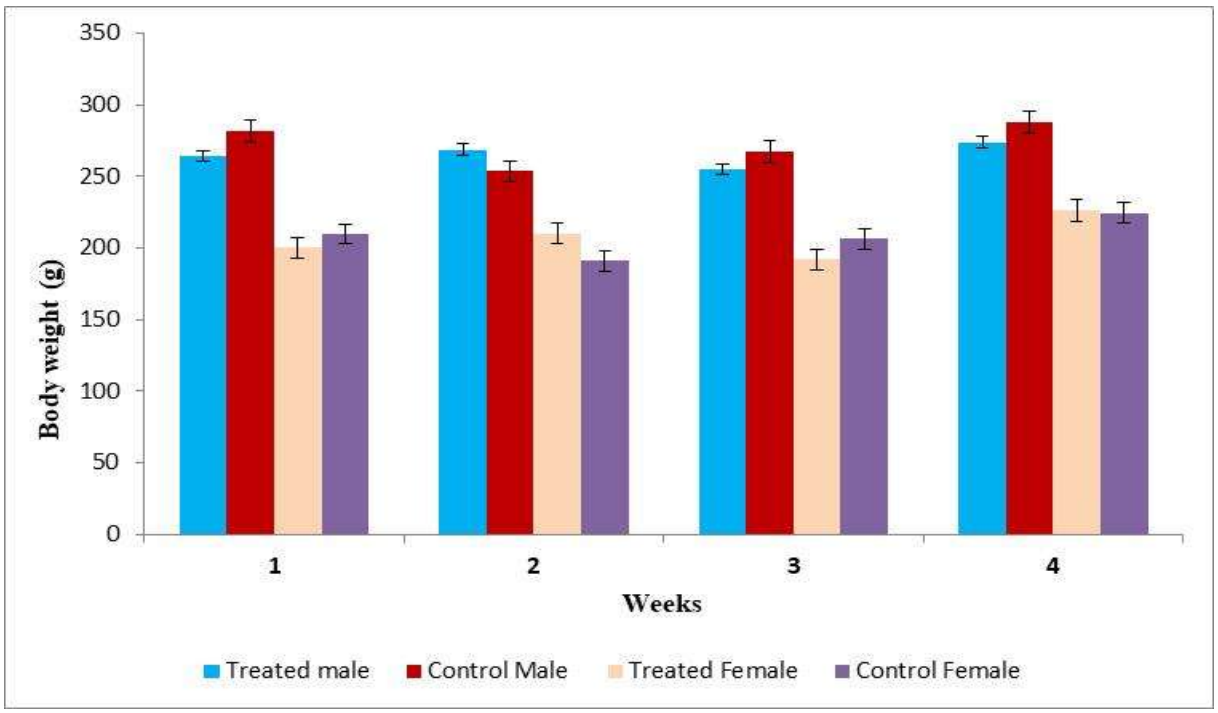

Table 1. Relative weight organs (g/100g body weight) of animals treated with alum stone compared to control.

	Organs	
	Liver	**Kidneys**
Control male	3.45±0.23	0.69±0.09
Treated male	3.79±0.09	0.69±0.04
Control female	3.53±0.29	0.66±0.4
Treated Female	3.8±0.18	0.69±0.24

Values are expressed as means ± SEM with significant difference (P ≤ 0.05) compared to control group.

Table2. Effect of dermal application on biochemical parameters of treated and control animals.

	Unit	**Control male**	**Treated male (1000mg/kg)**	**Control Female**	**Treated female (1000mg/kg)**
ALT	U/L	83.4±13.86	66.2±6.73	53.5±2.9	59±6.81
AST	U/L	132.8±14.38	121.6±10.55	107.25±8.93	124±9.17
Cholesterol	Mmol/L	0.71±0.09	0.63±0.06	0.76±0.09	0.77±0.09
Sodium	Mmol/L	143.6±6.73	143.2±1.35	140.25±1.03	137.75±1.1
potassium	Mmol/L	7.46±0.37	6.48±0.43	5.98±0.28	6.3±0.21
Triglyceride	Mmol/L	0.79±0.04	0.86±0.18	0.63±0.05	0.68±0.02
Total protein	g/L	78.8±1.38	77.35±1.39	81.17±2.24	78.1±2.55

Values are expressed as means ± SEM with significant difference *(P ≤ 0.05)* compared to control group.

Table3. Effect of dermal application on hematological parameters of treated and control animals

	Unit	Control male	Treated male	Control Female	Treated female
Total white blood cells	10^9/L	14.44±0.71	13.89±1.76	14.21±2.48	7.84±0.6
Total red blood cells	10^{12}/L	9.4±0.1	9.39±0.13	9.05±0.28	8.38±0.31
Hemoglobin	g/L	16.48±0.31	16.45±0.39	16.07±0.6	15.52±0.42
Hematocrit	%	44.44±0.85	44.17±1.17	44.27±2.02	43.87±1.28
Mean corpuscular volume	fL	47.24±0.83	47.06±1.99	48.92±1.66	52.57±1.08
Mean corpuscular Hb	pg	17.52±0.24	17.52±0.42	17.75±0.52	18.55±0.29
Mean corpuscular Hb conc	g/L	37.08±0.31	37.26±0.65	36.32±0.27	35.40±0.21
Platelets	10^9/L	469.6±0.09	498.90±113.23	685±115.4	448±42.01
Neutrophils	%	4.52±1.08	5.21±1.35	2.35±0.89	1.80±0.18
Lymphocytes	%	10.02±0.46	8.42±0.93	8.68±1.73	4.09±0.40
Monocytes	%	1.48±0.06	0.85±0.3	1.50±0.32	0.74±0.19

Values are expressed as means ± SEM with significant difference ($P \leq 0.05$) compared to control group.