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Effect of different extracts of *R. emodi* on hemato-biochemical parameters in rabbit wound healing model

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Abstract
The present study was aimed to evaluate the toxicity effect of different extracts of *R. emodi*, used for treatment of excisional full-thickness skin wounds.
Method: Thirty clinically healthy rabbits were divided into 4 equal groups. Two excisional wounds (1.5×1.5 cm) on the dorsal spine were created in all under general anesthesia. Except for the first group treated with povidone iodine which served as control, all the other groups were treated *R. emodi* extracts (ethanolic extract, aqueous extract and petroleum-ether extract). Evaluation of its effects on different hematological and biochemical parameters were recorded at different intervals of the study.
Results: The haematological indices and biochemical indices of rabbits in treatment group returned to normal level much earlier than the control group. In the petroleum ether treated group the increase in protein values was found earlier. Also the glucose values were low in all treated group with lowest being in ethanolic extract treated group.
Conclusion: No hepato toxicity and nephro toxicity was reported in *R. emodi* extract treated groups. 10% ethanolic, aqueous and P. ether extract ointments of *R. emodi* are safe to use in full-thickness wounds in rabbits.

Keywords: Wound Healing, Rhubarb, Rabbit, Haemato-biochemical, Extracts

1. Introduction
Different herbal drugs are been used and evaluated for their wound healing efficacy. Rhubarb, being one of the medicinal plants which, has been cultivated for over 5000 years for its medicinal purposes, originating in the mountains of the North-western provinces of China and Tibet and was first listed as a purgative and stomachic [1]. The botanical name of Himalayan rhubarb is *Rheum emodi* Wall. ex meissn. (Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Caryophyllales; Family: Polygonaceae; Genus: Rheum L.) [2]. Indian Rhubarb, which is official in the Indian Pharmacopoeia, consists of the dried rhizomes of *R. emodi* [3]. *Rheum emodi,* in Kashmiree called *Pambh challan* is a household herb, believed to have many potential theraupetic characteristics including wound healing. Previous studies suggest that roots of Rheum sps and their different extracts have wound healing, antioxidant, antibacterial, anti-fungal and antiviral activities [4].
Most herbal products in the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Some of them contain mercury, lead, arsenic, corticosteroids and poisonous organic substances in harmful amount [5]. Some medicinal plants are inherently toxic.in fact, the adverse effects of phytomedicines, as well as its adulteration, toxicity and drug interaction are common problems related to public health. There is very less information available about the effects of *R. emodi* and its different extracts on different hemato-biochemical parameters of body. Concerns by medicinal personnel indicate that herbal medicines may be harmful to vital organs such as liver and kidneys. The toxic effects of herbal plants may manifest in number of organs such as liver, kidney, stomach, nervous system and blood. We hereby design this study to verify the effects of *R. emodi* and its different extracts on hematological and biochemical parameters when used as a cutaneous wound healing agent.

2. Materials and Methods
2.1 Selection and grouping of animals
Twenty four clinically healthy rabbits of either sex, 9-15 months age, with body weight ranging between 2-3 kg were used for the study. The rabbits were randomly divided into 4 equal groups, of 6 animals each. The animals were kept off-feed for 6 hours and off-water for

2 hours. The dorsal thoraco-lumbar portion was shaved, cleaned and prepared for aseptic surgical procedures. General anesthesia was induced in animals for creation of wounds. Each animal was given xylazine @ 10 mg/kg I/M, left in calm environment for 5 minutes and then administered Ketamine Hydrochloride @ 50 mg/kg I/M.

2.2 Creation of wounds and treatment of wounds

Full thickness cutaneous wounds (1.5 x 1.5cm) were created on either side of dorsal spine in the thoraco-lumbar region. A locally fabricated metal marker (Plate 1) was used to demarcate the wound margins. The wounds were named as R and L on right and left side of vertebral column respectively.

All wounds were flushed with normal saline solution followed by treatment with medicaments, as shown in Table 1. The medicines were applied daily for 12 days and then on alternate days till complete healing of wound.

Table 1: Showing the grouping of animals based on application of different medicaments

Group	No. of animals	Treatment
Group 1	6	Flushing of wound with Normal saline solution + Povidone Iodine Ointment.
Group 2	6	Flushing of wound with Normal saline solution + 10% Ethanolic Extract Ointment (*Rheum emodi*).
Group 3	6	Flushing of wound with Normal saline solution + 10% Aqueous Extract Ointment (*Rheum emodi).*
Group 4	6	Flushing of wound with Normal saline solution + 10% Petroleum Ether Extract Ointment (*Rheum emodi).*

2.3 Evaluation of Hemato-biochemical parameters

The hemato-biochemical parameters were estimated pre-operatively, 24hr, 48hr, 72hr, 7th day, 14th day and on 21st day post wounding.

Haematological parameters: Total Erthrocytic Count (x 10^6/cu.mm), Total Leukocyte Count (x 10^3/cu.mm) and Differential Leukocyte Count (%) were calculated manually using standard technique (Schalm *et al.,* 1975). Haemoglobin (gm%) was estimated by using the standard technique by Sahli's acid haematin method and Packed cell volume (%) was estimated by using microhaematocrit method (Schalm *et al.,* 1975).

Biochemical parameters: Plasma Total protein (g/dl) was estimated using Biuret method and Plasma Albumin (g/dl) was estimated using BCG Dye Binding method. Glucose estimation (mg/dl) was done on immediately after collection of blood and was estimated using Accu-Checck Active Glucose meter. Urea estimation was done using DAM method and Serum Creatinine was estimated using modified Jaffe`s method. Aspartate aminotransferase (AST) also known as Serum glutamic oxaloacetic transaminase (SGOT) was estimated by modified IFCC method and Alanine transaminase (ALT) also known as Serum glutamic pyruvic transaminase (SGPT) was estimated by modified Reitman and Frankel's method.

Stastical analysis: Data obtained is presented as Mean ± standard error. Statistical analysis of the data was done using 17.0 SPSS software for windows.

3. Results

3.1 Haematological Parameters

The haematological parameters (Mean ± S.E) recorded in rabbits included in this study have been presented Tables 2-10. The hemoglobin content in the animals of the control group decreased significantly (p<0.05) immediately after wounding and remained so throughout the study period from the corresponding base values, and from treatment groups at all observation intervals. A significant (p<0.05) increase in PCV was observed only in group 1, which continued till day 7. Comparison among the groups revealed significantly (p<0.05) higher values of PCV in the animals of control group from those of treated groups at all observation intervals from day 1st to day 7 post wounding. TEC values change was significant only in the animals of control group, in which the values continued to decrease up to day 7, after which the values started increasing and reached to normal base values by the end of study period. Comparison among the groups revealed significant (p<0.05) decrease in TEC values of control group from the treated groups on day 2, 3 and 7 post wounding. Significant (p<0.05) changes in TLC values were seen on day 1 in group 4 animals, day 2 in animals of groups 2, and 4 and on day 3 in control group. While comparing among the groups TLC values of group 4 animals were significantly (p<0.05) higher from 0 day to 2nd day post-wounding.

The values of neurtophils highly increased significantly (p<0.05) post wounding in all the groups on day 1. The values were significantly higher from day 1 upto day 14 in all the groups except group 1 where significantly higher values were seen upto day 7. Comparison among the groups revealed significantly (p<0.05) lower value on day 3 of group 2. Post wounding the lymphocytes dropped significantly (p<0.05) on day 1. The values remained significantly (p<0.05) lower than the corresponding base values upto day 7 in group 1 and day 14 in groups 2, 3 and 4. Comparison among the groups revealed significant difference on day 3 where the values of group 1 animals were significantly (p<0.05) lower than that of 3rd group. Monocyte values showed fluctuations during the study period. Sometimes the fluctuations were significant (p<0.05). The changes in basophil values, though sometimes significant (p<0.05), didn't show any specific trend during the study period. Like basophils, the eosinophil values also fluctuated without any specific trend.

3.2 Biochemical parameters

The biochemical parameters (Mean ± S.E) recorded in rabbits included in this study have been presented Tables 11-17. Significant reduction in Total Protein contents was seen on day 1 in the animals of group 2, on day 2 in groups 1 and 4 and on day 3 in the animals of group 3. The serum albumin values (g/dl) showed a significant (p<0.05) decrease in the animals of group 1 on day 1 post wounding from their corresponding base values and continued with same trend till day 7 in groups 1 and till day 3 post wounding in group 3. The animals of group 3 and 4 showed significant (p<0.05) decrease in serum albumin values on day 2 post wounding while group 2 animals decrease was significant on day 3 post-wounding. The glucose values decreased significantly (p<0.05) on day 1st in animals of groups 2-4. Comparison among the groups revealed that the base value of group 4 animals was significantly (p<0.05) higher than those of other groups.

The BUN values of groups 3 and 4 animals showed significantly (p<0.05) higher values than those of other groups. In group 3 the values on 21st day were significantly (p<0.05) lower than the base value. Comparison among the groups revealed significantly (p<0.05) higher blood urea nitrogen values in animals of group 1 on day 2 and 3 post wounding. In group 1, the creatinine values increased significantly (p<0.05) till day 7 post-wounding and thereafter dropped abruptly to near normal values on day 21. In rest of the groups the values dropped slowly to reach near normal values by 21st day post-wounding except in group 2 in which the values could not reach to the base value. Comparison among the groups revealed significantly (p<0.05) higher values in group 1 those of other groups throughout study period. In control group the AST values decreased significantly (p<0.05) on day 2 post wounding. Thereafter, the values increased slowly to reach the corresponding base values by 21st day post-wounding. Post-wounding, the ALT values increased significantly (p<0.05) on day 1 and remained higher till day 7 in the animals of control group. The ALT values remained within normal range at all observation intervals in all the treated groups with minor fluctuations from corresponding base values except group 4 in which significantly (p<0.05) higher values were recorded only on day 7.

4. Discussion

Hematological parameters are considered as markers for presence of inflammation, infection and stress, esp. TLC and DLC. The control group showed significantly lower Hb values as compared to the corresponding base values. This is due to the decrease in serum content of lipid peroxidase and increase in the content of super dismutase activity in erythrocytes caused by rhubarb which leads to the improvement in the hemoglobin content of the animal [6]. In present study, Rhubarb extracts showed significant effect on TEC values in rabbits which started returning to base values earlier. This is on contrary with the findings of Mudhir [7] who reported no significant effect of Rhubarb on TEC values. The TLC values were in accordance with findings of Mudhir [7], who found significant increase in values after treating the animals with rhubarb.

In present study, the neutrophils showed an increase post-wounding reaching maximum on 24-48 hours. In case of treated groups there was a decrease in the values of neutrophil earlier that is on day 3 as compared to day 7 in control group. This may be attributed to the anti-inflammatory potential of rhubarb [8]. Lymphocytes are important indicators of infection. They play an important role in humoral antibody formation and cell- mediated immunity. There was a decrease in values of lymphocytes post-wounding which started returning to normal values towards the end of study period. A low normal lymphocyte concentration is associated with increased rates of infection after surgery and trauma. These facts explain the basis for the maximum values for neutrophil and minimum values for lymphocytes noted in present study from day 1 till day 3 in all groups. Monocytes, basophils and eosinophils constitute a very small portion in the total count of leucocytes. Any significant change in such small values may not be considered. However, in present study the monocytes showed an increase on day 1 post wounding in all groups, but showed a decrease below the corresponding base values on day 2 and 3 i.e. in the peak hours of inflammation. This could be a transitory decrease in monocytic count. This observation is in consonance with another study [9] which reported that acute inflammation leads to transient decrease in the monocytic count. The basophils increase at the time of allergic reactions [10]. In present study basophils didn't show any significant change. Rhubarb is reported to have an anti-allergic effect, hence no increase in basophils was reported.

The advent of the inflammatory process including infection, trauma, surgery, burns, and other wounds elicits the acute phase response. During this acute phase response, these proteins decline and as such are called as negative acute phase reactants [11]. Decrease in the values of total protein and albumin from day 1 till day 2-3 post wounding was observed in present study. This could be attributed to the inflammatory processes and to decreased food intake due to pain. This decrease in total protein and albumin after wounding could also be because of surgical stress and high temperature at the study area. Furthermore, there was increase in total protein and albumin towards the end of study period; this could be attributed to increased synthesis of amino acids and proteins in the remodeling phase of wound healing. In the petroleum ether treated group i.e. group 4 the increase in protein values was found earlier, this could be attributed to the presence of proteins and amino acids in the petroleum ether extract [12]. In present study on day 1st post-wounding the glucose level of all the groups decreased, which could be attributed to decreased feed intake due to pain in animals of all groups. Cortisol which is released in response to injury, accelerate gluconeogenesis and ketogenesis leading to increased blood glucose levels [13]. In all other groups in which rhubarb extracts were used they showed decreased values of plasma glucose, this may be attributed to the presence of glycoside E in rhubarb roots, which have an hypoglycemic effect [14]. Since glycosides are present strongly in ethanolic extract of rhubarb, the group shows lowest values of glucose. Urea and creatinine represent the two main nitrogenous components that are eventually excreted by the kidney, therefore changes in their levels in the blood stream would reflect the changes in kidney functions [15, 16]. The presence of increasing urea and creatinine concentration in the blood suggest the inability of the kidney to excrete these products, which further suggest a decrease in glomerular filtration rate [17]. Increase in creatinine levels is more faster than urea levels at the start of disease, and also decrease more quickly when an improvement takes place, thus being a more specific indicator of kidney injury than BUN [18]. In the present study the concentration of BUN and creatinine was found within normal range. Rhubarb has been studied for the management of renal function disorders [19] and has reported rhubarb to be a nephron protective drug [20]. Nephro protective effect of water soluble and water insoluble fractions of ethanolic extracts of rhubarb have been reported [21]. Nephro protective effect of rhubarb has been attributed to tannins present in rhubarb which significantly improve BUN and creatinine, glomerular filtration rate, renal plasma flow and renal blood flow [22]. Increase in creatinine values control group may be due to absence of any nephroprotectant and also due to stress in animals post wounding which caused the increase in values. The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. AST and ALT remained within normal range in all the treated groups showing that there was no toxic effect of drug on liver. This observation is in consonance with a previous study [23] which reported that *R. emodi* is an hepatoprotective plant. However during the present study the values increased significantly in the control group. This leads to inference that *R. emodi* extracts have hepatoprotective

effect, which was lacking in control group and higher values of ALT.

5. Conclusion

R. emodi extract treated groups showed no significant effect on hematological parameters. There was no decrease in hemoglobin value and T.E.C count as compared to control group. Except petroleum ether extract treated group, other two extract treated groups showed significant and earlier return of total protein and albumin levels that had decreased following surgery. Glucose values in all extract treated groups although remained within normal range but were observed to be lower than the base values, and started increasing slowly in control group only. *R. emodi* treated groups showed no signs of hepatotoxicity and nephrotoxicity, this may be attributed to the fact that rhubarb is hepatoprotective and nephro protective medicinal plant. The rhubarb treated groups showed better wound healing properties.

Table 2: Mean±SE of hemoglobin (g%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	12.80 ±0.16^{aA}	9.6 ±0.13^{aB}	9.38 ±0.49 ^{aB}	9.90 ±0.14^{aBC}	9.96 ±0.41 ^{aBC}	10.55 ±0.30^{aC}	10.63 ±0.08^{aC}
2	12.41 ±0.23 ^{aA}	12.03 ±0.31 ^{bA}	12.10 ±0.27^{bA}	12.23 ±0.24 ^{bA}	12.28 ±0.14^{bA}	12.38 ±0.11^{bA}	12.60 ±0.18^{bA}
3	12.36 ±0.12^{aA}	12.35 ±0.11 ^{bA}	12.15 ±0.02^{bA}	12.16 ±0.02 ^{bA}	12.18 ±0.03^{bA}	12.20 ±0.33 ^{bA}	12.30 ±0.05^{bA}
4	12.33 ±0.07 ^{aAB}	12.03 ±0.03 ^{bA}	12.16 ±0.15^{bAB}	12.16 ±0.15 ^{bAB}	12.16 ±0.15 ^{bAB}	12.23 ±0.16^{bAB}	12.53 ±0.03^{bB}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 3: Mean±SE of PCV (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	34.35 ±0.04^{aA}	39.76 ±0.50^{aB}	39.05 ±0.14^{aB}	39.26 ±0.09 ^{aB}	38.43 ±0.12^{aB}	32.40 ±0.60^{aC}	34.38 ±1.08^{aA}
2	34.50 ±0.88^{aAB}	37.73 ±0.90^{aB}	34.41 ±1.03^{bAB}	35.58 ±1.07 ^{bAB}	34.21 ±1.19^{bAB}	31.96 ±1.52^{aA}	33.13 ±1.56^{aA}
3	34.70 ±0.32^{aAB}	36.60 ±0.60^{aB}	33.46 ±1.13^{bAB}	33.88 ±1.46$^{b\ AB}$	33.51 ±1.57^{bAB}	34.53 ±1.12^{abAB}	32.75 ±0.84^{aA}
4	34.50 ±0.02 ^{aA}	36.00 ±1.26^{aA}	33.86 ±0.71^{bA}	34.33 ±1.07$^{b\ A}$	33.55 ±0.08^{bA}	35.63 ±0.12^{bA}	33.56 ±1.08^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 4: Mean±SE of TEC (x10^6 cu.mm) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	6.26 ±0.25^{aA}	6.01 ±0.01^{aABC}	5.96 ±0.00^{aABC}	5.88 ±0.00^{aBC}	5.73 ±0.01^{aC}	6.18 ±0.03^{aAB}	6.26 ±0.02^{aA}
2	6.23 ±0.30^{aA}	6.01 ±0.17^{aA}	6.18 ±0.04^{aA}	6.18 ±0.04^{bA}	6.20 ±0.06^{bA}	6.21 ±0.06^{aA}	6.24 ±0.06^{aA}
3	6.35 ±0.14^{aA}	6.03 ±0.16^{aA}	6.23 ±0.23^{aA}	6.25 ±0.18^{bA}	6.26 ±0.18^{bA}	6.30 ±0.16^{aA}	6.33 ±0.07^{aA}
4	6.38 ±0.44^{aA}	6.01 ±0.41^{aA}	6.20 ±0.02^{aA}	6.25 ±0.05^{bA}	6.28 ±0.02^{bA}	6.31 ±0.07^{aA}	6.36 ±0.06^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 5: Mean±SE of TLC (x10^3 cu.mm) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	5.93 ±0.27^{aA}	6.38 ±0.28^{aA}	7.06 ±0.38^{aAB}	8.21 ±0.62^{aB}	7.23 ±0.51^{aAB}	6.45 ±0.41^{aA}	6.05 ±0.28^{aA}
2	6.41 ±0.18^{Aa}	6.60 ±0.14^{aA}	7.43 ±0.36^{abB}	8.13 ±0.24^{aC}	7.36 ±0.26^{aB}	6.98 ±0.27^{aA}	6.30 ±0.15^{aA}
3	7.68 ±0.74^{bA}	8.13 ±0.77^{cA}	8.68 ±0.74^{bA}	9.21 ±0.84^{aA}	8.18 ±0.83^{aA}	7.73 ±0.84^{aA}	7.25 ±0.82^{aA}
4	7.00 ±0.33^{abAB}	7.81 ±0.22^{bcCD}	8.33 ±0.15^{abDE}	8.33 ±0.17^{aE}	7.36 ±0.12^{aBC}	6.78 ±0.08^{aA}	6.50 ±0.09^{aaA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 6: Mean±SE of neutrophils (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	32.16 ±1.27^{aA}	70.00 ±2.03^{aB}	72.83 ±1.16^{aB}	76.33 ±2.33^{aB}	52.33 ±6.99^{aC}	37.83 ±3.04^{aA}	32.50 ±1.45^{aA}
2	36.33 ±2.26^{aA}	66.67 ±1.99^{aB}	68.66 ±1.11^{aB}	69.33 ±1.76^{cB}	46.66 ±5.49^{aC}	46.33 ±3.73^{aC}	32.83 ±2.97^{aA}
3	28.35 ±3.98^{aA}	70.50 ±2.07^{aB}	71.33 ±1.97^{aB}	67.50 ±2.30^{bcB}	53.00 ±3.19^{aC}	39.83 ±2.68^{aD}	27.66 ±3.82^{aA}
4	34.33 ±2.56^{aA}	68.00 ±1.29^{aB}	69.50 ±1.36^{aB}	70.66 ±1.76^{acB}	52.50 ±2.32^{aC}	42.16 ±2.94^{aD}	31.83 ±4.55^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 7: Mean±SE of Lymphocytes (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	63.66 ±2.27^{aA}	25.83 ±2.50^{aB}	26.33 ±1.33^{aB}	25.50 ±1.99^{aB}	45.5 ±7.56^{aC}	56.83 ±3.97^{aA}	67.83 ±2.45^{aA}
2	61.50 ±2.48^{aAB}	31.16 ±2.10^{aD}	30.66 ±0.76^{aD}	30.30 ±1.74^{abD}	53.00 ±5.52^{aAC}	50.83 ±4.26^{aC}	65.50 ±2.96^{aB}
3	70.16 ±3.55^{aA}	27.83 ±3.04^{aB}	28.50 ±2.66^{aB}	31.16 ±1.16^{bB}	47.33 ±3.52^{aC}	58.50 ±1.94^{aD}	70.34 ±2.33^{aA}
4	65.33 ±2.70^{aA}	31.33 ±1.30^{aB}	29.66 ±1.20^{aB}	29.00 ±1.48^{abB}	47.50 ±2.32^{aC}	57.16 ±2.42^{aD}	67.33 ±4.15^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 8: Mean±SE of Monocytes (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	1.00 ±0.36^{aA}	2.16 ±0.87^{aA}	0.50 ±0.34^{abA}	0.16 ±0.16^{aA}	1.50 ±1.31^{abA}	1.33 ±0.66^{aA}	0.83 ±0.54^{aA}
2	1.00 ±0.36^{aAB}	1.33 ±0.49^{abB}	0.16 ±0.16^{aAC}	0.16 ±0.16^{aAC}	0.00 ±0.00^{aC}	0.33 ±0.33^{aAC}	0.16 ±0.16^{aAC}
3	1.00 ±0.51^{aAB}	2.16 ±0.74^{aB}	0.66 ±0.33^{abAB}	1.16 ±0.83^{aAB}	0.16 ±0.16^{aA}	1.00 ±0.81^{aAB}	O.83 ±0.4^{aAB}
4	0.00 ±0.00^{aA}	0.16 ±0.16^{bA}	0.00 ±0.00^{aA}	0.33 ±0.33^{aA}	0.00 ±0.00^{aA}	0.66 ±0.66^{aA}	0.50 ±0.50^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 9: Mean±SE of basophils (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	0.83 ±0.65^{aA}	0.16 ±0.16^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.16 ±0.16^{aA}	0.16 ±0.16^{aA}
2	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.16 ±0.16^{aA}	0.00 ±0.00^{aA}
3	0.50 ±0.22^{aA}	0.67 ±0.33^{bA}	0.50 ±0.34^{bA}	0.50 ±0.34^{bA}	0.16 ±0.16^{aA}	0.50 ±0.34^{aA}	0.83 ±0.47^{bA}
4	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 10: Mean±SE of Eosinophils (%) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	1.66 ±0.42^{aA}	1.33 ±0.42^{aAB}	0.50 ±0.34^{aAB}	0.00 ±0.00^{aB}	0.50 ±0.34^{aAB}	1.50 ±0.76^{aA}	0.66 ±0.42^{aAB}
2	0.83 ±0.54^{abA}	0.66 ±0.42^{abA}	0.00 ±0.00^{bA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	1.16 ±0.83^{aA}	0.00 ±0.00^{aA}
3	0.00 ±0.00^{bA}	0.33 ±0.33^{bA}	0.00 ±0.00^{bA}	0.50 ±0.34^{aA}	0.16 ±0.16^{aA}	0.50 ±0.34^{aA}	0.33 ±0.33^{aA}
4	0.00 ±0.00^{bA}	0.00 ±0.00^{bA}	0.00 ±0.00^{bA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}	0.00 ±0.00^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table-11: Mean±SE of total protein (g/dl) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	5.51 ±0.098^{aA}	5.35 ±0.05^{aAB}	5.30 ±0.09^{aB}	5.23 ±0.02^{aB}	5.29 ±0.03^{aB}	5.34 ±0.03^{aAB}	5.42 ±0.04^{aAB}
2	5.62 ±0.06^{aA}	5.38 ±0.06^{aB}	5.18 ±0.05^{aCD}	5.14 ±0.03^{aC}	5.35 ±0.05^{cBD}	5.50 ±0.00^{abAB}	5.6 ±0.11^{aA}
3	5.47 ±0.105^{aAB}	5.39 ±0.10^{aABC}	5.28 ±0.09^{aAC}	5.21 ±0.06^{aC}	5.30 ±0.04^{dAC}	5.54 ±0.03^{abB}	5.52 ±0.07^{aAB}
4	5.69 ±0.065^{aA}	5.49 ±0.09^{aAB}	5.35 ±0.08^{aB}	5.42 ±0.09^{bAB}	5.42 ±0.06^{aAB}	5.68 ±0.17^{bA}	5.57 ±0.05^{aAB}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 12: Mean±SE of albumin (g/dl) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	3.27 ±0.07^{aA}	2.88 ±0.16^{aB}	2.9 ±0.00^{aB}	3.06 ±0.02^{aAB}	3.01 ±0.04^{abAB}	3.09 ±0.09^{abAB}	3.26 ±0.18^{abA}
2	3.56 ±0.18^{aAB}	3.20 ±0.02^{bBC}	3.20 ±0.00^{cBC}	3.10 ±0.00^{aC}	3.15 ±0.06^{cC}	3.40 ±0.11^{acABC}	3.64 ±0.15^{aA}
3	3.24 ±0.04^{aA}	3.05 ±0.03^{aAB}	2.95 ±0.06^{abB}	2.90 ±0.00^{aB}	2.90 ±0.00^{bB}	2.95 ±0.06^{aB}	3.19 ±0.12^{bA}
4	3.43 ±0.10^{aAB}	3.30 ±0.06^{bA}	3.10 ±0.00^{bcC}	3.0 ±0.00^{aC}	3.10 ±0.00^{acC}	3.30 ±0.00^{bcA}	3.48 ±0.08^{abA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 13: Mean±SE values of Glucose (mg/dl) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	111.83 ±8.46^{aA}	105.0 ±10.56^{aA}	114.50 ±11.00^{aA}	122.16 ±6.79^{aA}	122.0 ±0.00^{aA}	122.50 ±6.60^{aA}	126.16 ±0.16^{aA}
2	130.16 ±0.10^{abA}	106.66 ±1.58^{aB}	109.16 ±3.52^{aB}	125.18 ±1.93^{aA}	128.33 ±6.29^{aA}	126.0 ±3.47^{aA}	129.16 ±0.10^{aA}
3	120.50 ±8.15^{abA}	101.66 ±4.97^{aB}	116.16 ±6.47^{aAB}	127.83 ±2.68^{aA}	129.50 ±5.90^{aA}	128.0 ±0.00^{aA}	125.83 ±5.02^{aA}
4	134.50 ±1.58^{bA}	113.50 ±3.24^{aB}	112.33 ±0.04^{aB}	128.50 ±4.37^{aAC}	128.83 ±0.47^{aAC}	129.55 ±0.13^{aAC}	126.16 ±3.68^{aC}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 14: Mean±SE values of BUN (mmol/l) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	4.57 ±0.07^{abA}	4.71 ±0.05^{aAB}	5.08 ±0.04^{aB}	4.98 ±0.01^{aAB}	4.59 ±0.27^{aAB}	4.52 ±0.14^{aA}	4.51 ±0.26^{aA}
2	4.55 ±0.05^{abA}	4.66 ±0.04^{aABC}	4.73 ±0.02^{bBC}	4.78 ±0.04^{bB}	4.61 ±0.03^{aAC}	4.58 ±0.06^{aA}	4.53 ±0.03^{aA}
3	4.60 ±0.00^{bAB}	4.68 ±0.03^{aAC}	4.63 ±0.04^{bAB}	4.73 ±0.02^{bC}	4.65 ±0.02^{aAC}	4.55 ±0.02^{aBD}	4.50 ±0.04^{aD}
4	4.66 ±0.03^{bAB}	4.65 ±0.04^{aAB}	4.73 ±0.04^{bA}	4.75 ±0.02^{bA}	4.65 ±0.02^{aAB}	4.63 ±0.03^{aAB}	4.60 ±0.05^{aB}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 15: Mean±SE values of Creatinine (µmol/l) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	80.50 ±0.34^{aA}	89.83 ±1.53^{aB}	92.66 ±0.49^{aC}	93.5 ±0.61^{aC}	95.83 ±0.40^{aD}	84.16 ±0.16^{aE}	81.33 ±0.33^{aA}
2	75.16 ±0.16^{bA}	77.83 ±0.30^{cB}	77.66 ±0.34^{cB}	77.66 ±0.21^{cB}	77.16 ±0.16^{cB}	76.33 ±0.33^{cC}	76.33 ±0.33^{cdC}
3	75.33 ±0.21^{bA}	78.61 ±0.19^{cB}	78.66 ±0.21^{dB}	77.81 ±0.16^{cC}	77.10 ±0.37^{cD}	76.33 ±0.21^{cE}	75.66 ±0.21^{cAE}
4	77.66 ±0.42^{cAB}	79.66 ±0.21^{bcC}	79.66 ±0.21^{cC}	78.80 ±0.05^{dD}	77.80 ±0.34^{cA}	77.83 ±0.16^{dA}	77.01 ±0.01^{dB}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 16: Mean±SE values of AST (IU/L) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	46.76 ±3.42^{aA}	42.30 ±2.52^{aAB}	39.90 ±0.57^{aB}	38.83 ±2.49^{aB}	39.81 ±0.47^{aB}	40.33 ±0.33^{aB}	42.6 ±0.25^{aAB}
2	38.90 ±4.34^{aA}	42.03 ±4.58^{aA}	38.78 ±1.27^{aA}	39.00 ±0.00^{aA}	42.83 ±0.40^{aA}	41.33 ±0.42^{aA}	40.53 ±1.28^{aA}
3	35.31 ±2.95^{aA}	37.28 ±2.79^{aA}	36.73 ±3.17^{aA}	36.95 ±3.27^{aA}	35.27 ±2.95^{aA}	35.51 ±2.89^{aA}	33.57 ±2.79^{aA}
4	35.18 ±0.95^{aA}	36.43 ±1.93^{aA}	35.16 ±2.16^{aA}	37.25 ±1.49^{aA}	39.18 ±1.61^{aA}	36.98 ±1.21^{aA}	35.43 ±1.69^{aA}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

Table 17: Mean±SE values of ALT (IU/L) in the animals of different groups at different observation intervals

Group No.	Observation Intervals (Days)						
	0	1	2	3	7	14	21
1	29.02 ±1.13^{abA}	59.85 ±2.17^{aB}	57.83 ±5.52^{aBC}	50.42 ±2.57^{aC}	39.95 ±2.18^{aD}	29.21 ±1.21^{aA}	28.36 ±1.30^{aA}
2	37.80 ±9.27^{bcA}	40.60 ±8.69^{cdA}	39.70 ±8.59^{cadA}	41.23 ±8.41^{aA}	26.04 ±4.97^{bA}	26.20 ±4.81^{aA}	23.39 ±3.66^{aA}
3	24.85 ±6.17^{abA}	25.29 ±6.16^{bcA}	25.39 ±6.23^{bcA}	25.71 ±6.23^{bA}	25.36 ±6.14^{bA}	25.46 ±6.36^{aA}	23.50 ±5.72^{aA}
4	48.69 ±2.46^{cA}	49.26 ±2.46^{adA}	50.44 ±2.40^{adAB}	52.78 ±1.32^{aAB}	56.36 ±1.99^{cB}	52.63 ±0.45^{bAB}	51.59 ±0.77^{bAB}

Figures with different superscript (small letters) differ significantly between groups
Figures with different superscript (capital letters) differ significantly between days within the groups
n = 6 animals in each group

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