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EFFECT OF *MORINGA OLEIFERA* LEAVES ON HEMATOLOGICAL AND SERUM CHEMISTRY ATTRIBUTES OF APPARENTLY HEALTHY RABBIT DOES

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ABSTRACT

The study was designed to assess the effect of *Moringa oleifera* leaf extract on various hematological and serum chemistry attributes (liver and kidney function test enzymes) of apparently healthy rabbit does (n=24), either positive or negative. The animals were divided in four groups namely A, B, C and D (n=6 rabbits/group). First group was considered as control group provided with basal diet and water without any supplementation. Group A, B and C were considered as treatment groups which were provided with basal diet, water and supplementation of *M. oleifera* crude leaf aqueous extract at the dose rates of 150mg/kg, 300mg/kg, and 450 mg/kg, respectively. On day 10, 20, 30 and 40, blood samples were collected from all animals. The blood samples (n=96) were analyzed through hematology analyzer and semi-automatic chemistry analyzer. Results regarding hematological attributes revealed that the Mitotic Index was significantly (P≤0.05) higher in control group whereas Red Cell Distribution Width Count was significantly (P≤0.05) higher in Group C as compared to their counterpart groups. Similarly, Total Erythrocytic Count was significantly (P≤0.05) higher in Group B. The creatinine, urea and blood urea nitrogen differed significantly (P≤0.05) while Uric acid showed non-significant (P≥0.05) difference between the groups. It is concluded that varying doses of Moringa do not exert any untoward effect on hematochemical attributes of rabbits and hence their health. Rather, positive effects were noticed at dose rate of 150mg/kg.

Key Words: *M. oleifera;* Hematology; Liver Function Test; Kidney Function Test

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INTRODUCTION

Domestic rabbit (*Oryctolagus cuniculus*) is a beneficial livestock species. Rabbits are small mammals belonging to family Leporidae and order Legomarpha. They are acknowledged ruminant research model used in various biomedical research fields including *in-vitro* treatment, neurophysiology, embryology organ rearrangement, and cardiovascular research. The studies of toxicology and analyses of effects of drugs on the embryos and development of fetus have been carried out extensively on the rabbits (Bourdon *et al*. 2018).

Herbal extracts and leaves are extensively being used in supplementation of animal feed for the improvement of animal growth and health. Recently, *Moringa oleifera* has been a center of attention for researchers worldwide. *M. oleifera* is a natural species of monogeneric family Moringaceae and native in India, Pakistan, Asia-Minor and Africa. It is known as 'drumsticks tree' or 'horse radish tree' in various regions of the world. In Pakistan, *M. oleifera* is grown all over the country and is commonly known as 'Sohanjna' (Anwar, Ashraf, and Bhanger, 2005). Studies have revealed that *M. oleifera* is rich in protein, desirable amino acids, vitamins, minerals and is an important source of crucial nutrients (Falowo *et al*. 2018). The extracts of various portions of Moringa plant contain vital minerals such as calcium, iron, zinc, copper, manganese, magnesium and potassium. It also contains large number of natural anti-oxidant molecules like flavonoids, phenolics, ascorbic acid and carotenoids (Dillard and German, 2000; Siddhuraju & Becker, 2003). Other beneficial healing properties of the *M. oleifera* plant include antispasmodic, anti-hypertensive, antioxidant, hepatoprotective, antifungal and antibacterial actions (Amaglo *et al*., 2010; Mbikay, 2012). *M. oleifera* is used for the cure of various diseases in the native medicine system (Rockwood, Anderson, and Casamatta, 2013), particularly in South Asia (Anwar and Rashid, 2007).

Previously, it has been disclosed that *M. oleifera* exerts positive effect on growth rate and microbial preservation and digestive tract with high suitability of flourishing rabbits (El-Kholy *et al*. 2018). In Pakistan, work has been conducted on assessment of antioxidant and nephroprotective effects of *M. oleifera* in paracetamol toxic albino rabbits (Ijaz *et al*., 2016), the spasmolytic and hypotensive effects of clean compounds from *M. oleifera* and their pharmacological studies have also been conducted (Gilani *et al*. 1994). However, there is a scarcity of literature regarding the effect of *M.*

oleifera on apparently healthy rabbit does. Therefore, the present study has been conducted with an objective to assess the effect of *M. oleifera* leaves on various hematological and serum chemistry attributes *viz*. liver and renal function test (LFT and RFT) enzymes of apparently healthy rabbit does.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Zoology and Department of Physiology, the Islamia University of Bahawalpur, Pakistan. Bahawalpur. The city lies at 69º52′and 75º24′E longitude and 27º42′and 29º45′N latitude; at an altitude of 112meter above sea level. The climate is arid, monsoonal, high temperature, with high rate of evaporation, low relative humidity and strong summer winds. It is among the driest and hottest areas of Pakistan, with summer spanning May through October. The annual mean temperature of area is 28.33°C with average rainfall of 180 mm per year (Farooq, Qayyum, Samad, Chaudhry, & Ahmad, 2010).

Moringa leaf extract preparation: Leaves of the *M. oleifera* plant were taken and air-dried under shade at room temperature. The dry leaves finally were ground into powder which was sifted by 1mm iron mesh and stored in air tight plastic jars at room temperature. Weighed amounts of 150mg, 300mg and 450mg of the powdered leaves were taken for making aqueous solution that was orally administered (20mL) with the help of syringe without needle.

Animals and their management: Rabbit does (n= 24) of 6-8 months and belonging to homogenous litters were purchased from the local vendor. All the rabbit does were active and quite healthy as assessed through visual inspection and through anamnesis from the vendor. They were kept in the cages and were acclimatized to their housing and feeding pattern for 1 week prior to the initiation of the experimental trial. All the animals were given pelleted feed (17.7 CP, 12.13 MJ/kg DE) and *ad libitum* alfalafa hay.

Treatment procedure: The experimental animals were divided into four groups. First group was considered as control whereas Groups A, B and C were the treatment groups (n=6 rabbits/group) fed 150mg/kg, 300mg/kg, and 450 mg/kg live weight of *oleifera* crude leaf extract, respectively.

Blood sampling and processing: Blood sampling was carried out on day 10, 20, 30 and 40 post-administration of Moringa crude leaf extract. A total of 96 samples were hence taken. Blood samples were obtained aseptically using a 5mL disposable syringe through the jugular vein into well labeled EDTA- added vacutainers and in yellow topped serum activating gel vacutainers. Upon clot formation, serum was extracted through appropriate centrifugation using Sigma 1-14 (Sigma Laboratories GmbH, Germany). The obtained serum was stored at 4ºC until further analyzed.

Hematological analyses: All the samples were analyzed through an automated veterinary hematology analyzer (Junior Abacus Vet-5, USA) for determination of hematological indices including total leukocyte count (TLC), total erythrocyte count (TEC), granulocytes % (GRA), lymphocytes (LY), mitotic index (MI), granulocytes number (GR), red cell distribution width counts (RDWc), platelet count (PLT), procalcitonin test (PCT), mean platelet volume (MPV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and platelet distribution width counts (PDWc).

Serum chemistry analyses: The LFT enzymes *viz*. Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST), Alkaline Phosphatase (ALP), bilirubin and albumin and RFT enzymes *viz*. Creatinine, Urea, Blood Urea Nitrogen (BUN) and Uric Acid were determined through semi-automatic chemistry analyzer (Biosystem BTS350, Barcelona, Spain) using commercial kits as per details given below:

Urea, BUN, Uric Acid and Albumin: Bioassay Systems QuantiChromTM Assay Kit (DICL-250), Bioassay Systems, Hayward CA, USA. Creatinine, Bilirubin and AST: RANDOX Kits (CR 510, BE 454 and AS 101), RANDOX Laboratories Ltd. Antrim, UK. ALT and ALP: Ecoline ® 125, Diagnostica Merck Kit (KGaA 64271), Dramstadt, Germany.

Statistical analysis: Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS for Windows Version 17, SPSS Inc., IL. USA). Results were expressed as Mean (± SE). The difference between time-based collections within the groups was ascertained through Repeated Measures Analysis of Variance (MANOVA), followed by Bonferroni *post-hoc* test. Significance was considered as $P \leq 0.05$.

RESULTS

Overall mean (± SE) values for hematological parameters in apparently healthy rabbit does (n=24) fed various levels of *M. oleifera* is presented in Table 1. Results showed that the MI, TEC and RDWc were significantly ($P \leq 0.05$) affected by varying doses of *M. oleifera* whereas remaining all other attributes remained unaltered. The MI was significantly ($P \leq 0.05$) higher in control group but decreased in all the *M. oleifera* fed treatment groups. The TEC remained same for all groups except for Group B in which a significant ($P \leq 0.05$) .

Table 1: Overall mean (± SE) values for hematological parameters in apparently healthy rabbit does (n=24) fed various levels of *M. oleifera.*

Parameters	Groups				Overall
	Ctrl	A	B	C	
Total Leukocyte Count (10^9/L)	6.8±0.3	5.8±0.3	6.6±0.4	6.5±0.3	6.4±0.1
Granulocytes (%)	1.7±0.1	1.3±0.1	1.3±0.2	1.6±0.2	1.5±0.1
Lymphocytes (%)	70.0±2.0	61.0±3.1	65.2±3.2	63.0±3.3	64.8±1.5
Mitotic Index (%)	5.4±1.5^{a}	2.1±0.4^{b}	1.9±0.2^{b}	2.2±0.3^{b}	2.9±0.4
Granulocytes Number (10^9/L)	16.0±1.5	19.0±2.5	17.8±2.9	20.8±2.8	18.4±1.2
Total Erythrocyte Count (10^12/L)	4.6±0.0^{b}	4.7±0.0^{b}	5.1±0.1^{a}	4.6±0.0^{b}	4.8±0.0
Hemoglobin (g/L)	110.0±0.2	108±0.2	116±0.2	113±0.3	112±0.1
Packed Cell Volume (Fraction)	0.335±1.3	0.334±1.2	0.354±1.3	0.33 ±1.1	0.339±0.6
Mean Corpuscular Volume (fL)	66.1±1.6	63.7±1.1	64.3±1.4	64.1±1.3	64.6±0.7
Mean Corpuscular Hemoglobin (pg)	22.8±1.5	21.0±0.6	21.2±0.4	20.9±0.5	21.5±0.4
Mean Corpuscular Hemoglobin Concentration (mmol /L)	3.34±1.5	3.35±1.4	3.35±1.5	3.34±1.4	3.35±0.7
Red Cell Distribution Width Counts (%)	14.6±0.7^{b}	14.9±0.7^{b}	14.6±0.7^{b}	22.7±2.8^{a}	16.7±0.8
Platelet Count (10^9/L)	581.5±43.6	504.3±43.6	543.9±54.5	584.3±29.7	553.5±21.8
Procalcitonin test (%)	0.3±0.0	0.3±0.0	0.3±0.0	1.7±1.4	0.7±0.3
Mean Platelet Volume (fL)	6.9±0.2	6.9±0.1	6.9±0.2	6.8±0.1	6.9±0.1
Platelet Distribution Width Counts (%)	13.0±1.7	13.0±1.7	13.2±1.8	12.8±1.8	13.0±0.8

*Different superscripts ($^{a, b}$) within rows differ at P≤0.05.

Table 2: Overall mean (±SE) values for liver function test enzymes in apparently healthy rabbit does (n=24) fed various level of *M. oleifera.*

Parameters	Groups				Overall
	Ctrl	A	B	C	
Alanine Aminotransaminase (U/L)	10.8±2.2	13.8±2.9	11.4±1.9	9.3±2.1	11.2±1.1
Aspartate Aminotransaminase (U/L)	38.1±5.8	49.5±6.9	38.0±5.6	45.3±7.7	42.8±3.2
Alkaline Phosphatase(U/L)	240.2±26.2	169.2±27.5	212.4±27.0	252.9±33.4	219.4±14.5
Bilirubin (µmol/L)	5.4±0.8	6.8±0.9	4.6±0.6	7.8±2.2	6.2±0.6
Albumin (g/L)	43.0±2.8	40.2±1.6	42.4±2.1	44.0±2.7	42.4±1.2

Table 3: Overall mean (±SE) values for renal function test enzymes in apparently healthy rabbit does (n=24) fed various level of *M. oleifera.*

Parameters	Groups				Overall
	Ctrl	A	B	C	
Creatinine (µmol/L)	48.0±6.2^{a}	42.9±4.3^{a}	57.4±6.9^{b}	43.0±4.4^{c}	47.6±2.8
Urea (mmol/L)	6.0±1.0^{a}	4.4±0.9^{b}	7.0±0.9^{a}	6.4±0.8^{a}	5.9±0.4
BUN (mmol/L)	2.8±0.5^{a}	2.1±0.4^{b}	2.3±0.2^{b}	3.0±0.4^{b}	2.8±0.2
Uric acid (mmol/L)	0.14±0.01^{a}	0.30±0.08^{b}	0.15±0.04^{a}	0.18±0.03^{a}	0.2±0.02

*Different superscripts (^{a,b}) within rows differ at P≤0.05.

increase was noticed. Similarly, the RDWc remained same for all studied groups but significantly (P≤0.05) increased in Group C.

Overall mean (±SE) values for LFT enzymes in apparently healthy rabbit does (n=24) fed various level of *M. oleifera* are presented in Table 2. Results indicated that all the LFT enzymes were statistically non-significant (*P≥0.05*) for all groups.

Overall mean (±SE) values for RFT enzymes in apparently healthy rabbit does (n=24) fed various level of *M. oleifera* is presented in Table 3. Results revealed that creatinine significantly (P≤0.05) increased in Group B and decreased in Group C as compared to control and Group A. Urea significantly (*P≤0.05)* decreased in group A as compared to other study groups. The BUN significantly (P≤0.05) decreased in Group A and B as compared to control and Group C.

DISCUSSION

Blood investigation is a method for evaluating clinical and wellbeing status of animals and humans. Agreeing to Oyedemi *et al*., , (2010) the valuation of hematological factors could be beneficial to study the harmful effect of some substances present in plant extracts on the blood composition of various animals and humans. It also reveals the physical and functional response of the organisms in its surroundings Unung et al. 2019). The present work is a novel one as it targets apparently healthy rabbit does as models for study of effect of *M. oleifera* leave extract on various hematological and serum chemistry attributes.

The results of hematological parameters in our study depicted that TEC increased in all the treatment groups fed various levels of *M. oleifera* compared to the control group. However, a significant increase was noticed for group B which was fed 300mg/kg of *M. oleifera*. The higher level of TEC examined in this research is similar to the results obtained earlier in a work by Osman, Shayoub, and Babiker, (2012) conducted to assess the effect of *M. Oleifera* leaves on blood parameters and body weights of albino rats and rabbits These results are also in line with the previous study conducted on rabbits El-Gindy, Zeweil, and Hamad, (2017). This increase in TEC for Moringa fed rabbits could be attributed to an increased erythrogenesis and an increased bone marrow functioning by Ikwunze *et al.,* (2016). In contrast, no significant change in TEC was observed by Ahemen *et al.,* (2013) and Jiwuba *et al.,* (2016) who studied physiological responses of rabbits fed graded levels of *M. oleifera* leaf meal (MOLM) on hematological and serum biochemical indices of growing rabbits. The results of this study are related to the previous researches but differences in the results were also observed as compared to many studies. The differences in the results of this study from previous may be due to the different breeds of rabbits used in different studies, difference in climate or difference in dosage forms of Moringa.

The MI was significantly lower in all Moringa fed treatment groups as compared to the control group in our study. The MI, in fact, represents the total number of dividing cells in contrast to the number of cells being analyzed and is the percentage of cells undergoing mitosis. A decreased number in Moringa fed treatment groups in our study is a beneficial effect of Moringa as illustrated previously Sharayu and Asmita, (2017).

The RDWc was significantly higher in Group C as compared to control and all other treatment groups. Similar results have been reported for anemic human patients by Suzana *et al,.* (2017) and for albino rabbits fed Moringa Ofem *et al.,* (2015). The RDWc is a range of volume and size of RBCs and is a valid indicator of anisocytosis of RBCs. Though an increase in this hematological parameter is considered as some kind of nutrient deficiency Constantino, (2013), however, in our study its increase can be valued in respect to increase in TEC for Moringa fed rabbits.

The results of LFT enzymes in our study were non-significant in all Moringa fed treatment groups and control group. Similar results have been reported for growing rabbits fed graded levels of *M. oleifera* leaf meal (MOLM) (Ahemen *et al*; 2013, E. Ewuola, Jimoh, Atuma, and Soipe; 2012). The results examined were similar to the results obtained by Nuhu, (2010) conducted to evaluate the effect of MOLM on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. The non-significant effect of Moringa leaf on AST, ALT and ALP is an indicator that the treatments have no untoward effect on the health status of the rabbits. The values for all the LFT enzymes were within the normal physiological ranges. However, the results of our study were in contrast to the earlier findings in which physiological responses of rabbits fed graded levels of MOLM on hematological and serum biochemical indices of growing rabbits were studied Jiwuba *et al*. (2016). The difference in the results of this study may be due to the different breeds of rabbits, difference in climate or difference in dosage forms of Moringa.

The results of RFT enzymes showed that creatinine was significantly higher in Group B (fed with 300mg/(kg) live weight of Moringa leaf extract) and lower in Group C (fed with 450mg/kg of Moringa leaf extract) as compared to control and group A. However, all the values were within the reference ranges described for rabbits in previous study by Melillo, (2007). The result of our study is in agreement with the previous study conducted in Egypt on the treatment of *Ulva lactuca* (sea lettuce) by Jiwuba *et al,.* (2016). The result of present study was in agreement with the results of study performed in Nigeria on rats Achuba, Ubogu, and Ekute, (2016) and on broiler chicken by Tijani *et al.,*

(2016). The creatinine is produced from muscle creatine and is excreted through the kidneys at a constant rate. An increase beyond the reference values is an indicator of impaired kidney disease. However, as the values of present study were within the reference range for rabbits, hence, the increase in Group B fed with 300mg/kg Moringa could be attributed to stress to dehydration or seasons. Regarding urea, its value was significantly decreased in Group A fed with 150mg/kg of Moringa, as compared to other study groups whereas BUN was significantly lower both for Group A and B. The results are in agreement with earlier experiments conducted in (Nigeria E. O. Ewuola, Sokunbi, Sanni, Oyedemi, and Lawal, 2015; Jiwuba *et al*., 2016). The attributes of urea and creatinine are valued separately as well as in a ratio for assessment of renal functioning of individuals. All the values of RFT enzymes in present study were within physiological range for rabbits which indicates no hazardous effect of Moringa on the studied animals.

Conclusion: The results of this study revealed that *M. oleifera* leaf extract exerted positive effect on hematological and serum chemistry attributes of apparently healthy rabbit does at an optimal dose rate of 150mg/kg. This study was a preliminary study conducted on hematological and serum chemistry attributes of Moringa fed apparently healthy rabbit does. Because of the similarities between rabbits and humans, the benefit of this study is to see the adverse effects (if any) of crude leaf extract of *M. oliefera* on hematological and serum chemistry attributes in humans. However, it directs for future studies with differing Moringa dosages and larger sample size. We also recommend a detailed study regarding the effect of Moringa with a larger sample/population number on physiological, hormonal and reproductive traits of rabbit does.

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