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Toxic Effects of Chloroform and Aqueous Extracts of *Peganum harmala* on Hematological and Growth Parameters in Rabbits

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Abstract.- The primary objective of the study was to evaluate the toxic effects of *Peganum harmala* extracts on hematological and growth parameters using rabbit model. Thirty five rabbits (1200-1700 g) of either sex were randomly divided into 3 groups D, E and F. The rabbits in group D were given *P. harmala* chloroform extract, while the members in group E were administered *P. harmala* water extract. The animals in group F were given normal saline. The groups D and E were further divided into three sub-groups of 5 each receiving 1, 5 and 10 mg/ml of extract. Blood samples from each rabbit were collected at day 0 (pre-medication), day 9 and day 30 (post-medication). Feed efficiency and daily weight gain in group D3 and E3 on day 30, was significantly lower ($P<0.05$) compared to control group F. Erythrocytes count in group D3 was significantly lower ($P<0.05$) than control group F on day 30. A non-significant difference ($P>0.05$) was observed in hemoglobin concentration and leukocyte count in rabbits of all treatments groups. It was concluded that parenteral administration of chloroform extract of *P. harmala* at 10mg/kg had more deleterious pronounced effect on growth rate and erythrocyte count in rabbits compared to aqueous extract of the plant.

Key words: *Peganum harmala*, chloroform extract, aqueous extract, RBC count.

INTRODUCTION

Assessment of therapeutic potency of plants is increasing day by day to discover new unconventional therapies and to fulfill the emerging therapeutic trend of natural product in modern world. There is growing trend on new therapeutic agents for the treatment of protozoan diseases in animals. Natural plant products that can be vital source, in search of compounds and new agents with therapeutic properties, are still not known (Derakhshanfar and Mirzaei, 2008).

Peganum harmala (Zygophyllaceae), locally known as "harmal" is a plant, commonly habitant to arid and semiarid area of central Asian deserts, had multipurpose medicinal activities (Rehman *et al.,* 2009). Most common and important beta carboline alkaloids of *P. harmala* extracts were identified as tetrahydroharmine, harmalol, harmine, harmol and harmaline (Herraiz *et al.,* 2010). Purification of *P. harmala* seeds using bioassay led to the isolation of harmaline, deoxyvasicinone, harmine and vasicinone. Harmaline and harmine displayed a moderate *in-vitro* anti-plasmodial effect against *Plasmodium falciparum.* Vasicinone demonstrated a vasorelaxant activity when applied as a treatment of phenylephrine-induced contraction of isolated rat aorta (Quinazoline alkaloid). *P. harmala* and its alkaloid's activity against the protozoa and antibiotic resistant isolates of bacteria have been proved in earlier studies (Arshad *et al.,* 2008). *P. harmala* derived protein (15KD) effectively alleviated the oxidative stress in erythrocytes, testes and brain of the experimental laboratory animals (Soliman and Fahmy, 2011). *P. harmala* derived 15KD protein had effective antioxidant effect almost similar to that of vitamin C effect (Farouk *et al.,* 2008). Some alkaloids of *P. harmala* have been found, responsible

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for the acetylcholinesterase (AChE) inhibitory effect (Adhami *et al.*, 2011). Similarly anti-diabetic and anti-oxidative properties of the plant seeds have been reported (Singh *et al.*, 2012).

In Pakistan, this multi-purpose plant is being widely used in traditional medicine. There is lack of study on the toxicity evaluation of the *P. harmala.* It is necessary to determine the toxic effects of the plant on health parameters. This paper describes the effect of parenteral administered chloroform and aqueous extract of *P. harmala* on hemogram and growth rate in rabbits.

MATERIALS AND METHODS

Collection of plant samples and identification

Fresh flowery buds of *P. harmala* were collected from the periphery of Lahore, Pakistan, identified and authenticated by a botanist by comparing with specimen (voucher number 4092) stored in the herbarium of Botany Department, Punjab University, Lahore, Pakistan. Plant samples were dried in shade at room temperature (25 ^{o}C) and were grounded to a fine powder by wily mill (standard model 4), stored in glass bottle and kept at room temperature (25^{o}C) for further usage.

Extraction by Soxhlet apparatus

Representative ground samples of the *P. harmala* were subjected to sequential (non-polar to polar) extraction with n–hexane, chloroform and ethanol solvents using soxhlet apparatus and then aqueous extraction of the *P. harmala* samples. The 150 g of dried powdered material was packed in a thimble made of Whatmann's filter paper and placed in soxhlet apparatus (5 Liter capacity). In each step 2.5 Liter of solvents (hexane, chloroform and ethanol) were used. After the completion of each extraction, powder was dried in a shade and weighed before its use for the next step. Similarly flask after each extraction was emptied by thoroughly taking out all extracted material. For extraction in solvents (n-hexane and chloroform) 50^{o}C temperatures was provided till the completion of extraction. During the ethanol extraction temperature was maintained at 72^{o}C. However for the crude aqueous extract (CAE) of the reference plant's, flowers powder, of 100 g was mixed with 600 ml of distilled water in a 1.5 liter flask and boiled for 1.5 h. Following cooling to 40°C, the 'brew' was filtered using Whatman No.1 filter paper.

Only filtrate obtained in CAE and extracted material from chloroform extraction of *P. harmala* was concentrated in vacuum rotary evaporator. The concentrated material was finally stored at 4^{o}C.

Preparation of medicinal plant solutions for the assessment of toxicity in rabbits

Solutions of extracted material in chloroform and water of *P. harmal* was made with normal saline at concentration of 2 mg /ml. In solution of chloroform extract of *P. harmal* was 1st diluted with DMSO (Dimethyl sulpha oxide) at 0.98 % of its total volume in graduated media bottles size of 100 ml. After that final volume was made with normal saline. In case of water soluble extract, initially it was diluted with normal saline and then DMSO was added at a concentration of 0.98 % of total volume. Prepared solutions were kept at 4^{o}C for 24 hr. Un-dissolved extracted particles in the solutions were filtered using 0.22µm syringe filter in a sterilized media bottles. Bottles were kept at 4^{o}C temperature for future uses.

Source and maintenance of rabbits

Rabbits (1200-1700 g BW) were purchased from Animal House of UVAS, Lahore. Rabbits were kept in cages for 15 days for the acclimatization, before commencement of the experiment. Every rabbit was tagged for its identification.

Rabbits were kept in clean metallic tier cages with soft bedding of wheat straw. In each cage partition was supported by two sheets, just beneath the rabbits it was comfortable having well pores to pass down the urine, feces and wastes on the second pore free iron sheets. Cages and iron sheets were routinely cleaned and washed and were daily sprayed with disinfectants (phenol) properly. They were supplied with clean food and water *ad libitum.* They were provided with 12 h light/dark light cycle.

Experimental design

A total of 35 rabbits of either sex were

randomly distributed in to 3 major groups (D, E and F). The F, group was kept as a control group and others groups i.e. D and E were further subdivided into three sub-groups *viz.* D1, D2, D3, E1, E2 and E3, each of five rabbits, which received i.m. dose of 1, 5 and 10 mg/dl of chloroform extract (D1, D2 and D3) and aqueous extract (E1, E2 and E3) on alternate days. All groups therefore, received 5 doses in all.

Blood sampling of rabbits

Blood samples were drawn from the jugular vein each rabbit at day 0 (pre-medication), day 9 and day 30 (post-medication) using disposable syringe and was immediately transferred into EDTA (Ethylene diaminetetra acetate) coated vacutainer for hematological study.

Haematological parameters estimation

Total RBC count, total WBC count and hemoglobin content were measured using auto hematology analyzer of Pathology Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Clinical examination of experimental rabbits

Signs and symptoms like anorexia, active, dull or depressed states were observed after administration of drugs after every 4 h for a period of 10 days. Weight of each individual was recorded daily by using digital balance. Toxicity signs including nervous signs, diarrhoea, muscle spasm, frothy discharge and labored breathing, if any were noted during every observation period. Body weight and feed intake of experimental rabbits were measured on day 10, 20 and 30 post-drug administrations. Feed conversion (FC) was determined using an equation as described by Ensmingar (1980).

FC= feed intake (g) during the specific period/gain weight (g) during the same period.

At the end of experiment, average daily weight gain, feed consumption and feed efficiency were determined. Mortality rate was also recorded after administration of medicinal plant extract in rabbits till the end of experiment.

Statistical analysis

Comparison of various treatments for different parameters was carried out by one way analysis of variance followed by post hoc test (Duncan's multiple range test) using SAS program (SAS SYSTEM, SAS Inst., Cary, North Carolina, v 9.1).

RESULTS

Toxicity

Observations of clinical signs during the experiment did not reveal any toxicity signs in rabbits treated with either extract of *P. harmala* at any concentration. No mortality was recorded in any group of rabbits.

Effect on feed consumption

Daily feed intake, feed consumption of rabbits treated with chloroform and aqueous extracts of *P. harmala* are given in Table I, while feed efficiency has been shown in Table II.

Feed consumption in rabbits of different treatment groups remained unchanged and in comparison to control there was no significant difference ($P>0.05$).

It was found that both extracts of the *P. harmala* at each concentration had a non-significant difference ($P>0.05$) in daily feed consumption compared to control group. A non-significant ($P>0.05$) difference in daily feed consumption was also seen among various treatment groups.

A non-significantly difference ($P>0.05$) in feed efficiency was observed in rabbits of groups D1, D2, E1 and E2 on day 30. Feed efficiency in group D3 and E3 on day 30, was significantly lower ($P<0.05$) than control group F. In comparison to control group F, a significantly lower ($P<0.05$) net feed efficiency was seen in D3 and E3 groups.

Weight gain of rabbits

Data on daily weight gain and net weight gain is shown in Table III. A significantly lower ($P<0.05$) weight gain was recorded in groups D3 and E3 at day 30 of experiment compared to control group F. A non-significant difference ($P>0.05$) was seen in weight gain of rabbits in groups D1, D2, E1 and E2 compared to control group (F) at day 30. A significantly lower ($P<0.05$) mean daily weight gain was recorded in groups D2, D3, E2 and E3

Table I.- Feed consumption of rabbits treated with various concentrations of *P. harmala* extracts in comparison to control*.

Treatments groups	Feed consumption (g) after			Total feed consumption (g)	Daily feed consumption (g)
	10 days	20 days	30 days		
D1 *(n=5)*	549.58±17.10^{+}	611.44±22.47^{+}	592.98±43.21^{+}	1754.00±76.25^{+}	58.46±2.54^{+}
D2 *(n=5)*	512.34±12.31^{+}	598.68±16.27^{+}	616.08±26.06^{+}	1727.10±41.01^{+}	57.56±1.36^{+}
D3 *(n=5)*	444.02±16.47§	530.18±13.08^{-}	611.60±22.05^{+}	1585.80±27.04^{+}	52.85±0.90^{+}
E1 *(n=5)*	540.34±20.68^{+}	601.70±18.85^{+}	568.76±33.43^{+}	1710.80±70.23^{+}	57.02±2.34^{+}
E2 *(n=5)*	507.40±12.71^{+-}	572.44±13.59^{+-}	619.76±11.92^{+}	1699.60±30.30^{+}	56.85±0.97^{+}
E3 *(n=5)*	460.10±16.57$^{-\S}$	535.53±12.75^{-}	609.37±33.30^{+}	1605.00±57.27^{+}	53.49±1.90^{+}
F *(n=5)*	557.52±18.59^{+}	589.40±11.52^{+}	564.08±24.67^{+}	1711.00±31.21^{+}	57.03±1.03^{+}

*Values are presented as mean ±SE. Mean having different superscripts (+, - and §) in same column are significantly different (*P<0.05*).
For detail of treatment groups, see legend of Figure 1.

Table II.- Feed efficiency in rabbits treated with various concentrations of *P. harmala* extracts in comparison to control*.

Treatment groups	Feed efficiency after			Net feed efficiency
	10 days	20 days	30 days	
D1 *(n=5)*	0.083±0.002^{+}	0.079±0.003^{+}	0.085±0.007^{+-}	0.082±0.003^{+}
D2 *(n=5)*	0.080±0.006^{+-}	0.075±0.005^{+}	0.075±0.008^{+-}	0.077±0.006^{+}
D3 *(n=5)*	0.056±0.004§	0.056±0.003^{-}	0.046±0.005§	0.052±0.002§
E1 *(n=5)*	0.088±0.008^{+}	0.083±0.007^{+}	0.092±0.008^{+}	0.088±0.004^{+}
E2 *(n=5)*	0.079±0.002^{+-}	0.078±0.004^{+}	0.079±0.005^{+-}	0.078±0.003^{+}
E3 *(n=5)*	0.066±0.003$^{-\S}$	0.061±0.003^{-}	0.066±0.008$^{-\S}$	0.065±0.004^{-}
F *(n=5)*	0.086±0.002^{+}	0.086±0.003^{+}	0.095±0.003^{+}	0.089±0.001^{+}

*Values are presented as mean ±SE. Mean having different superscripts (+, - and §) in same column are significantly different (*P<0.05*).
For detail of treatment groups, see legend of Figure 1.

Table III.- Mean weight gain (g) in rabbits treated with various concentrations of *P. harmala* extracts in comparison to control *.

Treatment group	Mean weight gain (gm) after			Daily weight gain (g)	Net weight gain (g)
	10 days	20 days	30 days		
D1 *(n=5)*	46.37±2.29^{+-}	48.92±2.58^{+}	50.02±3.46^{+-}	4.83±0.24^{+-}	145.31±7.37^{+-}
D2 *(n=5)*	41.57±2.90^{+-}	45.47±2.75^{+}	45.91±4.57^{+-}	4.45±0.29^{-}	133.76±8.88^{-}
D3 *(n=5)*	25.08±1.68§	29.69±1.68^{-}	28.79±4.08§	2.78±0.15§	83.57±4.68§§
E1*(n=5)*	47.33±3.48^{+-}	49.98±3.57^{+}	52.32±4.62^{+}	4.98±0.12^{+-}	149.63±3.70^{+-}
E2 *(n=5)*	40.22±1.17^{-}	45.07±1.94^{+}	49.54±4.01^{+-}	4.49±0.18^{-}	134.84±5.52^{-}
E3 *(n=5)*	30.61±1.80§	35.26±1.27^{-}	39.70±3.11$^{-\S}$	3.51±0.17§§	105.57±5.22§
F *(n=5)*	48.75± 2.50^{+}	51.47±2.14^{+}	53.72±2.70^{+}	5.12±0.09^{+}	153.95±2.87^{+}

*Values are presented as mean ±SE. Mean having different superscripts (+,-, § and §§) in same column are significantly different (P<*0.05*).
For detail of treatment groups, see legend of Figure 1.

compared with control group F. A non-significant difference (*P>0.05*) was seen in daily weight gain of treatment groups D1 (4.83±0.24 g/day) and E1 (4.98±0.12 g/day) as compared to control group F (5.12±0.09).

A significantly lower (*P<0.05*) mean net weight gain was seen in groups (D2, D3, E2 and E3) compared with control, while a non-significantly

lower (*P>0.05*) weight gain was seen in treatment groups D1 and E1 (Fig. 1).

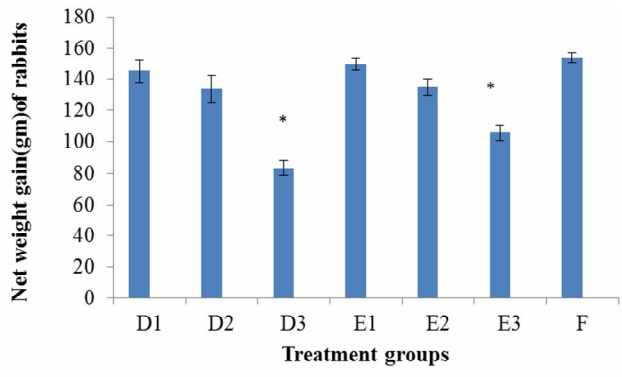

Fig. 1. Comparison of mean net weight gain (g) in rabbits of *P. harmala* extracts treated and control group. D1, *P. harmala* chloroform extract at 1mg/kg, BW; D2, *P. harmala* chloroform extract at 5mg/kg BW; D3, *P. harmala* water extract at 10mg/kg BW; E1, *P. harmala* water extract at 1mg/kg BW; E2, *P. harmala* water extract at 5mg/kg BW; E3, *P. harmala* water extract at 10mg/kg BW; F, Normal saline at 5m/kg BW

Hematological parameters

Comparison of various hematological parameters such as RBC count, WBC count and Hb (g/dl) in different groups is shown in Table IV.

RBC count in group D3 on day 30 of experiment was significantly lower (*P<0.05*) compared to control group. A non-significant difference (*P>0.05*) in RBC count among treatment groups D1, D2, D3, E1, E2 and E3 was recorded at day 0, 9 and 30 of experiment.

Mean values of WBC were 7.11±0.21, 6.69±0.63, 7.01±0.39, 6.56±0.58, 6.89±0.10, 6.57±0.38 and 7.14±0.37 X 10^3 at the end of day 30 of experiment in groups D1, D2, D3, E1, E2, E3 and F, respectively. A non-significant difference (*P>0.05*) was seen in WBC count in rabbits of all experimental groups on various days of experiment.

Mean values of hemoglobin concentration were 12.99±0.54, 12.73±0.79, 12.17±0.89, 13.16±0.63, 12.70±0.84, 12.14±0.65 and13.66±0.54 on day 30 in groups D1, D2, D3, E1, E2, E3 and F, respectively. Among all treatment groups hemoglobin values were non-significantly different (*P>0.05*) throughout the experimental period.

DISCUSSION

Medicinal drugs are mostly considered to be harmless but several researchers have documented the renal toxicity and hepatic toxicity (Colson and De Broe, 2005; Corns, 2003; Langmead and Rampton, 2001). In numerous countries, *Peganum harmala* is very famous in traditional medicine, despite having variety of alkaloids and toxic effect for animals (An *et al.*, 2010).

In present study clinically no toxicity of either extract of the *P. harmala* was recorded. Current findings did not reveal any inflammatory or allergic reaction at injection site and so intramuscular route was considered as appropriate and useful. In contrast to present study observations, some researchers recorded (Zuhair *et al.*, 2008) convulsions and muscle tremors. They also reported in their study findings inflammatory response following aqueous extract administration. The prospective of toxicity difference could exist in plants of same types or in different parts of one plant as reported earlier by researchers (Bhatti *et al.*, 2011; Wen *et al.*, 2012).

Plant extracts were evaluated for feed intake in rabbits. It is assumed that plant extract contains toxic ingredients and will affect feed metabolism in rabbits. A non–significant difference (*P>0.05*) was seen in feed intake in rabbits of all groups treated with extracts of *P. harmala* compared to control. In the present study feed conversion was also recorded to determine effects of treatments in rabbit's health. Findings showed significantly lesser (*P<0.05*) net feed efficiency in D3 and E3 group compared with control group. Weight gain reflects the status of the animal health, and is a useful indicator to check the toxicity effect of the certain drugs or harmful things. Although sex effect on weight gain in rabbits was not evaluated in present study, previous study (Abdel-azeem *et al.*, 2006) had shown no effect of sex on weight gain. Total weight gain (g) was significantly lower (*P<0.05*) in rabbits receiving 5 and 10 mg/kg BW of either extract of the plant. This indicated the plant extracts at higher doses have adverse effect on weight gain of animals. This may be attributed to ingredients present in plant extracts, at higher doses resulted in depression of thyroid activity which led to significant role in the

Table IV.- RBC (X 10^6), WBC count (X 10^3) and Hb g/dL concentration in rabbits treated with various concentrations of *P. harmala* extracts in comparison to control *.

Group (n=5)	RBC (X 10^6)			WBC (X 10^3)			Hb (g/Dl)		
	Day 0	Day 9	Day 30	Day 0	Day 9	Day 30	Day 0	Day 9	Day 30
D1	4.93±0.07^a	5.60±0.17^a	5.18±0.15^{ab}	6.80±0.39^a	7.22±0.43^a	7.11±0.21^a	12.74±0.50^a	12.88±0.53^a	12.99±0.54^a
D2	4.51±0.08^a	5.30±0.21^a	5.18±0.29^{ab}	6.40±0.56^a	6.87±0.20^a	6.69±0.63^a	12.54±0.52^a	12.61±0.44^a	12.73±0.79^a
D3	4.79±0.31^a	4.86±0.31^a	4.55±0.32^b	6.58±0.48^a	6.95±0.38^a	7.01±0.39^a	12.88±0.66^a	12.06±0.84^a	12.17±0.89^a
E1	4.31±0.13^a	5.67±0.30^a	5.50±0.27^{ab}	6.18±0.18^a	6.58±0.64^a	6.56±0.58^a	12.46±0.48^a	12.64±0.94^a	13.16±0.63^a
E2	4.56±0.37^a	5.05±0.10^a	5.13±0.38^{ab}	6.51±0.42^a	6.89±0.24^a	6.89±0.10^a	12.90±0.80^a	12.64±0.75^a	12.70±0.84^a
E3	4.64±0.26^a	4.96±0.27^a	4.85±0.34^{ab}	6.36±0.20^a	6.84±0.43^a	6.57±0.38^a	13.08±0.0.33^a	12.60±0.48^a	12.14±0.65^a
F	4.64±0.12^a	5.80±0.49^a	5.66±0.22^a	6.60±0.34^a	7.14±0.33^a	7.14±0.37^a	12.92±0.92^a	13.32±0.75^a	13.66±0.54^a

*Values are presented as mean ±SE. Mean having different superscripts (a, b and c) in same column are significantly different (*P<0.05*).
For detail of treatment groups, see legend of Figure 1.

metabolism of fat, protein, mineral and carbohydrate. Decrease in weight gain may be due to alkaloids present in *P. harmala* which affected central nervous system as documented in earlier studies (Brunton *et al.*, 2005). In contrast to present study higher weight gain was recorded in a recent study done by Xiccato *et al.* (2012). In addition to plant toxicity effect great difference may be due to age variation, environmental difference, housing system and stress during the handling and parenteral administration of extract solution and blood sampling.

In order to determine the possible toxicity effect, hemoglobin amount, RBC and WBC count were estimated. Present study findings showed that the extracts of the *P. harmala* had no effect on the hematological parameters RBC X 10^6, WBC X10^3 and Hb g/dl in rabbits during the experimental period. Except *P. harmala* chloroform extract lead to significant decrease (*P<0.05*) in RBC count compared to control. This shows that at high dose of chloroform extract may cause anemia. But in contrast, non-significant difference (*P<0.05*) in RBC count was reported compared to control following parenteral administration of the aqueous extract of the plant (Zuhair *et al.,* 2008).. The study concluded that chloroform extract of the *P. harmala* at higher dose proved more toxic to growth parameters as well as to some hematological parameters as compared to water extract of the *P. harmala*.

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