ASSOCIATION OF *PEGANUM HARMALA* L. SUPPLEMENTATION WITH LIVER FUNCTION TEST OF BROILER CHICKS.

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ABSTRACT: To monitor the effects of various levels of methanolic extract of *Peganum harmala* (*P. harmala*) on liver function in broilers birds, 28-days feeding trial involving 240 one-week old broiler chicks was carried out. The birds were grouped into four dietary treatments of -0, 200,250 and 300 mgL^{-1} of drinking water, which were further replicated 6 times in completely randomized design. Standard management practices were adopted, feed and water were offered *ad libitum.* At the end of every week, one bird was randomly selected from each replicate, bled to aspirate blood for liver enzymatic activities i.e. Alanine transaminase (ALT), Asparate aminotransferase (AST), Alkaline phosphatase (ALP) and serum protein determination. The liver enzymes i.e. ALT, AST and ALP showed gradual reduction in their concentration under different level of *P. harmala* methanolic extract. Significantly lower ($P<0.05$) values for ALT were found for the group Ph-250 except at day-14, where it was significantly low for group Ph-200. The AST levels were significantly ($P<0.05$) decreased in treated groups as compared to control at all recorded stages. The minimum values were recorded for group Ph-250 at day-14 and 35, while at day-21 and 28 no significant change was observed among the treated groups. The ALP level was significantly ($P<0.05$) lower in treated groups at all recorded stages. No significant change was observed in serum protein of broiler chicks at any recorded stage. It is concluded that methanolic extract of *P. harmala* at the dose rate of 250 mgL^{-1} drinking water may be used to improve serum hepatic parameters in broiler chicks.

Key words: *Peganum harmala,* liver function, hepatoprotective, broilers.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human and animal ailments. Medicinal plants or herbs consists of many pharmacologically active chemical compounds which may act as diuretic (Vohra and Khan, 1981), as anthelmentic (Al-Khalil, 1995), as an appetizer (Al-Yahya, 1986), antibacterial (Desta, 1993) and antifungal (Rathee *et al*., 1982). Herbs are expected to serve as safer alternative as growth promoter due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimum health hazzards and environment friendliness (Devegowda, 1996; Hussain et al., 2011).

Peganum harmala (known as harmal) belongs to family Zygophylaceae and is a multipurpose medicinal plant (Herraiz *et al*., 2009). Harmaline, harmine, harmalol and harmol are the main beta carboline alkaloids in *P. harmala* extracts. (Herraiz *et al*., 2010). *P. harmala* has great variety of pharmacological and biological activities such as antibacterial, antifungal and MAO inhibition (Abdel-Fattah *et al*., 1997), dermatosis (Saad and Rifaie, 1980), hypothermic (Abdel- Fattah *et al*., 1995), anticancer (Adams, 1983), analgesic and anti-inflammatory (Monsef *et al*., 2004), disinfectant (Shahverdi *et al*., 2005) growth promoting (Walid, 2009), cholesterol lowering and hepatoprotective effects (Hamden *et al*., 2008). Limited published work is available regarding the impact of *P. harmala* on liver functions and its hepatoprotective effects in broiler chicks. The present study was designed to determine the impact of different levels of methanolic extract of *P. harmala* on the liver function in broilers.

MATERIALS AND METHODS

Present study was conducted to explore the potentials of methanolic extract of *Peganum harmala* (*P. harmala*) on liver functions and its hepatoprotective effects in broiler chicks at Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan.

Experimental design: A total of 300 day-old broiler chicks were obtained from commercial market and were reared for a pre-experimental period of 7 days. On day 8^{th}, two hundred and forty (240) broiler chicks of approximately the same weight and appearance were selected and divided into four treatment groups; Ph-0, Ph-200, Ph-250 and Ph-300 getting methanolic extract of *P.*

harmala at the rate of 0, 200, 250 and 300 mgL^{-1} of drinking water respectively. Each group was further sub divided into six replicates with 10 chicks/ replicate (Table 1). Chicks were reared in open sided shed in pens. Saw-dust was used as litter. Drinker, bulb, feeder and other necessary equipment were provided in each pen to maintain identical management. Experiment was carried out for 28 days.

Table-1. Layout for experiment

Group	Dose level (mgL^{-1})	Replicates					
		R1	R2	R3	R4	R5	R6
Ph-0	0	10	10	10	10	10	10
Ph-200	200	10	10	10	10	10	10
Ph-250	250	10	10	10	10	10	10
Ph-300	300	10	10	10	10	10	10

Preparation of extract: The methanolic extract was prepared from *P. harmala* seeds at Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. One kg of *P. harmala* seeds was dipped in 3 liters of 80% aqueous methanol for five days and filtered with filter paper. The methanolic extract of *P. harmala* was used for further experiment after evaporating the methanol by using rotary evaporator (BÜCHI Labortechnik AG.1998, Switzerland) under low pressure.

Serum hepatic parameters: At the end of every week, one bird from each replicate was selected randomly for blood sampling and biochemical analysis. Blood samples were collected at 7:00-8:00 a.m. with fasting period of 2 hours. Blood samples were centrifuged at 4,000 rpm for 10 minutes for serum separation. Serum samples were analyzed for hepatic parameters i.e. AST (Asparate aminotransferase), ALT (Alanine transaminase), ALP (Alkaline phosphatase) and serum protein according to the IFFC (International Federation of Clinical Chemistry and Laboratory Medicine).

Statistical analysis of data: The data were statistically analyzed with the standard procedure of analysis of variance (ANOVA) by using Completely Randomized Design. Means were compared for significance of differences by least significant difference (LSD) as described by Steel et al. (1997). Statistical package SAS (1998) was used to perform the above analysis on computer.

Statistical Model;

$Y_{ij} = \mu + \alpha_j + E_{ij}$

Where;

Y_{ij} = Yield or response variable subjected to i^{th} chick and j^{th} treatment; yield comprises AST, ALT, ALP and serum protein

μ = Population mean

α_j = Treatment effect; treatment comprises 0, 200, 250 and 300 mg *P. harmala* extract L^{-1} of drinking water

E_{ij} = Random error subjected to i^{th} chick and j^{th} treatment

RESULTS AND DISCUSSION

Serum hepatic parameters

Alanine aminotransferase (ALT): Significant differences ($P<0.05$) were found between control and treated groups at all recorded stages (Table 2). Methanolic extract of *P. harmala* has significantly decreased ALT values as compared to control. The least values for ALT were found for the group Ph-250 except at day-14, where it was least for group Ph-200. The ALT values has shown a gradual significant ($P<0.05$) increase when dose level was increased to 300 mg L^{-1} drinking water.

The hepatoprotective potential of *P. harmala* may be due to presence of substances that are phenolic in nature, and may decrease the free radical lipid peroxidation level which leads to the stabilization of membrane structures (Hamden *et al*., 2008).

Similar results have been reported by Hamden *et al.,* (2008) who reported that ethanol and chloroform extract of *P. harmala* significantly reduced ALT level in thiourea treated rats. Our finding are contrary to Al-Hazmi (2002), who reported highly significant ($P<0.05$) increase in ALT level in albino mice treated with aqueous extract of *P. harmala*. Our findings are contrary to Walid (2009), who reported that neither ALT nor AST activity was changed in broiler birds when fed 10% harmala leaves in diet. The contradiction may be assigned to difference in dose level *P. harmala* and experimental animal used.

Aspartate aminotransferase (AST): The AST levels were significantly ($P<0.05$) decreased in treated groups as compared to control at all recorded stages. The minimum values were recorded for group Ph-250 at day-14 and 35, while at day-21 and 28 no significant difference was observed among the treated groups (Table 3).

P. harmala methanolic extract significantly reduced AST level in broiler chicks. Our findings are in agreement with Hamden *et al*., (2008) who reported protective effect of ethanolic and chloroform extract of *P. harmala* on thiourea induced liver toxicity in rats by maintaining AST and ALT activities.

Table-2. Effect of administration of different levels of methanolic extract of *Peganum harmala* on alanine aminotransferase (ALT) in broiler chicks

Group	Day-14		Day-21		Day-28		Day-35	
	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %
Ph-0	29.06^{a} ±1.49	12.56	30.09^{a}±1.01	8.24	27.91^{ab}±0.80	7.03	28.95^{a}±0.71	6.04
Ph-200	24.40^{c}±1.01	10.14	25.76^{bc}±0.98	9.37	29.10^{a}±0.40	3.36	26.27^{b}±0.84	7.84
Ph-250	26.74^{b}±0.95	8.71	25.36^{c}±1.00	9.75	26.09^{b}±0.59	5.53	23.46^{c}±1.37	14.31
Ph-300	28.76^{ab}±0.66	5.67	28.22^{ab}±0.34	2.97	29.58^{a}±0.73	6.01	26.24^{b}±0.84	7.83
LSD	2.02		2.58		2.10		1.76	

Means within a column with different superscripts are significantly different at α = 0.05
Ph = *Peganum harmala* levels; 0-300 = 0-300 mgL^{-1} of water
LSD= Least significant difference

Our findings are in agreement with Lamien *et al*., (2005), who reported a significant decrease in AST level by feeding decoctions from galls of *Guiera senegalensis* an alkaloid containing plant. Our findings are contrary to Al-Hazmi (2002), who reported significant increase (P<0.001) in AST level of albino rats treated with aqueous extract of *P. harmala*. Our findings are also contrary to Walid (2009), who reported that inclusion of 10% *P. harmala* leaves in the diet of broiler did not alter the AST or ALT levels. Zuhair *et al.,* (2008) reported that aqueous extract of *P. harmala* has no apparent toxic effect on liver and kidneys in rats. The contradiction may be assigned to difference in dose level *P. harmala* and specie of experimental animal used.

Alkaline phosphatase (ALP): Significant (P< 0.05) differences in ALP values between the control and treated groups were observed at all recorded stages. However, among the treated groups, non-significant differences (P<0.05) were observed at all recorded stages except at day-21 (Table 4).

The decrease in the level of ALP may be attributed to the inhibition of ALP secretion from the cells or it may be due to the inhibition of its synthesis at translational level or transcriptional level or inhibition of its secretion from cells by alkaloids (Varley, 1975; Zantop, 1997). Our findings are in agreement with Zuhair *et al.,* (2008), who observed gradual decrease in the ALP level by aqueous extract of *P. harmala* in rats, however, this decrease was not statistically significant. Hamden *et al.,* (2008) also reported that ethanol and chloroform extracts of *P. harmala* has hepatoprotective effect in thiourea induced rats. Our findings are in agreement with that of Arshad *et al*., (2008), who reported that continuous feeding of extract of *P. harmala* for 6 weeks to broilers resulted in decrease in ALP level.

Table-3. Effect of administration of different levels of methanolic extract of *Peganum harmala* on aspartate aminotransferase (AST) in broiler chicks

Group	Day-14		Day-21		Day-28		Day-35	
	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %
Ph-0	28.89^{a}±0.79	6.70	26.58^{a}±1.03	9.51	28.59^{a}±0.71	6.10	28.74^{a}±0.98	8.35
Ph-200	24.95^{b}±1.27	12.44	17.02^{b}±1.56	16.68	16.66^{b}±0.79	11.61	22.24^{b}±1.20	13.21
Ph-250	21.26^{c}±0.98	11.32	19.25^{b}±0.92	11.71	18.54^{b}±1.42	18.71	16.82^{c}±1.37	19.95
Ph-300	22.72^{bc}±1.44	15.55	17.17^{b}±1.63	23.31	17.23^{b}±0.73	10.42	19.98^{bc}±0.73	9.01
LSD	3.40		3.01		2.62		3.32	

Means within a column with different superscripts are significantly different at α = 0.05
Ph = *Peganum harmala* levels; 0-300 = 0-300 mgL^{-1} of water
LSD= Least significant difference.

Our findings are in agreement with Al-Hazmi (2002), who reported that aqueous extract of *P. harmala* significantly decreased ALP level in mice. Our findings are also supported by Lala *et al.,* (2004), who reported that harmine is one of the active alkaloid of *P. harmala* that is non-hepatotoxic and non-nephrotoxic in nature.

Serum protein: Non-significant differences in serum protein values were observed between the treated and control group and among the treated groups (Table 5). Our findings are in agreement with Abaza (2001), who reported that supplementation of natural feed additives to the diet of broilers had no significant effect on the serum protein level of the broilers.

Our findings are contrary to Abaza (2001), who found significant difference in serum protein values in broilers when fed *P. harmala* seeds at rate of 2.5 kg/ton of feed. Our findings are also contrary to Walid (2009), who reported hypoproteinemia in broiler chicks fed 10% *P. harmala* leaves in feed. Our findings are contrary to

Arshad *et al*., (2008), who reported that continuous feeding of crude extract of *P. harmala* to broiler chicks for 6 weeks resulted in depletion of serum protein level. This difference in the findings of present study and that of other workers might be due to the dose of extract used or duration for which the dose was given.

Table-4. Effect of administration of different levels of methanolic extract of *Peganum harmala* on alkaline phosphatase (ALP) in broiler chicks

Group	Day-14		Day-21		Day-28		Day-35	
	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %
Ph-0	2570.83^{a}±52.36	4.99	2514.00^{a}±59.63	5.81	2565.67^{a}±30.48	2.91	2479.67^{a}±40.87	4.04
Ph-200	2180.50^{b}±48.62	5.46	2220.50^{bc}±39.78	4.39	2180.50^{b}±50.07	5.62	2159.83^{b}±46.77	5.30
Ph-250	2267.50^{b}±48.85	5.28	2098.00^{c}±37.48	4.38	2206.83^{b}±32.21	3.57	2209.83^{b}±49.13	5.45
Ph-300	2278.00^{b}±39.09	4.20	2256.17^{b}±31.43	3.41	2225.67^{b}±41.67	4.59	2175.50^{b}±69.28	7.80
LSD	149.01		128.24		120.37		141.76	

Means within a column with different superscripts are significantly different at $\alpha = 0.05$
Ph = *Peganum harmala* levels; 0-300 = 0-300 mgL^{-1} of water
LSD= Least significant difference

Table-5. Effect of administration of different levels of methanolic extract of *Peganum harmala* on serum protein in broiler chicks

Group	Day-14		Day-21		Day-28		Day-35	
	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %	Mean ± SE	CV %
Ph-0	4.03±0.07	4.35	3.87±0.10	6.37	3.81±0.20	12.85	3.96±0.10	5.95
Ph-200	4.05±0.11	6.50	3.82±0.18	11.27	3.75±0.16	10.35	3.93±0.08	5.26
Ph-250	4.03±0.12	7.34	3.98±0.13	8.18	3.91±0.09	5.70	3.87±0.17	10.75
Ph-300	3.94±0.09	5.84	3.93±0.09	5.30	3.94±0.12	7.30	3.93±0.13	7.81
LSD	0.32		0.37		0.38		0.32	

Ph = *Peganum harmala* levels; 0-300 = 0-300 mgL^{-1} of water
LSD= Least significant difference.

Conclusion: It is concluded that serum hepatic parameters i.e. ALT, AST and ALP were improved by methanolic extract of *P. harmala.* The dose rate of 250 mgL^{-1} of drinking water has shown significant hepatoprotective effects in broilers.

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