

PARTHENIN INDUCED SERUM BIOCHEMICAL CHANGES IN *RATTUS NORVEGICUS*

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The present investigation was conducted to assess the toxicity (Median lethal dose, LD₅₀) of parthenin (extracted from the leaves of *Parthenium hysterophorus*) and its effect on blood biochemistry of *Rattus norvegicus*. The intramuscular and oral LD₅₀ of parthenin in *Rattus norvegicus* were found as 47.78 mg/kg b.wt. and 192.1 mg/kg b.wt., respectively. The parthenin was intramuscularly administered to *Rattus norvegicus*, at 40 mg/kg b. wt., for acute and 10 mg/kg b. wt. for sub-chronic treatments respectively, to study its impact on haematobiochemical parameters. The levels of serum total proteins registered a significant alteration after both the treatments. The levels of serum albumins, globulins and cholinesterase show significant decrease after acute treatment. Parthenin induces prominent acute changes rather sub-chronic treatment.

Keywords : Median lethal dose (LD₅₀); Parthenin; *Rattus norvegicus*; Serum albumins; Serum A/G ratio; Serum cholinesterase; Serum globulins; Serum total proteins.

Introduction

Parthenin, a major sesquiterpene lactone, is present in almost all parts of exotic weed *Parthenium hysterophorus* (Congress grass) of family Asteraceae. The plant is a native of North and South America and now has been introduced to different parts of the world including India. Chemical analysis of *Parthenium* reveals that almost all the plant parts contain various kinds of sesquiterpene lactones and phenolic acids (parthenin, caffeic acid, vanillic acid, p-aisic acid, chlorogenic acid, p-hydroxy benzoic acid etc.) of which parthenin [1,6 β-dihydroxy-4-oxo-10α H-ambrosa-2,11(13)-dien-12-oic acid-γ-lactone] is the major constituent approximately amounting 8 % of the total dry weight of the plant¹.

Both adverse and beneficial effects of parthenin have been reported in past by a few workers. Toxicity effects of parthenin include asthma, hayfever², non-air borne contact dermatitis³ and air borne contact dermatitis⁴. On the other hand, parthenin has been proved to exhibit antitumour activity in mice⁵, hypoglycaemic effect in alloxan induced diabetic rats⁶ and a major role in the field of nanotechnology⁷.

Albino rat, *Rattus norvegicus* has been selected as an experimental animal for the present study because of their easy maintenance in laboratory and system similarity with other higher mammals so the results can more closely be applied on human beings. Blood plays a

vital role in coordinating the activities of various tissues through distribution of nutrients, metabolites and other substances to maintain homeostasis. Total serum proteins maintain the normal distribution of water between blood and the tissue fluid. Serum albumin contributes 70-80 % of osmotic pressure while serum globulins are necessary for immune system. A/G ratio may be of help in diagnosis of diseases. Serum proteins, albumins, globulins, A/G ratio and cholinesterase reflect tissue metabolism, nervous transmission and the state of health of an animal. The present study, therefore, is aimed to assess the toxicity of parthenin and its effect on blood biochemistry of *Rattus norvegicus* after acute and sub-chronic intoxication.

Material and Methods

Extraction of Parthenin: Leaves of *P. hysterophorus* were collected from areas around the residential colonies and campus area of M.S.J. College, Bharatpur, Rajasthan, India in the month of July-September, 2008. The plants were identified by a botanical taxonomist at Botany Department, M.S.J. College, Bharatpur.

The parthenin was extracted from the leaves of *P. hysterophorus* by using the method of Patil and Hegde⁸ with slight modifications. The effluent was obtained in the form of white crystalline residue and was tested for purity by analysing its melting point and TLC and was characterized as parthenin by NMR done at Department of Chemistry, University of Rajasthan, Jaipur, India.

Table 1. Toxicity evaluation of parthenin in *Rattus norvegicus*.

Mode of Administration	Regression Equation derived by Log-Probit	LD ₅₀ (mg/kg b. wt.)	Fiducial Limits (95 % Confidence level)
Intramuscular	$Y=4.6271+6.8840(x-1.6251)$	47.78	47.42-48.15 mg/kg b. wt.
Oral	$Y=4.6991+4.4362(x-2.2156)$	192.1	189.4-194.7 mg/kg b. wt.

Experimental animal: Albino rats, *Rattus norvegicus* were procured and acclimatized in laboratory conditions. Rats were continuously given attention to monitor good health and hygienic conditions. They were fed on pellet diet, bread and vegetables at times and provided water *ad libitum*.

Toxicity Evaluation: In order to evaluate toxicity, the Median Lethal Dose (LD₅₀), single doses of different concentrations of parthenin were prepared by dissolving the required amount in distilled water and administered in the rats by oral and intramuscular routes. The rats were divided into 2 groups viz. oral and intramuscular group. Each group was further sub-divided into 6 sets (one control and five test), each consisting of 8 individuals. The five sets of rats of intramuscular group were given doses of 8, 16, 32, 64 and 128 mg per kg of body wt., respectively while the rats of oral group were given doses of 30, 60, 120, 240 and 480 mg per kg of body wt., respectively. The percentage mortality and toxicity symptoms were recorded upto 96 hours for each set of both groups including the control sets. The LD₅₀ value was determined by log-dose probit analysis method⁹.

Dose Determination: Sub-lethal doses were selected after the determination of LD₅₀ for experimentation.

Experimental Protocol: Sixty healthy rats irrespective of sex were selected for experimentation. The rats were weighed and divided randomly into four sets-one acute of 6 rats, one sub-chronic of 24 rats, and two control of 6 and 24 rats for acute and sub-chronic treatments, respectively. The experimental groups were given intramuscular injection of parthenin in sub-lethal doses of 40 mg/kg body wt. and 10 mg/kg body wt. for acute (one day) and sub-chronic (3, 7, 15 and 30 days) treatments, respectively. Twenty-four rats of sub-chronic group were given four fractionated sub-lethal doses (6 rats for each fractionated dose) on 1st, 3rd, 7th and 15th day of experimental period. The controls were given vehicle treatment only using a similar amount of diluent through intramuscular injection.

Blood samples: The rats were euthanized and the blood was drawn by exsanguination after one day (acute) and

after 3, 7, 15 and 30 days (sub-chronic) from ventricle of the heart into the test tubes without anticoagulant for serum separation. Serum was separated by centrifugation at 3000 rpm for 20 minutes.

Biochemical estimation: Serum total proteins¹⁰⁻¹³, albumins^{13,14}, globulins, A/G ratio and cholinesterase¹⁵ were estimated by the methods devised by various workers.

Analysis of data : Data were subjected to statistical evaluation using standard statistical procedures including student's t-test.

Results and Discussion

In the present investigation, the toxicity of parthenin has been evaluated on the basis of percentage mortality observed at different dose levels and is found to be route dependent as has also been reported by earlier workers^{16,17}. The intramuscular and oral LD₅₀ of parthenin in *Rattus norvegicus* were found as 47.78 mg/kg body wt. and 192.1 mg/kg body wt., respectively (Table 1).

The toxicity due to parthenin observed in the present investigation supports the findings of earlier workers that sesquiterpene lactones contribute to the toxicity of several poisonous species of Asteraceae to livestock. Narsimhan *et al.*¹⁶ reported the toxicity of parthenin in rats, while Singh and Gupta¹⁸ observed the toxicity of *P. hysterophorus* leaf extract in *Rattus rattus*. The toxicity symptoms like locomotory disability, itching sensation, labored breathing and dizziness were observed with varying intensity, which lasted for 2-3 hours. The toxicity symptoms observed in the treated rats are in agreement to the works of Singh and Gupta¹⁸ and Vijaylakshmi *et al.*¹⁹.

The blood biochemical changes observed in the treated rats after acute and sub-chronic intoxication of parthenin are depicted in Table 2.

From the present study, it is demonstrated that the acute toxicity of parthenin causes a significant decrease in serum proteins of treated rats after one day acute treatment and 7 days of sub-chronic treatment while the same exhibited significant increase after 15 days sub-chronic treatment.

Hypoproteinaemia may be attributed to two probable

Table 2. Serum Biochemical changes after parthenin intoxication in *Rattus norvegicus*.

Treatment and Dose	Days	Serum Total Proteins (g/dl)		Serum Albumins (g/dl)		Serum Globulins (g/dl)		Serum A/G Ratio		Serum Cholinesterase (U/L)	
		Mean ± S.E.		Mean ± S.E.		Mean ± S.E.		Mean ± S.E.		Mean ± S.E.	
		C	T	C	T	C	T	C	T	C	T
Acute (40 mg/kg b.wt.)	1	6.20 ± 0.06	5.42 ± 0.16 B	3.37 ± 0.06	3.02 ± 0.10 C	2.83 ± 0.07	2.40 ± 0.09 B	1.19 ± 0.04	1.26 ± 0.05 NS	1333.38 ± 8.80	739.06 ± 9.07 A
	3	6.32 ± 0.06	6.23 ± 0.06 NS	3.37 ± 0.04	3.33 ± 0.06 NS	2.95 ± 0.07	2.90 ± 0.04 NS	1.15 ± 0.04	1.15 ± 0.03 NS	1338.40 ± 8.87	1343.26 ± 9.30 NS
	7	6.27 ± 0.11	5.85 ± 0.11 C	3.27 ± 0.06	3.12 ± 0.06 NS	3.00 ± 0.15	2.73 ± 0.08 NS	1.11 ± 0.08	1.14 ± 0.03 NS	1278.09 ± 9.22	1304.25 ± 7.80 NS
Sub-chronic (10 mg/kg b.wt.)	15	6.33 ± 0.06	6.58 ± 0.08 C	3.38 ± 0.08	3.30 ± 0.12 NS	2.95 ± 0.07	3.28 ± 0.20 NS	1.15 ± 0.04	1.04 ± 0.11 NS	1282.36 ± 8.65	1305.92 ± 6.19 NS
	30	6.55 ± 0.12	6.22 ± 0.29 NS	3.40 ± 0.09	3.45 ± 0.18 NS	3.15 ± 0.11	2.77 ± 0.15 NS	1.09 ± 0.06	1.26 ± 0.07 NS	1294.89 ± 9.48	1318.21 ± 6.13 NS

C = Control, T = Treated, NS = Non-significant, A = p < 0.001, B = p < 0.01, C = p < 0.05

reasons-(a) Haemodilution where both albumin and globulins are decreased and A/G ratio remain unchanged, (b) low albumin level in serum with a decrease in A/G ratio²⁰. In the present study, a significant decrease in the serum total proteins seems to be an outcome of the decrease in the level of both albumins and globulins (Table 2).

Liver is usually considered to be the site of formation of the plasma proteins²¹. The decline in serum proteins accordingly be attributed to liver injury²² caused by parthenin treatment. Alternately, the decreased synthesis of protein might be due to non-availability of the precursor compounds probably by malabsorption of proteins²⁰. Accordingly, the significantly reduced concentration of serum proteins would suggest that the rate of synthesis of the proteins in the liver and/or their release into the blood is affected by parthenin treatment.

The observed hypoproteinaemia in the present investigation may further be attributed to the possible reduction in the reserve material for the protein formation in body. The protein forming reserve, protein synthesis and the protein loss, exhibit a dynamic equilibrium in body²³. It is thus suggested that parthenin may cause disruption of this dynamic and delicate physiological equilibrium after the treatment, shifting it towards either reduced synthesis or towards the protein loss. The above findings are in agreement to Singh¹⁷ and Ahmad *et al.*²⁴, while in contradiction to Jimoh *et al.*²⁵.

Furthermore, the hyperproteinaemia in rats that has been observed after day 15 shows that the compensatory physiology in body has been set up to build up the normal serum protein level. It is noteworthy that plasma protein depletion (as occur after early sub-chronic treatment) causes rapid mitosis of the hepatic cells and growth of the liver to a larger size; these effects are coupled with rapid outcome of plasma proteins²⁶ thus increasing the total serum protein level in rats.

A significant decline in the serum albumins level of the treated rats is evident after one day acute treatment. The fall in the amount of serum albumins in the treated rats may be attributed to the dysfunction of the liver parenchyma, as the liver parenchyma is the seat of synthesis of the entire albumin in body²⁷.

Liver dysfunction has also been documented in rats after *P. hysterophorus* leaf extract exposure²⁸. In hepatocellular damage and dysfunction the liver is unable to synthesize albumin at the normal rate, while the catabolism of albumin proceeds as usual resulting in hypoalbuminaemia²⁷.

The amount of serum globulins in the present investigation decreased significantly after one day acute

treatment. The observed hypoglobulinaemia reflects a possible dysfunction of liver and/or the decreased level of immunoglobulins in the serum caused by the parthenin treatment. It is to recall that all the albumin and fibrinogen of the plasma proteins as well as 50-80 % of the globulins are formed in the liver and the remainder of the globulins (especially the α -globulins) are formed in the lymphoid tissues²⁶.

Serum A/G ratio, in the present investigation, increased non-significantly in the rats acutely treated with parthenin which indicates a relative decrease of serum globulins in relation to albumins, which decreases to a lesser extent than globulins.

The serum cholinesterase activity in the present investigation has declined significantly in the rats after acute parthenin intoxication. The probable reason for the decreased level of serum cholinesterase is the dysfunction of liver, the site for synthesis of cholinesterase enzyme²⁹, caused by parthenin treatment.

In conclusion, parthenin is toxic and its exposure shows acute changes rather than sub-chronic. Further, it is suggested that histo-pathological examination of liver be done for confirmation of the probable liver dysfunction caused by parthenin.

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