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PRE-CLINICAL TOXICITY STUDIES OF NEEM [*AZADIRACHTA INDICA*] IN MICE AND RATS

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ABSTRACT

The present study has been conducted to assess the Pre-Clinical safety and efficacy of Neem [*Azadirachta indica*] leaves in mice and rat model. Albino mice (Swiss) were used in graded doses of *Azadirachta indica* by oral routes. Acute toxicity of *Azadirachta indica* showed normal behaviour and no mortality up to the 14th day. Effect of *Azadirachta indica* on haematological, biochemical and histopathological parameters in sub –acute toxicity was carried out. Sub-acute toxicity of *Azadirachta indica* was found no significant changes in haematological, biochemical parameters and histopathological examinations.

KEYWORDS: Azadirachta indica: Acute- toxicity: Sub- Acute-toxicity: Histopathology.

1. INTRODUCTION

Azadirachta indica [*Family*:Meliaceae] is commonly known as *Neem*, which is found in various parts of India. *Azadirachta indica* have employed as a folk medicine remedy for hypoglycemic, antiseptic, antiulcerogenic[1], anti-inflammatory[2,9], antioxidant.[3] immunomodulatory [4] and adaptogenic activities.[5] *Azadirachta indica* has a role in anxiolytic activity[6] bronchial asthma and active anaphylaxis.[7]. *Azadirachta indica* has safe in acute toxicity[8]and antipyretic activities.[9].The present investigation was conducted to toxicity study in detail *Azadirachta indica* in view of its medicinal importance in folklore medicine.

2. MATERIALS AND METHODS

2.1 Animal and Drug Administration

After approval of Institutional Animal Ethical Committee (IAEC), the present study was conducted in the Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi on inbred Albino mice (Swiss) 20-40g and Albino rats (Wistar Strain)100-200g .They were kept in the departmental animal house in individual cages at an ambient temperature of 25 ± 3^0C and 60- 70% relative humidity with 12h:12h light:dark cycles. They had fee access to standard rodent pellet diet and drinking water (Kinley) during the entire study period. The food was withdrawn 18h prior to experimentation, however, water was allowed *ad libitum.*

2.2.1 Plant Material

The *Azadirachta indica* fresh green leaves was collected from the campus of Institute of Medical Sciences , BHU, Varanasi. Fresh leaves were separated from stem and crushed with the help of pestle and mortar to make a paste and then squeezed to get the juice. The juice was directly used for experimentation because Ayurvedic literature suggests in this form.

2.3.1 Acute-Toxicity Studies on Mice

Albino mice (Swiss) weighing 20-40 g were divided into 7 groups. These animals were fasted 18 h prior to the experimentation. Both the test and control groups were received in a same volume of drug or vehicle control as per body weight. Experiments were conducted as per OECD guidelines-423(Acute-Oral Toxicity-Single Dose).[10] The each group contains equal male and female mice (5 male + 5 female) were given graded doses of *Azadirachta indica* leaves (10, 100, 200, 500, 1000 & 2000 mg / Kg, p.o.). Group –I received double distilled water as control. The animals were kept in observation for 96 h upto 14 days for any gross behavioural changes and mortality. The animals were observed for symptoms viz. writhing pilo-erection, salivation fur, lacrimation, convulsion, hyperreactivity, etc continuously for the first 4h after dosing. The numbers of survival were noted after 24h.These animals were then maintained and observed daily for 14 days for further any toxicity. Complete postmortem was done on all survivors or if any animal found dead or moribund condition during the study period. Histopathological examination was performed on all collected tissues of individual animals.

2.3.2. Sub-Acute Toxicity on Rats

Albino rats (Wistar strain) weighing 100-200g were divided into 4 groups. The each group contains equal male and female (5M + 5F=10), was treated with *Azadirachta indica* at the dose level of 200, 100and 50 mg/kg daily for 28 days. Group I received double distilled water in same ratio served as control (vehicle). The mortality rate, behavioral changes, if any was recorded during the experimentation. The body weight of animals, measured food and water were recorded weekly upto 28 days. Investigation of all animals in each group for the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC) and blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) on 28th day. All animals in each group have been sacrificed on 30th day the following vital organs *viz*., liver, kidney, lungs, spleen, ovaries, testes, stomach and intestine were separated, weighed for histopathological investigation of toxicity of the drugs, if any.

2.4 Statistical Analysis

All the data was analyzed by student's t-test followed by ANOVA.

3. RESULTS AND DISCUSSION

3.1Acute-Toxicity Studies on Mice

The animal treated with doses of 10, 100, 200, 500 &1000 mg / Kg, no mortality recorded upto 14 days.. The higher dose 2000mg/kg showed 10% mortality was recorded within 96h. After postmortem ,histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.2 Sub-Acute-Toxicity Studies on Rats

Azadirachta indica shows no significant effect in the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC), blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) body weight, weight of vital organs in comparison to control. The histological characters also showed no abnormal features in tissues studied. It is concluded that there is no specific pathological change detected in slides prepared in above said dose studied.

Table 1: The body wt. of animals (g) treated with *Azadirachta indica*.Values are mean ±SE Figures in parentheses indicate number of animals used.

Group & Dose (mg/kg, p.o.)	Initial wt. [g]	1st week	2nd week	3rd week	4th week
Control (vehicle)	130.0 ± 5.22 (10)	145.0 ± 5.11 (10)	162.0 ± 4.45 (10)	164.0 ± 2.88 (10)	167.0 ± 9.72 (10)
A. indica 200	121.0 ± 3.33 (10)	137.0 ± 4.60 (10)	140.0 ± 6.47 (10)	148.0 ± 7.17 (10)	149.0 ± 6.05 (10)
100	133.0 ± 3.56 (10)	147.0 ± 3.00 (10)	155.0 ± 5.43 (10)	164.0 ±1.25 (10)	179.0 ± 5.56 (10)
50	124.0 ± 5.62 (10)	142.0 ± 5.32 (10)	148.0 ± 6.50 (10)	150.0 ±1.82 (10)	165.0 ± 4.14 (10)

Table 2: Sub acute toxicity: Food (g) and water (ml) consumption weekly treated with *Azadirachta indica*. Values are mean ± S.E. Figure in parentheses denotes the number of animals used.

Group & Dose (mg/kg, p.o.)	Food Consumption (Weekly)				Water Consumption (Weekly)			
	I	II	III	IV	I	II	III	IV
Control (Vehicle)	26.14 ± 1.52 (10)	34.23 ± 3.06 (10)	52.65 ± 6.12 (10)	57.53 ± 4.01 (10)	59.57 ± 3.44 (10)	55.11 ± 5.21 (10)	160.0 ± 9.28 (10)	155.52 ± 6.03 (10)
A.indica 200	32.83 ± 5.60 (10)	37.11 ± 3.02 (10)	50.0 ± 10.35 (10)	52.85 ± 6.54 (10)	55.05± 3.26 (10)	60.77 ± 3.99 (10)	161.40 ± 5.65 (10)	155.75 ± 9.60 (10)
100	32.85 ± 9.11 (10)	35.22 ± 4.02 (10)	41.32 ± 4.03 (10)	51.72 ± 1.65 (10)	59.24 ± 3.39 (10)	66.52 ± 3.25 (10)	157.85 ± 6.01 (10)	146.85 ± 7.54 (10)
50	37.12 ± 11.25 (10)	31.40 ± 1.19 (10)	57.55 ± 8.32 (10)	61.32 ± 4.77 (10)	57.65 ± 4.18 (10)	57.35 ± 2.62 (10)	150.75 ± 6.72 (10)	147.57 ± 6.32 (10)

Table 3: Sub-Acute Toxicity: Investigations of hematology of rat blood treated with *Azadirachta indica* on 28th day. Values are mean ± S.E.Figure in parentheses denoted the number of animals used.

Groups & Dose (mg/kg p. o.)	Hb% g/dl	WBC 10^3 /mm^3	RBC 10^6 /mm^3	DC %				
				L	N	M	E	B
Control (Vehicle)	19.89±1.27 (10)	6800.00±255.34 (10)	6.54±037 (10)	61.8±2.71 (10)	32.10±2.27 (10)	1.40±0.63 (10)	3.3±0.64 (10)	0.40±0.25 (10)
A.indica 200	16.27±0.95 (10)	6800.00±243.57 (10)	6.42±0.39 (10)	63.7±1.65 (10)	31.2±2.17 (10)	0.80±0.70 (10)	4.50±1.22 (10)	0.40±0.23 (10)
100	18.59±1.03 (10)	6457.00±197.00 (10)	4.75±0.75 (10)	62.8±2.25 (10)	31.5±1.07 (10)	0.80±0.70 (10)	4.65±0.65 (10)	1.0±0.46 (10)
50	19.70±0.48 (10)	6920.00±38.14 (10)	6.58±0.33 (10)	60.30±2.27 (10)	32.20±2.08 (10)	1.62±1.12 (10)	4.42±0.54 (10)	1.0±0.36 (10)

Table 4: Sub-Acute Toxicity: Investigations of Blood Biochemistry of rat blood (serum) treated with *Azadirachta indica* on 28th day. Values are mean ± S.E.Figure in parentheses denoted the number of animals used.

Groups & Dose mg/kg of b wt.	Total Bilirubin mg/dl	SGOT U/l	SGPT U/l	Albumin g/dl	Serum Creatinine mg/dl	Alkaline Phospatase U / l	Total Protein g/dl
Control (vehicle)	0.95 ± 0.12 (10)	115.2 ± 1.22 (10)	36.7 ± 5.40 (10)	3.69 ±0.25 (10)	0.54 ± 0.03 (10)	133.75 ± 7.65 (10)	6.93 ±0.23 (10)
A.indica 200	0.67 ± 0.25 (10)	142.4 ± 9.41 (10)	37.5 ± 4.66 (10)	4.15± 0.15 (10)	0.55 ± 0.02 (10)	175.4 ± 40.77 (10)	7.58± 0.32 (10)
100	0.46 ± 0.05 (10)	112.7 ±20.5 (10)	35.65 ±1.73 (10)	3.76 ± 0.11 (10)	0.54± 0.05 (10)	137.6 ± 30.11 (10)	6.55 ±0.29 (10)
50	0.79 ± 0.13 (10)	128.35±15.9 (10)	42.75 ±1.65 (10)	3.75 ± 0.07 (10)	0.56± 0.02 (10)	178.0 ± 22.20 (10)	7.04 ±0.31 (10)

Table 5: Sub-Acute Toxicity: The Weight of Vital Organ [g] treated with *Azadirachta indica* on 28th day. Values are weight in [g] mean ± S.E. Figure in parentheses denoted the number of animals used.

Groups & Dose mg/kg, p.o.	Heart	Kidney	Adrenal	Spleen	Liver
Control (Vehicle)	0.57±0.02 (10)	1.17±0.05 (10)	0.040 ±0.004 (10)	0.35±0.02 (10)	4.99±0.36 (10)
A. indica 200	0.56±0.01 (10)	0.95±0.03 (10)	0.038±0.003 (10)	0.33±0.01 (10)	4.17±0.15 (10)
100	0.55±0.02 (10)	1.15±0.07 (10)	0.047±0.006 (10)	0.36±0.03 (10)	4.78±0.38 (10)
50	0.53±0.008 (10)	1.09±0.05 (10)	0.039±0.005 (10)	0.31±0.007 (10)	4.45±0.33 (10)

CONCLUSION

Acute toxicity of *Azadirachta indica* has safe upto the doses of 2000 mg/kg and caused no mortality and normal behavior. The results of Sub-acute toxicity reveal that *Azadirachta indica* shows no significant effect in the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC), blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) body weight, weight of vital organs in comparison to control. *Azadirachta indica* is rich in alkaloids and flavanoids. *Azadirachta indica*,apart from divers uses in folk medicine, has recently been shown to possess anti- inflammatory, analgesic and antioxidant properties.[2,,3,8,9] The acute and sub-acute toxicity studies indicate that *Azadirachta indica* have a significant margin of safety in mice and rats.

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