Safety evaluation of *Azadirachta indica* seed oil, a herbal wound dressing agent

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SUMMARY. *The oil of* A. indica *given orally to mice showed low toxicity. It was non-irritant to the skin of rabbits in primary dermal irritation test. In sub-acute dermal toxicity, rabbits exposed daily to* A. indica *oil for 21 days showed no significant changes in body weight and organ/ body weight ratio. Serum oxalo-acetic transaminase, serum pyruvic transaminase levels, blood glucose and blood urea nitrogen values were found to be unaltered. No treatment-related histopathological changes were observed. The study suggested that* A. indica *oil is devoid of any adverse effect on kidneys and liver, is non-irritant to the skin of rabbits and is, therefore, relatively safe for external application in wounds.*

Key words: *Azadirachta indica*; acute toxicity; sub-acute dermal toxicity.

Oil from *Azadirachta indica* (H-Neem) has been reported for its antidiabetic,[1-4] spermicidal,[5] antifertility,[6] antibacterial[7] and wound healing[8] properties. It is generally considered that herbal drugs described in traditional system of medicine are safe and devoid of any undue toxicity. However, very few of these drugs have been subjected to a systematic toxicological evaluation. As no information on the toxicity of *A. indica* oil is available in the literature, it was planned to investigate its toxicity ranging from acute toxicity to subdermal toxicity.

EXPERIMENTAL

Plant material. A. indica ripe fruits collected from the Institute campus in July-August were identified by Botany Department of Bareilly College, Bareilly. A voucher specimen was deposited at the Pharmacology and Toxicology Division of this Institute.

Extraction of oil. Seeds were separated from fruits, shade dried, powdered and Soxhlet extracted with petrol (60-80°C). Petrol extract was filtered, petrol was removed by distillation whereupon light coloured seed oil was obtained. The yield of the oil was 41% on dry matter basis.

Experimental animals. Swiss albino mice of either sex (18-22 g) and white New-Zealand rabbits of either sex, were procured from the Laboratory Animal Resource Section of the Institute. The animals had free access to food and water. All animals were acclimatized to laboratory conditions before use.

Acute toxicity. Albino mice of either sex were divided into six groups of 6 animals each and fasted for 16 h with free access to water before experimentation. Oil was administered orally in doses ranging from 1.0-28.19 g/kg using a constant multiplying factor of 1.95.

Doses beyond this could not be administered because of large volume. Observations for toxic symptoms and mortality were made upto 72 h.

Primary skin irritation test. Six rabbits of either sex (1-1.5 kg) were used. The primary irritation of skin was measured by patch test technique.[9] The test material was spread in a dose of 0.5 ml on a shaved area of 6 cm.[2] Three sites of administration were assigned, respectively to test substance, 1% sodium lauryl sulphate and normal saline. Sodium lauryl sulphate and normal saline served as positive and negative controls. The entire trunk area was wrapped with elastic bandage. Observations were recorded at 24, 48 and 72 h of drug application. Reactions were evaluated after 24 h and substances were categorised.[10]

Sub-acute dermal toxicity. Rabbits of either sex (1.2-1.5 kg) were used. Pre-treatment biochemical profile was determined and the animals were then randomised into experimental and control groups. Application of the test material was made by inunction on the shaved area of the intact skin which was approximately 10% of the total body surface of the animal. The oil (2 ml) was applied at the demarcated site daily for 21 days, by mild pressure using a glass rod. Similar application was made by using normal saline. Following final exposure, each animal was observed daily for a period of two weeks for clinical signs, behavioural changes, local toxicity at the site of application as well as on the remote tissues (progressive systemic effects) and for mortality, if any.

Individual body weight was recorded before and after the termination of the experiment. Blood samples were collected for the biochemical parameters by cardiac puncture at the term of exposure of drugs. Blood urea nitrogen [11] and blood glucose [12] levels were determined. Glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) were determined from serum.[13]

Necropsy examination was performed by slaughtering all the surviving animals at the termination of the study. Liver, kidneys, spleen, heart and lungs were weighed and organ-body weight ratios were also determined. Portions of organs and skin were preserved in 10% formalin and processed for histopathological examination using haematoxylin and eosin stain.

Statistical analysis. Student's t test was followed for determining the difference between control and treatment values.

RESULTS AND DISCUSSION

The behaviour of the mice was not affected by the administration of *A. indica* oil up to 7.41 g/kg. However, hyperexcitability, convulsive jerks and rough coat in animals receiving 14.45 g/kg and 28.19 g/kg of oil were observed during the first hour. At third hour, symptoms were intensified which included hyperexci-

Group*	Terminal body weight kg	Heart Weight g	Heart BW ratio %	Kidney Weight g	Kidney BW ratio %	Spleen Weight g	Spleen BW ratio %	Liver Weight g	Liver BW ratio %	Lung Weight g	Lung BW ratio %
Control (normal saline)	1.48 ±0.08	5.15 ±0.38	0.34 ±0.01	11.1 ±0.41	0.75 ±0.54	0.74 ±0.07	0.04 ±0.005	62.00 ±4.06	4.27 ±0.47	10.86 ±0.94	0.74 ±0.07
A. indica (oil)	1.38 ±0.06	4.80 ±0.43	0.36 ±0.02	10.48 ±0.57	0.77 ±0.07	0.64 ±0.05	0.05 ±0.003	67.5 ±1.52	4.97 ±0.28	10.38 ±1.63	0.64 ±0.06

* 6 animals in each group.

Table 1 - Effect of *A. indica* oil on body and organ weights of rabbit. Mean ± S.E.

Groups*	Pre-treatment				Post-treatment			
	BG	BUN	SGOT	SGPT	BG	BUN	SGOT	SGPT
Control (normal saline) *	71.27 ±5.73	17.02 ±1.38	71.33 ±6.68	91.19 ±14.39	73.42 ±7.12	18.05 ±1.56	77.10 ±9.13	90.47 ±13.62
A. indica (oil)	73.32 ±6.01	17.61 ±1.49	65.87 ±6.97	89.61 ±11.48	72.38 ±3.41	17.26 ±0.67	62.29 ±6.29	94.57 ±6.29

BG = Blood glucose and BUN = Blood urea nitrogen (mg/100 ml of blood)
SGOT = Serum glutamic transaminase and SGPT = Serum pyruvic transaminase
(μMol of pyruvate hydrolysed/minute/litre)
* 6 animals in each group.

Table 2 - Effect of *A. indica* oil on selective biochemical parameters in rabbits. Mean ± S.E.

tability to sound and touch, convulsive jerks and laboured respiration. One animal died in both the groups. During 72 h observation period, three mice died in the group receiving highest dose of the oil.

Application of 0.5 ml of the oil on the skin of the rabbits elicited no adverse reaction and this classified the substance as non-irritant. The primary dermal irritation index of positive control, sodium lauryl sulphate, was 1.10 ± 0.31. Normal saline did not produce any adverse reaction.

On sub-acute dermal exposure to the oil of *A. indica* in rabbits, no mortality was observed. Body weights, organ weights and organ/body weight ratio of treated rabbits were not statistically different from those of controls (Table 1). The difference observed were sporadic and attributed to normal variation in rabbits.

Histopathological examination of different organs revealed no remarkable lesions in control as well as in oil-treated rabbits. The oil revealed no local reaction at the site of application and gross histopathological examination indicated that oil was non-irritant on prolonged exposure to the skin.

There were no statistically significant differences in the blood glucose and blood urea nitrogen levels between control and treated rabbits (Table 2). Blood glucose levels reflect a dynamic steady state between production of blood glucose by liver and its utilization by other tissues.[14] Hyperglycaemia has been observed in severe nephritis, pancreatic diseases and hepatic disorders. Low blood sugar levels were also found as a result of deficiency of adrenal cortex, anterior pituitary, glucagon release, tumor of pancreas and hypothyroidism.[15] No alteration in blood glucose levels on application of oil suggests that balance between glucogenolysis and glycogenesis, between glycolysis and gluconeogenesis and lipogenesis is unaffected. Estimation of blood urea nitrogen levels has been recognised as a simple and reliable test to assess the kidney function.[16] In kidney diseases, the products of protein metabolism are not excreted but they are retained in blood and hence the measurement of blood urea nitrogen level gives fairly accurate idea of the health of the kidney. In the present study, the oil possessed no adverse effect on kidneys.

The present study revealed a non significant decrease in SGOT and increase in SGPT level following application of the oil. However, these values were within normal limits. Serum transaminase activity is known to alter in certain patho-

logical conditions associated with cellular damage of hepatic, cardiac or skeletal muscles.[17-19] It is further suggested that SGPT might possibly be more specific index for liver cell damage than SGOT because of its high concentration in hepatic tissues.[20,21] This emphasises the importance of liver function test and use of serum enzyme changes in the toxicity study.[22-24] The findings of the present study indicate that probably the hepatic cells were not damaged following the application of oil in rabbits. The non significant changes in the SGOT and SGPT enzymes, as observed, were further confirmed by no pathological changes of the liver. In conclusion, the study reveals that mice tolerated higher doses of the oil, and no toxicological and histopathological treatment-related effects were observed in rabbits following sub-acute exposure to *A. indica* oil. The oil was not irritant to rabbit skin, thus making it safe for external use in wound dressing.

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