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MAMMALIAN TOXICITY STUDIES OF NIMBOKIL-60 EC

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Nimbokil-60 EC, an expelled neem (*Azadirachta indica* A. Juss) seed oil based insecticide was tested to determine its mammalian toxicity. Tests for acute and percentaneous toxicity, acute inhalation, skin and eye irritation, sub-chronic effects and effect on fertility were carried out. Results revealed that 'Nimbokil' had no adverse or ill effects on the test animals under administered doses. LD-50 was found to be 16 ml kg^{-1} body-weight of the animals. Autopsy observation showed no gross changes in vital organs i.e. heart, lungs, liver, ovary and testicles of the test animals. When applied as such (100% concentration) completely within 72 h. However, at lower concentrations, no eye irritation was noticed. It is therefore, concluded that Nimbokil-60EC be classified as non-toxic'.

Key words: Neem, Nimbokil, Toxicology, Phytochemicals, Botanical pesticides.

Introduction

The earlier insecticides, composed of phyto-chemicals as nicotine, rotenone, ryanodine alkaloids and pyrethrum were important insecticides until the discovery of DDT initiated 'Dawn' of synthetic pesticides (Bowers 1993). The use, from 1940 onward, of highly toxic, broad-spectrum, synthetic insecticides with superior efficacy and cost effectiveness increased agricultural yields resulting in surplus food. A few persistent synthetic pesticides threaten planetary ecology. Synthetic pesticides also effects the food constituents (Szymczak and Grajeta 1980; Zurawski *et al* 1980; Saad *et al* 1984; Parveen *et al* 1998) . Environment concern and rapid development of resistance by many pests to synthetic pesticides (Georghiou 1990 & 1994; Chaudhry and MacNicoll 1998) lead to intensive search seeking environmentally pacific strategies for plant and public health protection. Studies of insect biology and chemical ecology have revealed numerous points of attack that promise environmental compatibility. Included in this developing arsenal are insect growth, feeding and behaviour regulators optimised from insect hormones and phyto-chemicals (Bowers *et al* 1966). Plants are clearly capable of defending themselves not only by poisoning hungry insects, but by using their chemistry to subtly perturb discrete aspects of insect life. These subtle defences that interfere with insect growth, development, reproduction and behaviour may have no counterpart receptors in higher animal. Thus, these offer the opportunity to develop safe and environmentally pacific methods for the pest control.

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Naeem *(Azadirachta indica,* A. Juss*)* seed oil, mainly contains triglycerides of oleic, stearic, linoleic and palmitic acids ((Schmutterer 1995) that are bio-degradable (Cornish *et al* 1993). Chemically over 35 substances have been isolated, identified and elucidated so far from it (Jones *et al* 1988). The limonoids are the principal bio-active compounds (NRC 1992). The oil, along with suitable emulsifier, synergists and sunscreen was formulated to make "Nimbokil-60 EC", a broad spectrum botanical pesticide. The mammalian toxicity studies of this product were carried out to confirm its safety and results are reported here.

Materials and Methods

Nimbokil-60 EC, a brown thick oil having light bitter smell, was evaluated according to the Draize test modified procedures laid down by the USA code of Federal Regulations " 1972" ((Gorrod 19981) and methods described by Loomis 1975 & 1978; Litchfield 1962.

Acute toxicity. Male and female albino mice 25-30 g were housed separately, and observed for one week to record normal behavioural pattern. 60 albino mice were divided into ten groups, each group comprised of 6 animals (3 males and 3 females). Group 1-X received Nimbokil orally in Single dose 1-10 ml kg^{-1} body weight, respectively. 72 h observation for gross behavioural changes and mortality were recorded.

Acute percentaneous toxicity LD-50 and LD-100. Nimbokil was administered orally in different doses to ten groups albino mice, weighing 25-30 g each group comprised of 5 male and 5 femle. The animals were kept in isolation and observed for 24 h.

Acute inhalation. Acute pulmonary toxicity studies were conducted in adult and healthy albino mice, 6 male and 6 female, kept in a chamber. 40 ml of Nimbokil taken in a China dish, placed on a heater, was also kept in the chamber. Nimbokil was heated slowly for five minutes and animals were exposed to the fumes. After two hours post-exposure period animals were removed and placed in cages. Four animals were sacrificed and observed for gross changes in the lungs and other vital organs.

Skin irritations. The acute dermal toxicity test was performed on sixteen adult, healthy albino rabbits. The area over the back of each rabbit extending from the base of the back to the hind quarters was shaved. These animals were divided into two groups. about 2 square inch area of the bare skin from group one animal was abraded by making minor incisions through the surface layer of cells without producing bleeding and no incision was made on the group two. Nimbokil was applied (0.5 ml approx.) over the shaved area on the skin of both the groups. The treatment was repeated after 24, 48 and 72 h.

Eye irritation. Assessment of eye irritation was carried out on eight albino rabbits weighing between 2-3 kg. Different concentrations (5-100%) of Nimbokil were made in distilled water and 0.1 ml of each concentration was instilled in one eye of each animal by pulling the lower eye lid down away from the eye ball, separately. The lids were then gently held together for a period of one second before the animal was released. The other eye remained untreated and acted as the control. The eyes were examined and grade of ocular reactions recorded after 24, 48 and 72 h.

Short term oral administration (Sub Chronic Toxicity). For sub-chronic studies the experimental albino mice weighing between 25-30 g were divided into four groups. Each group comprised of 6 animals (3 males and 3 females). Group 1 to 3 received Nimbokil orally through Cannula, at the dose rate of 0.0025, 0.005 and 0.01 ml kg^{-1} body weight in single daily dose, upto 28 days. Group 4 was kept as control and received distilled water only. Physical check of behavioural changes were made daily and body weight was recorded, weekly.

After 28 days, autopsy was performed on one animal, selected at random, from each group to observe gross changes in the vital organs.

Effect on fertility. After 28 days feeding of Nimbokil male and female mice of similar dose groups were mixed together. Similarly, control group was also mixed to observe the effect on fertility.

Results and Discussion

Acute toxicity. Oral feeding of Nimbokil upto 8 ml kg^{-1} body weight showed no untoward effects during 72 h of observation period (Table 1) . The animals were found to be active alert and normal. Autopsy findings of experimental animals revealed no gross changes in the heart, lungs, liver, kidney, ovary and the testicles (Fig 2) compared to control animal (Fig 1)

Percentaneous toxicity (LD-50 and LD-100). These studies of Nimbokil revealed that oral administration of the product in doses upto 8 ml kg^{-1} body weight were practically non-toxic (Table 2) and showed no untoward signs and symptoms during 24 h of observation, where as the limit for the test is 5 ml kg^{-1} body weight.

Oral administration of Nimbokil above the doses of 14 ml kg^{-1} body weight showed toxicity symptoms. the severity and depth of symptoms were proportional to the concentrations of the

Table 1
Acute toxicity of Nimbokil-60 EC on albino mice

S.No.	No. of animals	Body weight (g)	Oral dose (ml kg^{-1} body weight)	Survival (%)	Mortality (%)	Toxicity effects
1	6	29 ± 1	1	100	0	Normal
2	6	28 ± 2	2	100	0	Normal
3	6	29 ± 2	3	100	0	Normal
4	6	28 ± 1	4	100	0	Normal
5	6	28 ± 1	5	100	0	Normal
6	6	29 ± 2	6	100	0	Normal
7	6	29 ± 2	7	100	0	Normal
8	6	29 ± 1	8	100	0	Normal
9	6	28 ± 2	9	100	0	Normal
10	6	28 ± 1	10	100	0	Normal

test product. The sings and symptoms of toxicity were similar in all the experimental animals. The toxicity manifestations were sedation, increased respiration, increased heart rate (Tachycardia), convulsions, ataxia (abnormal gait, disturbed equilibrium), paralysis of hind limbs and marked depression. the induction time ranges from 5 to 30 min depending upon the concentrations of the product, administered. LD-50 was obtained at 10 ml kg^{-1} body weight and LD-100 at 20 ml kg^{-1} body weight (Table 2). Yet, there was no gross change observed in the killed animal (Fig 3).

Acute inhalation. The experimental animals didn't show any ill effect after 2 continuous inhalation of the Nimbokil vapours. Even after 24 h of treatment, the test animals were normal and healthy. Autopsy findings revealed no gross changes in the lungs or other vital organs (Fig 4).

Skin irritations. In all the tested albino rabbits no erythema or oedema was observed i.e. the primary irritation score was zero showing that Nimbokil is non-toxic to the skin.

Eye irritations. The results indicated that Nimbokil does not produce any ill effects in the eyes of experimental

Table 2
LD-50 determination of Nimbokil-60 EC on mice post oral dose administration

S.No.	Body weight (g)	Dose (ml per kg body wt)	No of dead/ total (male & female)	Mortality (%)
1	27 ± 1	11	0/10	0.0
2	27 ± 2	12	0/10	0.0
3	27 ± 1	13	0/10	0.0
4	26 ± 1	14	1/10	10.0
5	26 ± 1	15	3/10	30.0
6	27 ± 2	16	5/10	50.0
7	27 ± 2	17	6/10	60.0
8	27 ± 1	18	8/10	80.0
9	26 ± 1	19	9/10	90.0
10	27 ± 1	20	10/10	100.0

Table3
Sub-chronic toxicity on Nimbokil-60 EC (in albino mice)

Group No.	No of Animals	Mean weight(g) pre-treatment	Oral dose (ml kg^{-1}) of nimbokil	Mean weight (g) post-treatment	Observation
1	6	27 ± 1	0.00250	26 ± 1	No mortality
2	6	27 ± 1	0.00500	27 ± 1	No mortality
3	6	27 ± 2	0.01000	26 ± 1	No mortality
4	6	26 ± 1	distilled water	27 ± 1	Normal

Fig 1. A disected mice of control group.

Fig 2. Nimbokil-60 EC seen in enlarged stomach of mice after 24 h of feeding. Vital organs are normal.

animals upto 10% concentration. The pesticide showed ocular reactions like redness from 25% conc. and above. At 75% concentration mild swelling and wetness (Water discharge) was observed. The product, as such, produced redness and mild swelling of the eye ball in test animal after one hour of application . The symptoms remained for 24 h. The ocular reaction gradually decreased and completely vanished after 72 h.

Table 4
Effect of Nimbokil-60 EC on fertility of the mice

S.NO	Observations	Nimbokil Oral dose ml kg^{-1} body weight			Control
		0.0025	0.0050	0.0100	
1	No. of female mice	3	3	3	3
2	No. of male mice	3	3	3	3
3	No. of mice delivered	2	1	2	2
4	Percentage fertility	66.6	33.3	66.6	66.6
5	Mortality	Nil	Nil	Nil	Nil

Short term oral administration ((Sub chronic toxicity). Oral feeding of the pesticide at tested doses, for 28 days, were found to impart no ill effects. No mortality or marked changes were observed in the experimental animals. The animals were found to be active alert, healthy and normal. The results are summarised in Table 3.

Autopsy findings of the respective test animals revealed that there was no gross or pathological changes on the heart, liver, lungs, kidneys, ovary and testicles.

Effect of fertility. No adverse effect on fertility was observed in all the experimental groups. The percentage of fertility was similar in test as well as control groups. The young's of test groups were found to be active and healthy like that of control group. Data is presented in Table 4. Our results of Nimbokil's mammalian toxicity tests showed that the product is non-toxic. However, Nimbokil had marked depressant action upon the central nervous system resulting in death at higher concentrations. These finding are in accordance with the studies made for ' Neem Oil' and other neem based products carried out elsewhere (Jacobson 1988; Larson 1988; NRC 1992; Schmutterer 1995). It is worth mentioning that Nimbokil

Fig 3. Nimbokil-60 EC killed (LD-100) mice showing no gross changes.

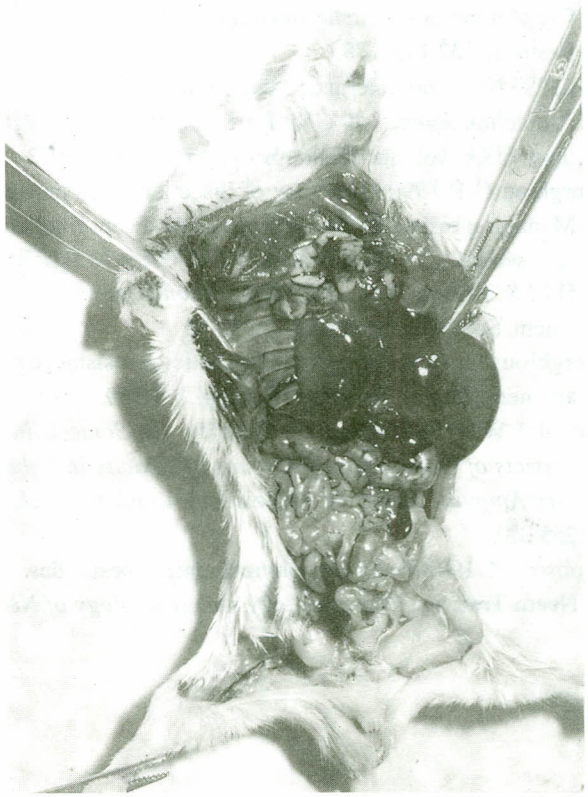

Fig 4. Sacrificed mice after 2 h inhalation test for Nimbokil-60 EC showing clear lungs.

has many naturally occurring components (e.g. fatty acids) of food plants that are part of normal human diet. Therefore, clarified neem oil has been given exemption by Environmental Protection Agency, USA (EPA 1995). The neem compounds compared to other natural insecticides like pyrethrin and rotenone are rather stable substances (Jarvis *et al* 1998). Its relative stability, together with its high potency, multiple mode of actions on insects and safety for non-target organisms, higher animals and human beings give high hopes for the future wide spread use in Pakistan.

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