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Acute and Sub-Acute Toxicity Evaluation of *Hydnocarpus laurifolia* Extract on Albino Rats

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Abstract

Synthetic pesticides are very effective to control agricultural pests. Increased use of synthetic pesticides adversely affects quality of the agricultural products and human health. Present studies reveal the health problems in usage of pesticides other important methods of pest control is very relevant. There comes the importance of natural pesticides which doesn't have any alternative negative effects to the non-target organisms. Present investigation points out the acute and sub-acute toxicity evaluation of *Hydnocarpus laurifolia*.

Keywords: Natural pesticides; *Hydnocarpus laurifolia*; *Leptocorisa acuta*

Introduction

Pest control is essential for maintain quality of agricultural products and human health. Cai explains the production of fruits, vegetables and cereals will be reduced by 78, 54 and 32% respectively if chemicals are not used in pest control [1]. But the hazardous nature of pesticides depends on the chemical and physiological properties, type of formulation, and method of application and characteristics of the receiving system. Most of the chemical pesticides are not applied to target organ as a pure material but rather as a formulation. Various reports revealed about severe problems in living beings due to the use of chemical pesticides. Rui et al. reported the risks in inhabitants of Changchun, Northeast China from organophosphorus pesticides used in vegetables [2]. Cord blood of 999 pregnant women was analyzed after the consumption of bean products, red meat, fish etc. which showed the presence of organochlorine pesticides [3]. Persistent organic pollutants and non-persistent pesticides causes diabetic related problems [4]. Since present studies reveals the health problems in usage of pesticides other important methods of pest control is very relevant. There comes the importance of natural pesticides which doesn't have any alternative negative effects to the non-target organisms.

Many synthetic pesticides are applied to control the bug. Carbaryl, Lambda-cyhalothrin, malathion and zeta-cypermethrin are the common long lasting and broad-spectrum pesticides used against the rice bugs. Kay et al. studied by bioassay technique, the efficacy of insecticides on *Eysarcoris trimaculatus* (Distant) and *L. acuta* (Thunberg) in north Queensland [5]. They applied Diazinon (280 g a.i. ha^{-1}), carbofuran (1000 g a.i. ha^{-1}) and dimethoate (135 g a.i. ha^{-1}). 100% mortality of *L. acuta* by acephate (750 g a.i. ha^{-1}) was recorded and not a consistent result on both the species by Trichlorfon (625 g a.i. ha^{-1}) and carbaryl (1040 g a.i. ha^{-1}). Monocrotophos (300 g a.i. ha^{-1}) killed 99% of *the pest* while fenthion (440 g a.i. ha^{-1}) showed 100% mortality. Endosulfan showed residual effect after two days (735 g a.i. ha^{-1}) and killed>99% of the insect on the day of spraying. Chlorpyrifos killed 100% at 500 g a.i. ha^{-1} and 750 g a.i. ha^{-1} ; 98% on the day of spraying using 250 g a.i. ha^{-1}. Finally, they concluded by recommending Chlorpyrifos (750 g a.i. ha^{-1}) as an effective pesticide to control *L. acuta* on rice.

Pangtev studied the efficiency of seven insecticides to control *L. acuta* in Nagaland [6]. Among them Malathion dust applied at 1 kg a.i./ha was found to be most effective insecticide. Other pesticides applied were BHC [HCH] dust applied at 1 kg a.i./ha (43.75 qt/ha), parathion-methyl dust applied at 1 kg a.i./ha (43.25 qt/ha), carbaryl dust applied at 1 kg a.i./ha (39.80 qt/ha) and endosulfan dust applied at 1 kg a.i./ha (39.34 qt/ha) showed effect reduction in pest.

H. laurifolia seeds reported to have well known activity in various studies as follows: Ethanolic extract of *H. laurifolia* possess anti-diabetic: chloroform extract possesses anti diabetic, anti-hyperglycemic, and anti-hyperlipidemic effect [7,8].

Zahir et al. reported oil is useful in skin problems as a mixture with lemon juice on burned skin, leprosy and in joint pain relief. Seed paste is used for the treatment of eczema, white patches, itching and infection.

Various extracts of *H. laurifolia* was given to rats after inducing diabetes by streptozotocin (30-50 mg/kg) intraperitoneally. Rats shown glucose level>250 mg/dl were selected for the study. Orally melformin given as hypoglycemic agent during the study period. Glucose tolerance test was conducted in eleven groups of rats (male rats 180-250 g wt). By conducting glucose-oxidase method blood glucose level is estimated. The study concluded as anti-hyperglycemic activity of the test drugs in diabetic rats significantly reduces the blood glucose levels ($p<0.0001$) at 1, 2 and 4 hr respectively. The results confirmed that *H. laurifolia* seed extracts have potent anti-diabetic activity for the treatment of diabetes mellitus [7].

Materials and Methods

Acute toxicity evaluation of *Hydnocarpus laurifolia*

Healthy albino rats weighing 80-120 g issued for the study. A group of eight rats of both sex served as one treatment. Different doses of methanol extract of HL seed (50, 100, 200, 400, 600, 800, 1000, 1200, 1500 mg/Kg body weight) is orally administered to the test animals (single dose). One group served as vehicle treated control. They were fed with pellet feed and water ad libitum. Mortality counts, behavior and appearance of the test animals were observed immediately after the administration of the extract and for 7 days at 24 h interval. This test gives dose response relationship and the LD 50 value [9] (Table 1).

Experimental design

For safety evaluation inbreed strains of Sprague-dawley albino rats of body weight ranging between 80-120 g maintained under standard laboratory conditions was selected from the department animal house for the study. The animals were caged in well ventilated iron metallic cages at room temperature. They were fed with normal rat feed (Lipton, India) and water ad libitum.

Group 1	Control 1 mL of 10% DMSO
Group 2	50 mg/kg body weight of HL in 10% DMSO
Group 3	100 mg/kg body weight of HL in 10% DMSO
Group 4	200 mg/kg body weight of HL in 10% DMSO
Group 5	400 mg/kg body weight of HL in 1 mL of 10% DMSO
Group 6	600 mg/kg body weight of HL in 1 mL of 10% DMSO
Group 7	800 mg/kg body weight of HL in 1 mL of 10% DMSO
Group 8	1000 mg/kg body weight of HL in 1 mL of 10% DMSO
Group 9	1200 mg/kg body weight of HL in 1 mL of 10% DMSO
Group 10	1500 mg/kg body weight of HL in 1 mL of 10% DMSO

Table 1: Toxicity evaluation on rats by administering different doses of methanol extract of HL seed.

The extracts were given once by oral gavages and maintained under laboratory conditions providing normal diet and water *ad libitum*. All the animals were observed for signs of toxicity like ataxia, circling, nasal hemorrhage, lacrimation, labored breath and paralysis immediately after oral administration for seven days at 24 h interval.

Sub-acute toxicity evaluation

In this study albino rats of body weight ranges from 100 to 120 g were maintained under laboratory conditions provided with normal diet and water ad libitum were used. Five groups having six animals in each is kept for the study.

Experimental design

The control group administered with DMSO, Group II was given 500 mg/kg body weight (high dose) for a period of 28 days (single dose per day) by gavage [10]. This experiment shows accurate result on the toxic effect on target organs by HL extract. Blood is collected to observe clinical parameters such as Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP) after 7, 14, 21 and 28 days. Total RBC and hemoglobin were estimated.

Serum Glutamic Oxaloacetic Transaminase (SGOT)

SGOT is analyzed by reading the colour of 2,4-dinitro- phenyl hydrazene formed in alkaline solution. Buffered substrate (0.5 mL) was added to the test and control tubes which are incubated at 37°C for 3 min. 0.1 mL sample was added to the test tube, mixed thoroughly and incubated for 60 min at 37°C. 0.5 mL 2,4-dinitro phenyl hydrazine was added and kept at room temperature for 20 min. After this 5 mL of 0.4N sodium hydroxide was added, vortexed and kept for 5 min. The optical density was measured at 520 nm. The working pyruvate standard (0.02 mg) was also measured similarly. The enzyme activity was calculated using the formula and the value. The value was expressed in IU/litre of serum.

$$\frac{OD\ of\ the\ test - OD\ of\ the\ control}{OD\ of\ the\ standard \times Con.\ of\ Std. \times Dilution\ factor}$$

Serum Glutamic Pyruvic Transaminase (SGPT)

Stock solutions prepared for the assay as follows: Phosphate buffer solution 0.2M of 7.4. Buffered substrate by dissolving 1.78 gm of L-alanine with 30 mg of Ketoglutaric acid in 20 mL of phosphate buffer containing 1.25 mL 0.4N sodium hydroxide. This is made up to 100 mL with phosphate buffer solution of pH 7.4 at 4°C. DNPH is prepared by dissolving 200 mg dissolved in hot 1N HCl and made up to 1000 mL with 1N HCI. Finally, Standard pyruvate prepared by dissolving 2.2 mg sodium pyruvate/10 mL in distilled water.

Buffered substrate (0.5 mL) was added to the test and control tubes and incubated at 37°C for 3 min. 0.1 mL sample was added to the test tube, mixed thoroughly and incubated for 30 min at 37°C. 0.5 mL of DNPH was added and kept at room temperature for 20 min. After this 5 mL of 0.4N sodium hydroxide was added, vortexed and remained for 5 min. The optical density was measured at 520 nm. Similarly, the working pyruvate standard (0.02 mg) was also measured. The enzyme activity was calculated using the formula:

$$\frac{OD\ of\ the\ test - OD\ of\ the\ control}{OD\ of\ the\ standard \times Con.\ of\ Std. \times Dilution\ factor}$$

Alkaline Phosphatase (ALP)

In presence of alkaline oxidizing agent amino antipyrine gives a red or purple colour with compounds containing phenolic group. This reaction has been used to determine the phenol produced by the action of phosphates on disodium phenyl phosphate, 2 mL of buffered substrate was added to the test and control tubes and were incubated at 37°C for 3 min in a water bath. 0.1 mL of the sample was added to the tubes (test) and incubated for 15 min at 37°C. After incubation 0.8 mL of 0.5N NaOH and 0.1 mL of sample was added to the control tube, followed by 1.0 mL of 4-Aminoantipyrine and 1.0 mL of potassium ferricyanide was added, mixed and the optical density was read under 520 nm. The activity of alkaline phosphatase was determined by the following formula and was expressed in mg.

The enzyme activity was calculated using the formula:

$$\frac{OD\ of\ the\ test - OD\ of\ the\ control}{OD\ of\ the\ standard \times Con.\ of\ Std. \times Dilution\ factor}$$

Hemoglobin

Hemoglobin content was estimated by following the procedure of Hawk 1965.

Blood cell count

Red blood cells and white blood cells was counted by using hemocytometre and expressed as cells per cubic millimeter.

Results

Acute toxicity evaluation of *H. laurifolia*

Test dose (mg/kg)	Immediate	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	7th day
50	N	N	N	N	N	N	N	
100	N	N	N	N	N	N	N	
200	N	N	N	N	N	N	N	
400	N	N	N	N	N	N	N	No death
600	N	N	N	N	N	N	N	
800	N	N	N	N	N	N	N	
1000	N	N	N	N	N	N	N	
1200	N	N	N	N	N	N	N	
1500	N	N	N	N	N	N	N	

Table 2: Acute toxicity evaluation of *H. laurifolia* on *L. acuta.*

Test dose	Atoxia	Circling	Lacrimation	Paralysis	Laboured breathing	Death
(mg/kg body weight)	0 h - 7th day	0 h - 7th day	0 h - 7th day	0 h- 7th day	0 h - 7th day	0 h - 7th day
50	Nil	Nil	Nil	Nil	Nil	Nil
100	Nil	Nil	Nil	Nil	Nil	Nil
200	Nil	Nil	Nil	Nil	Nil	Nil
400	Nil	Nil	Nil	Nil	Nil	Nil
600	Nil	Nil	Nil	Nil	Nil	Nil
800	Nil	Nil	Nil	Nil	Nil	Nil
1000	Nil	Nil	Nil	Nil	Nil	Nil
1200	Nil	Nil	Nil	Nil	Nil	Nil
1500	Nil	Nil	Nil	Nil	Nil	Nil

Table 3: Investigation of acute toxicity symptoms in rats after giving methanol extract of *H. laurifolia.*

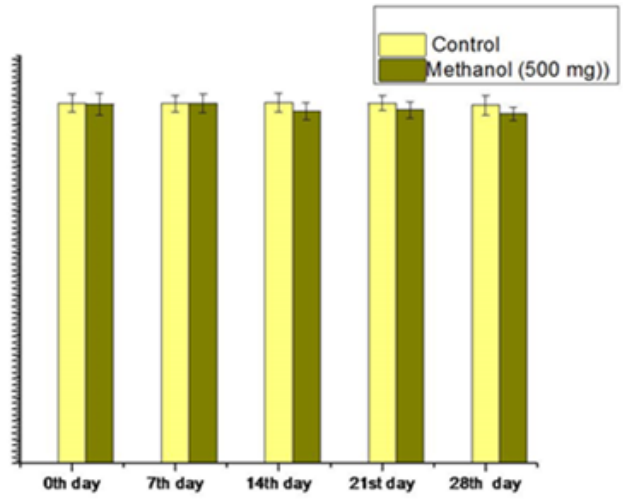

Figure 1: Effect of *H. laurifolia* extract on Serum Glutamic Oxaloacetic Transaminase (SGOT).

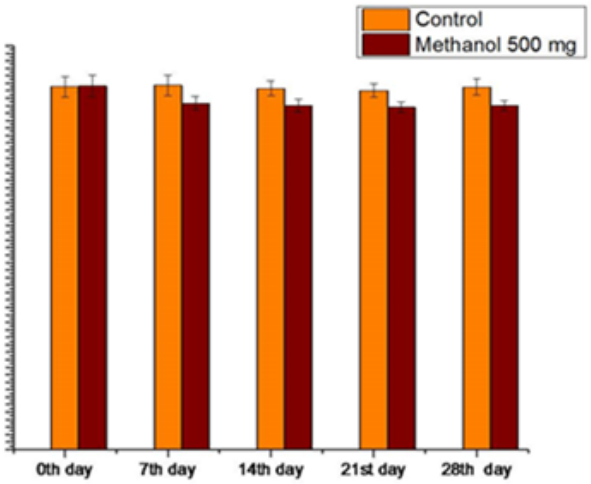

Figure 2: Effect of *H. laurifolia* extract on Serum Glutamic Pyruvic Transaminase (SGPT).

Alkaline Phosphatase (ALP)

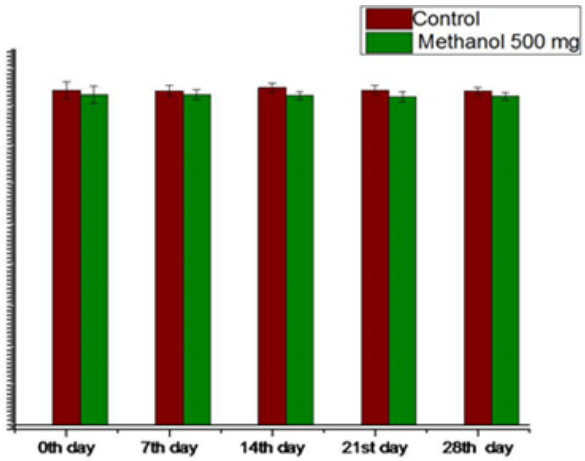

Figure 3: Effect of *H. laurifolia* extract on Alkaline Phosphatase.

Hemoglobin

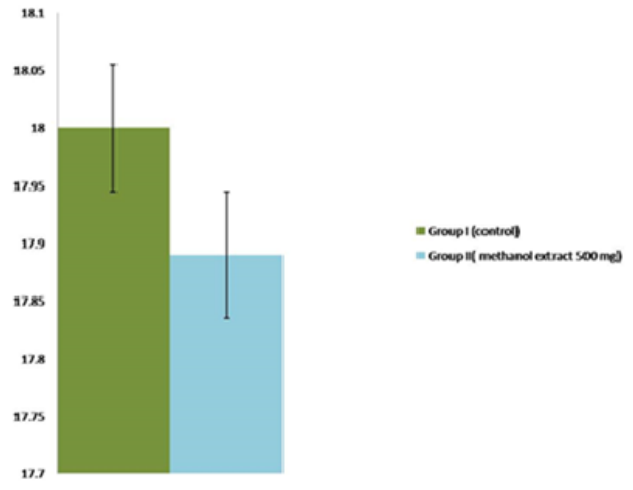

Figure 4: Hemoglobin content (g/dl).

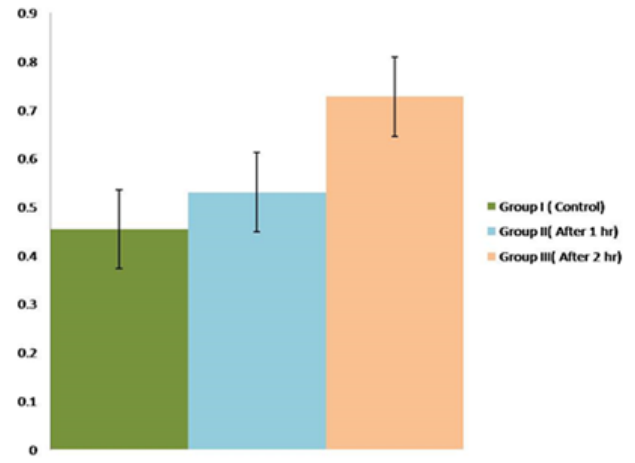

Figure 5: RBC count (cell/cubic millimetres).

VA Acute Toxicity Evaluation of *H. laurifolia*

Acute toxicity evaluation of *H. laurifolia* on *L. acuta* was done at different concentrations was shown in Table 2. After giving the dose, immediately and till 96th hour (four days) the rats were noticed for its behavioral changes, signs of toxicity and mortality. The result showed that the single oral dose of the extract showed no mortality of the rats even at higher dosage levels indicates safety of the extract. The result shown in Table 3 showed 1500 mg/kg given to the rats is nontoxic. Table 3 shows the acute toxicity symptoms in rats.

Sub-acute toxicity evaluation

Serum Glutamic Oxaloacetic Transaminase (SGOT): Serum Glutamic Oxaloacetic Transaminase is an enzyme released into blood when the liver or heart is damaged. By giving single dose of 500 mg/kg body weight of *H. laurifolia* extract in rats, SGOT was analyzed for 28 days. The result was shown in Figure 1. From the figure 0th day to 8th day the values are found to be very close with the control group (1 mL 10% DMSO). On initial day Group I (control) shown 59.67 g/dl and the Group II 59.51 g/L which ends in final day value as 59.31 g/L and 57.91 g/L for Group I and Group II respectively. From initial day to final day the value of Group I and Group II were very close to each other, this indicates that treating with the seed extract of *H. laurifolia* as an insecticide if safe.

Serum Glutamic Pyruvic Transaminase (SGPT): Serum Glutamic Pyruvic Transaminase is an enzyme produced in liver and when liver damage occurs its level in serum get increased and so it is a sensitive test for evaluating liver function. As the plant extract is given to the rats it was known from the results as shown in Figure 2. The values of the control and the sample treated was nearly the same. From the starting day to the 28th day of analysis the results of treated group are agreeing with the control group. Initial day value of Group I and Group II were 48.54 g/L and 48.64 g/L respectively. On 14th day the value of Group II is 46.02 g/L and Group I 48.31 g/L. From the results the treatment of *H. laurifolia* extract causes decrease in SGPT levels.

Alkaline Phosphatase (ALP): The result of alkaline phosphatase content is shown in Figure 3. From the initial day to the final day the

Group II values are less than the Group I as it indicates the positive effect of the extract. Values indicates control (Group I) ranging from 76.31 units/L to 76.02 units/L and the Group II 75.35 units/L to 74.88 units/L from first day to final day. Alkaline phosphatase known as cytosolic marker enzyme which reflects hepatocellular necrosis as they are released into the blood after cell membrane damage. The present study reflects that there were no significant differences in the activity of ALP after 28 days of rat's administration with the most effective plant extract at dose level of 500 mg/kg body weight.

Hemoglobin: Figure 4 shows the hemoglobin content in control and 500 mg methanol treated group.

Red blood cell count: Figure 5 shows the RBC content in control and 500 mg methanol treated group.

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