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Research Article

ACUTE ORAL TOXICITY STUDIES OF PONGAMIA PINNATA AND ANNONA SQUAMOSA ON ALBINO WISTER RATS

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ABSTRACT

The acute toxicity studies of crude seed extracts of Pongamia Pinnata and Annona Squamosa were studied in female Albino Wister rats. The studies included the gross observation such as changes in body weight and food intake. Rats are grouped as 5 rats in each group. The rats treated with pongamia pinnata with dose of 2000mg/kg body weight were safe. Where as in annona Squamosa treated rats with same dose mortality occurred. The dose of annona was reduced to 300mg/kg bw and observed for consecutive 14 days. This dose of Annona was safe. The changes in body weight and biochemical parameters were statistically significant and increasingly not significant when compared to that of control group of rats. It is proved by this study that Annona extract is very toxic than Pongamia extract.

Keywords: Acute toxicity, Pongamia Pinnata, Annona Squamosa and Albino Wister rats.

INTRODUCTION

Toxicology is the aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms. In order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. No drug is used clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animals toxicological studies may be three types such acute, sub-acute and chronic toxicological studies.

In **acute toxicity studies**, single dose of drug given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products.

In **sub-acute toxicity studies**, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues[1].

In **chronic toxicity studies**, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic Potential of drug.

Antioxidants

Antioxidants are molecules that prevent cell damage and serve as parts of Enzymes. There are many types of antioxidants found in nature. There are vitamin antioxidants that are known to be protective like vitamins A, C, and E. There are mineral antioxidants like selenium and zinc and there are pigments (colors). Some pigments in plants and animals are potent antioxidants. Antioxidants trap harmful forms of oxygen and prevent them from damaging cells. Antioxidants in the diet

enter the blood stream and act directly to protect cells of the body from damage. In addition, some antioxidants stimulate the immune system, and/or increase the activity of detoxifying enzymes in the liver[2].

Oxidation

The process of oxidation is a normal function of all living things. Oxidation of various compounds is the **primary means by which humans and other animals get energy**. There are special compounds in nature called oxidant catalysts that provide a stable environment for oxidation to occur safely. The two most common oxidant catalysts are copper and iron.

As charges are transferred from iron and copper to oxygen for energy release, new forms of oxygen are generated. Each of these new forms of oxygen has a free set of charges; these are called reactive oxygen species or ROS (Reactive oxygen species) . As charges are transferred from the catalyst to oxygen and new ROS are formed a destructive process of charging stable compounds continues to generate more ROS. Some ways that ROS are generated include: **inflammation, strenuous exercise, detoxification, chemical exposure, exposure to radiation, cigarette smoke, alcohol, pollutants, and high fat diets.** ROS are damaging to proteins, membranes, and DNA(deoxy ribonucleic acids).

Damage to DNA can be the beginning of cancer initiation**.** The damage from ROS accumulates over time and is the major reason for aging and age-related increases in diseases such as cancer. Since ROS are produced constantly by many different pathways, defense mechanisms have evolved against ROS.

Antioxidants are small molecules that pick up ROS and prevent them from causing damage; in addition antioxidant enzymes in the body can also inactivate ROS. Aging results in a decrease in the amount of antioxidant enzymes which results in an increased risk for developing cancer and an increased incidence of immune impairment. As antioxidant enzymes decline ROS accumulate and so does the damage caused by them.

ROS are only a problem when in excess, when they are not in excess they play a positive role in health and development**.** Oxidants are used to help a growing fetus develop; they can alter gene expression, and activate natural detoxification systems. They are also produced and use by immune cells to kill invading infective agents. Often with chronic infection, white blood cells can produce excessive amounts of ROS, which increases the antioxidant requirement.

The important point is that oxidative pathways have been implicated as a factor in a wide variety of disease states[2].

PONGAMIA PINNATA

(syn. Pongamia glabra, Papilionaceae family), locally known as karanja. It is also called Derris indica and Pongamia glabra[3].It is a medium-sized evergreen tree with a spreading crown and a short bole. The tree is planted for shade and is grown as ornamental tree. It is one of the few nitrogen fixing trees producing seeds containing 30-40% oil. It is commonly known as Karanj. The present review will possibly help to the bridge between traditional claims and modern therapy on P. pinnata. It has been recognized in different system of traditional medicines for the treatment of different disease & ailment off human being .The seeds & sprouts of pongamia pinnata were used in folk remedies for tumors, bronchitis, chronic fever, whooping cough and chronic skin diseases and painful rheumatic joints. Seed oil is used in scabies, leprosy, piles, ulcers, liver pain and lumbago[3].

ANNONA SQUAMOSA

(syn. Sweetsop, Sugar apple fruit), in some regions it is also known as Custard-Apple[3]. In India the crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. It is a semi-evergreen shrub or small tree reaching 6-8 m tall. The fruit is usually round or oval, slightly pine cone-like, with a scaly or lumpy skin. The fruit flesh is edible, white to light yellow, and resembles and tastes like custard.

The seeds are scattered through the fruit flesh; they are blackish-brown, hard and shiny.

MATERIALS AND METHODS

Collection of Plant Materials

The seed of plant were collected from Indian Institute of Botanical Sciences, Kolkata, and authenticated by Taxonomist of Indian Institute of Botanical Sciences, Kolkata. After authentication the seed taken from plant (1000gm) ; the extraction process is carried out by soxhlet apparatus.

Experimental Animals
The experiment was carried out on albino female Wister rats. They were 4 – 8 weeks old, weighing between 100 –120 grams. The experimental protocol has been approved by institutional animal ethics committee. Rajah Muthiah medical college, Annamalai University.

Grouping of Rats
10 rats which having average weight 110 gms were grouped in two 5 for pongamia pinnata and 5 for annona sqamosa. And 5 rats weighed 120 gms were used for control.

Administration of Sample
The samples were given as 2000 mg/kg body weight for all test rats. The compounds were dissolved in distilled water so that each ml contains 4 mg of sample. After the administration of sample (route of administration is oral) the test rats and control rats were observed for body weight and feed intake for 14 consecutive days. In the second day of observation 3 of 5 annona treated rats were died might because of dose sensitiveness or intolerance of climatic conditions after the administration of compound. Then 3 more rats are grouped and treated with 300 mg/kg body weight annona squamosa crude extract and observed for 14 days.

Investigation of Biochemical Parameters
Three from pongamia treated rats, 2 of annona treated rats (2000mg/kg bw), 3 of annona treated rats (300mg/kg bw) and 2 of control rats were sacrificed and all tissues were taken and kept in –850 C for few days. Tissues were taken out and perfusion was carried out. After centrifugation of homogenized tissue the supernatant was used for biochemical parameter testing. The readings were taken by using spectrophotometer and values applied for statistical tools like mean and standard error. The readings were given for the ANOVA[10] to determine the significant difference from control values and values were presented in tables.
Lipid peroxidation[4] was estimated by thiobarbituric acid (TBA) methods, GSH[5,6] was estimated by DTNB (5, 5-DITHIOBIS-2-NITROBENZOIC ACID) known as Ellman reagent and GST[5,6] by enzyme-catalyzed condensation of glutathione with the model substrate CDNB,
BSA protein was estimated by BSA and Folin-phenol reagent using Lowry method[7,8]

RESULTS
Pongamia Pinnata and Annona Squamosa seeds were extracted and the crude extract was administered in the dose of 2000mg/kg bw for 5 rats. Pongamia treated rats are safe but in the second day of 14 days observation 3 of Annona treated rats were died because of toxic nature of Annona extract. Then dose of Annona was Reduced to 300mg/ kg body weight and given to four more rats and observed for 14 days. This dose of Annona was safe. It is proved by this study that Annona extract is very toxic than Pongamia extract.

DISCUSSION
The present preliminary investigations show the acute oral toxicity of Annona Squamosa seeds although Pongamia Pinnata is inactive at the concentration (2000mg/kg) tested; higher concentrations remain to be studied. It is of interest to note that Annona Squamosa (seed extract) was effective at a short duration of time.

Changes in food intake and body weight:
During 14 days of observation the body weight and food intake of rats were weighed. Pongamia treated rats shown the less changes than the Annona treated rats. Feed intake amounts of treated rats were less as compared to the control rats. After the administration of compounds the rats got irritation, dullness. Three of Annona treated rats were died in the second day after treatment. Two of them show the brain hemorrhage after the dissection. The average body weight and feed intake values were made as tables 1, 2, 3. The treated values are compared with control values for pongamia, Annona (2000mg) and Annona (300mg) respectively.

Changes in the biochemical parameters of different tissues:
Parameters like Lipid Peroxidation, Reduced Glutathione (GSH) and oxidized glutathione (GST) were determined in different tissues like Liver, Lungs, Spleen, Kidney, Heart and brain. Lipid peroxidation values had shown the maximum significant difference from control as compared to other parameters. The major part of metabolism that Liver shown the difference in lipid peroxidation in all treated

rats. These indicate significant adverse effect of Pongamol and Annonin. The GST and GSH did not show any significant difference. It was found that these parameters slightly changed with respect to control group rats but remain within the normal range except Lipid peroxidation. The significant changes of different parameters in different tissues were shown in tables 4, 5 and 6.

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Table 1: Average Body Weight and Feed Intake of Pongamia Pinnata Treated Rats.

Parameter	No of days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Body weight (In grams)	123	121	122	128	119	125	131	135	135	138	134	144	145	145
Feed intake (In grams)	42	43	40	65	55	60	65	58	58	60	48	75	58	73
Mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Average Body Weight and Feed Intake of Annona Squamosa (2000mg/kg bw) Treated Rats.

Parameter	No of days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Body weight (in grams)	131	118	123	119	119	119	120	120	121	124	126	126	125	124
Feed intake (in grams)	45	20	14	15	21	15	17	16	20	23	22	21	17	18
Mortality	0	3	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Average Body Weight and Feed Intake of Annona Squamosa (300mg/kg bw) Treated Rats.

Parameter	No of days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Body weight (in grams)	125	128	129	132	132	133	135	137	139	141	143	144	144	145
Feed intake (in grams)	34	36	24	48	26	27	29	44	47	47	47	35	30	32
Mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Acute Oral Effect on Lipid Peroxidation in Different Tissues of Female Rats.

Tissues	Pongamia pinnata (2000mg/kg BW)		Annona squamosa (2000mg/kg BW)		Annona squamosa (300mg/kg BW)	
	Sample		Sample		Sample	
	Control	Treated	Control	Treated	Control	Treated
Liver	17.84 ± 0.23	39.51 ± 1.690*	17.84 ± 0.23	37.5 ± 5.67*	17.84 ± 0.23	47.50 ± 4.67*
Lung	17.57 ± 1.7	44.44 ± 11.44	17.57 ± 1.7	36.98 ± 6.63	17.84 ± 1.7	55.57 ± 2.62*
Brain	18.10 ± 0.954	44.94 ± 9.36*	18.10 ± 0 .954	30.80 ± 11.38	18.10 ± 0.954	48.94 ± 6.25*
Kidney	16.56 ± 0.176	55.68 ± 12.07*	16.56 ± 0.176	44.75 ± 4.91*	16.56 ± 0.176	55.15 ± 1.53*
Heart	18.16 ± 0.544	27.25 ± 7.70*	18.16 ± 0.54	28.07 ± 2.69*	18.16 ± 0.54	47.56 ± 6.08*
Spleen	17.99 ± 0.38	66.22 ± 2.60*	17.99 ± 0.38	45.94 ± 1.93*	17.99 ± 0.38	73.36 ± 7.47*

*Significantly different from control. Values are mean ± standard error from two rats.

Table 5: Acute oral effect on GST in different tissues of female rats

Tissues	Pongamia pinnata (2000mg/kg BW)		Annona squamosa (2000mg/kg BW)		Annona squamosa (300mg/kg BW)	
	Sample		Sample		Sample	
	Control	Treated	Control	Treated	Control	Treated
Liver	0.514 ± 0.053	1.094 ± 0.088*	0.514 ± 0.053	0.753 ± 0.306	0.514 ± 0.053	0.824 ± 0.147
Lung	0.191 ± 0.063	0.297 ± 0.035	0.191 ± 0.063	0.199 ± 0.003	0.191 ± 0.063	0.337 ± 0.065
Brain	0.495 ± 0.053	0.346 ± 0.079	0.495 ± 0.053	0.128 ± 0.033*	0.495 ± 0.053	0.428 ± 0.036
Kidney	0.273 ± 0.116	0.607 ± 0.311	0.273 ± 0.116	0.410 ± 0.116	0.273 ± 0.116	0.503 ± 0.053
Heart	0.142 ± 0.033	0.203 ± 0.057	0.142 ± 0.033	0.185 ± 0.053	0.142 ± 0.033	0.447 ± 0.038*
Spleen	0.212 ± 0.043	0.241 ± 0.033	0.212 ± 0.043	0.137 ± 0.032	0.212 ± 0.043	0.276 ± 0.123

*Significantly different from control. Values are mean ± standard error from two rats

Table 6: Acute oral effect on GSH in different tissues of female rats.

Tissues	Pongamia pinnata (2000mg/kg BW)		Annona squamosa (2000mg/kg BW)		Annona squamosa (300mg/kg BW)	
	Sample		Sample		Sample	
	Control	Treated	Control	Treated	Control	Tr Eated
Liver	3.37 ± 0.176	4.89 ± 0.806	3.37 ± 0.176	4.33 ± 0.226*	3.37 ± 0.176	5.39 ± 0.437
Lung	3.05 ± 0.169	4.19 ± 0.593	3.05 ± 0.169	3.57 ± 0.452	3.05 ± 0.169	4.17 ± 1.27
Brain	3.01 ± 0.226	4.29 ± 0.707	3.01 ± 0.226	3.63 ± 0.190	3.01 ± 0.226	5.24 ± 0.440
Kidney	3.48 ± 0.403	4.55 ± 0.852	3.48 ± 0.403	3.83 ± 0.021	3.48 ± 0.403	5.47 ± 0.140*
Heart	2.93 ± 0	4.15 ± 0.613	2.93 ± 0	3.86 ± 0.403	2.93 ± 0	5.26 ± 0.396*
Spleen	3.17 ± 0.113	4.59 ± 0.374*	3.17 ± 0.113	3.90 ± 0.671	3.17 ± 0.113	5.18 ± 0.294*

*Significantly different from control. Values are mean ± standard error from two rats

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