

Toxicological Evaluation of the Aqueous Extract of *Carica Papaya* Leaves on Wistar Female Rats

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ABSTRACT

Objective: The main objectives of this work is to determine the lethal dose (LD₅₀) effect of *Carica papaya* leaves and signs of toxicity wistar female rats in a short term period study (14 days).

Study Design: Observational study.

Place and Duration: Mature green leaves of *Carica papaya* plant were collected from *Carica papaya* garden, District Malir Karachi, in October 2017. The leaves samples were identified and belong to *Carica papaya* family; Caricaceae in the Department of Botany, University of Karachi.

Materials and Methods: Seventy six (76) large expanded green healthy leaves of male plant were washed carefully with water, cut into small pieces and were crush in electric grinder by adding water at room temperature. The blended material was squeezed and filtered with the help of a man filter paper 42 pore size making sure that the extract should be free from leaf particles. Different doses were prepared from this extract according to the body weight of the rats and dissolved in known amount of water (1-2) ml depending on the amount of lyophilized extract taken. Specific pathogen- free, healthy inbred lines, adult albino female Wistar non-pregnant rats of 12 week weighing between 110-140g were obtained from animal house of Dow university of Health and Sciences (DUHS).

Results: The main objectives of this work is to determine the lethal dose (LD₅₀) effect of *Carica papaya* leaves and signs of toxicity wistar female rats in a short term period study (14 days). Lyophilized aqueous extract of *Carica papaya* leaves was administered orally to female Wistar rats. The lethal dose was found to be 16g/kg of body weight. This dose in rats caused mainly reduced appetite, gradually decreased body weight, uncomfortable feeling, abdominal pain, shallow breathing and itching and coughing. All Rats survived at a dose of 4g/kg and 8g/kg of body weight. Histopathological studies show acute inflammation and fibrinous exudates in lung parenchyma and mildly dilated sinusoids, prominent kupffer cells along with cellular swelling at a dose of 16g/kg of body weight.

Conclusion: It can be concluded from this study that herbal medicines from *Carica papaya* leave extracts appear to be nontoxic as lethal dose is high (16g/kg of body weight) but phase I clinical trials on healthy volunteers are needed for safety testing of these medicines before their use for treatment of dengue fever patients.

Keywords: *Carica Papaya*, Medicinal plants, Toxicology, Inflammation, Fibrinous materials.

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INTRODUCTION

Carica papaya, commonly called, Papaya belonging to the family Caricaceae is in use for centuries as food or as a medication¹. *Carica papaya* plant is commonly grown in all tropical and subtropical regions of the world. The leaves and fruit of papaya produce proteins and alkaloids which can be used in different pharmaceutical products². Customarily leaves have been utilized for treatment of a wide scope of illnesses, as in treatment of jungle fever, dengue, jaundice, immunomodulatory and antiviral action. Youthful leaves are wealthy in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic mixes (ferulic corrosive, caffeic corrosive, chlorogenic corrosive), thecynogenetic mixes (benzylglucosinolate) found in leaves. Both leaf and product of the *Carica papaya* Linn., have carotenoids in particular β -carotene, lycopene,

anthraquinones glycoside, when contrasted with developed leaves and henceforth have restorative properties like calming hypoglycaemic, hostile to fruitfulness, abortifacient, hepatoprotective, injury recuperating, as of late its antihypertensive and antitumor exercises have additionally been established³.

Papaya leaf has a countless of advantages. Sanath Hettige et al, who directed the exploration on 70 dengue fever patients, said papaya leaf juice assists increment with whitening platelets and platelets, standardizes coagulating, and fixes the liver⁴. Ravi Kumar Pigli et al, in his examination that dengue fever or dengue hemorrhagic fever is the most pervasive viral sickness brought about by dengue infection a group of flavivirus transmitted through aedes aegypti mosquito. Around 2.5 billion individuals world wide influenced by this infection. Dengue is single stranded RNA infection, has four serotypes for example type 1, 2, 3 and 4. There are no endorsed antiviral specialists or antibody is accessible for the treatment of this infection which causes expanding the death rate everywhere throughout the world. As there are no engineered medications accessible, presently it is have to concentrate on therapeutic plants which are viewed as compelling, more secure and non-toxic⁵. V Krishna Prabha et al, detail a prepared to serve refreshment consolidate with papaya leaves and guava against Dengue fever. Papaya leaves contain different supplements and constituents like saponins, tannins, heart glycosides and alkaloids. These constituents can follow up on the bone marrow, forestall its devastation and improve its capacity to deliver platelets. The guava natural products are plentiful in nutrient C (ascorbic corrosive) content. It very well may be reasoned that the papaya leaves prompt the quick increment in platelet tally and immunity⁶. Swati patil et al, decided the impact of Carica papaya departs

watery concentrate in expanding the platelet include in thrombocytopenic rodent model. Fluid concentrate of Carica papaya departs at centralization of 400 mg/kg and 800 mg/kg were given to cyclophosphamide incited thrombocytopenic rodents for a time of fifteen days. Blood was pulled back at different time interims to decide the platelet check. Likewise, the coagulating time was resolved on the fifteenth day of the examination by slender strategy. Carica papaya leaf remove was found to build the platelet tally and furthermore to diminish the thickening time in rats⁷. Seeping of variable seriousness is seen primarily in the extreme type of dengue, i.e., dengue hemorrhagic fever. Pathogenesis of draining includes spillage of plasma from the veins. It is by all accounts because of the obliteration of platelets through supplement framework or melancholy of bone marrow because of dengue viral disease or both⁸. An expansion in platelet and white platelets (WBC) check inside 24 h has been demonstrated clinically with papaya leaf juice. It indicated increment in platelet check via carpaine acquired from papaya leaf⁹. Recently the, leaves of Carica papaya plant are used as platelet enhancer in dengue patients¹⁰. Literature survey indicated that people usually use aqueous extract of papaya leaves without knowing its side effects in dengue fever. Parts of plant are used as antimicrobial¹¹, antifungal¹², antimalarial¹³, anticancer¹⁴ and in treatment of many other diseases. Papaya leaves have medicinal value and the plant is rich in active antioxidant compounds, flavonoids, alkaloids, saponins, tannins and minerals¹⁵. The leaves contain effective proteolytic enzymes papain and chymopapain which are good for the production of human growth hormone¹⁶. It has high amount of niacin, fat, protein, vitamins, iron, fiber which are necessary for the regulation of body and activate immune system and increase the total antioxidant power in blood and reduce lipid

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peroxidation level¹⁷. Despite of its effective compounds, Papaya leaves and roots also contain cyanide in the form of cyanogenicglucosides and latex which produce undesirable effects on human health when taken in excess¹⁸. The herbal drug could cause severe adverse effects at high doses on lungs, liver, heart and even leading to death due to the presence of toxic compounds in it¹⁹. Detailed toxicological study is needed in order to make safe herbal drug². The acute toxicity of the aqueous extract of *Carica papaya* leaves was reported recently indicating that it did not show any toxicity up to 2g/kg¹⁰. The toxic effects and LD₅₀ of this extract in more detail on female rats and their vital organs have been determined in this study.

MATERIALS AND METHODS

PLANT MATERIAL

Mature green leaves of *Carica papaya* plant were collected from *Carica papaya* garden, District Malir Karachi, in October 2017. The leaves samples were identified and belong to *Carica papaya* family; Caricaceae in the Department of Botany, University of Karachi.

PLANT MATERIAL EXTRACTION

Seventy six (76) large expanded green healthy leaves of male plant were washed carefully with water, cut into small pieces and were crush in electric grinder by adding water at room temperature. The blended material was squeezed and filtered with the help of a man filter paper 42 pore size making sure that the extract should be free from leaf particles. The extract should not be heated otherwise it loses its activity. The extract was lyophilized at -53 °C (10 mTorr). The obtained lyophilized extract was approximately one hundred ninety grams. On average, a large male green healthy fresh leaf weighs 100-130 grams and yields 20-22ml pure extract and 2-2.5 gram lyophilized extract. Different doses were prepared from this extract according to the body weight of the rats and dissolved in

known amount of water (1-2) ml depending on the amount of lyophilized extract taken. Aqueous extract was used immediately and remaining lyophilized extract was preserved at 4 °C in a refrigerator.

ANIMALS

Specific pathogen- free, healthy inbred lines, adult albino female Wistar non-pregnant rats of 12 week weighing between 110-140g were obtained from animal house of Dow university of Health and Sciences (DUHS). All the experimental animals were provided good animal husbandry and care under the supervision of a veterinary doctor. Animals were maintained in a controlled environment, humidity, and room temperature at 20-25°C. Rodent food and water were provided *adlibitum*. The study was approved from Institutional Animals Ethics and Care Committee.

ACUTE TOXICITY STUDY

Acute toxicity studies were carried out according to the Organization of Economic Co-Operation and Development (OECD) Guidelines (OECD, 1995). The sixteen rats were divided into four groups (each of 4 rats) with one control group and three experimental groups (1-3). Each experimental group received a single dose of 4 (Group 1), 8 (Group 2) and 16g/kg (Group 3) of body weight daily while the control group received only water vehicle. The extract was administered through intubation tube carefully. All animals were monitored for apparent signs of toxicity for 14 days.

PHYSICAL OBSERVATION AND MORTALITY

Physical observations like itching, coughing, diarrhea, sleeping, coma, abdominal pain and mortality were observed in all the rats after the administration of the extract orally.

EFFECTS OF EXTRACT ON VITAL ORGANS

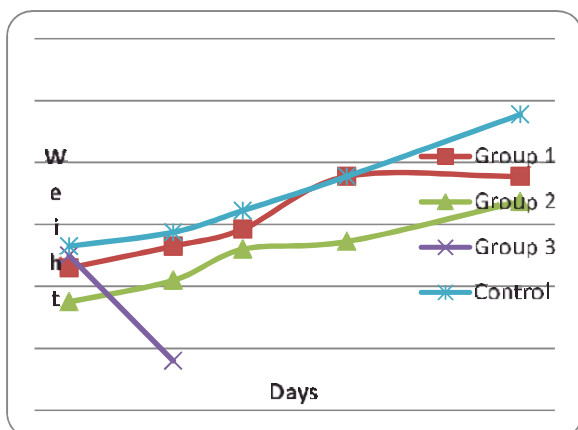
The Lethal Dose LD₅₀ of the aqueous extract of *Carica papaya* and its effect on animal organs was studied for a short term period of 14 days. All the control and experimental rats were sacrificed on 14th day. All the organs including liver, heart, kidneys, and lungs were fixed in 10% neutral buffer formalin for histopathological studies.

RESULTS

ACUTE TOXICITY STUDY

The treatment at 16g/kg body weight caused reduced appetite in all the rats. Other observed

Table 1. Physical Observations



DISCUSSION

Carica papaya plant origins are still in use since the ages as mainstay for the treatment of serious human ailments like cancer, malaria, viral and microbial infections, inflammation, and wound healing. In order to make safe Herbal Formulations, detailed toxicological studies are needed. The main objectives of this work is to determine the lethal dose (LD₅₀) effect of *Carica papaya* leaves and signs of toxicity wistar female rats in a short term period study (14 days). Lyophilized aqueous extract of *Carica papaya* leaves was administered orally to

behavioral and physical changes included weakness, loss of hair from the sides of ear and eyes, shallow breathing or feeling difficulty in breathing, increased respiratory rate and gradual decrease in body weight (Table 1) but no diarrhea was observed in all the treated groups. Mortality was recorded at the dose of 16g/kg of body weight in 4-5 days (Table 2) as half of the population expired on the 4th day and all animals expired on 5th day at this dose. Animals receiving doses of the extract of 4.0 and 8.0g/kg of body weight remained normal up to 14 days. The lethal dose (LD₅₀) was estimated to be 16g/kg of body weight orally in rats.

It shows that group 1 was normal as control group while the body weight of group 2 receiving 8g/kg of body weight decreased considerably compared to the control group. The body weight of group 3 receiving 16g/kg of body weight decreased sharply.

female Wistar rats. The lethal dose was found to be 16g/kg of body weight. This dose in rats caused mainly reduced appetite, gradually decreased body weight, uncomfortable feeling, abdominal pain, shallow breathing and itching and coughing. All Rats survived at a dose of 4g/kg and 8g/kg of body weight. Histopathological studies show acute inflammation and fibrinous exudates in lung parenchyma and mildly dilated sinusoids, prominent kupffer cells along with cellular swelling at a dose of 16g/kg of body weight. It should high lethal dose and cause mortality. However the low dose of aqueous extract of *Carica papaya* leaves is apparently not harmful and therefore may be used in phase 1 clinical trials need to be done to show its safety in humans.

Table 2. Mortality was recorded on 4th and 5th day after extract administration at a dose of 16g/kg of body weight.

Doses of aqueous extract of CP leaves	Rats			Mortality observed	Physical Effects
	#	E/T	Sex		
Control (0g/kg)	4		0/4 Female	none	Healthy
GP 1(4kg/kg)	4	0/4	Female	none	Normal as control.
GP 2 (8kg/kg)	4	0/4	Female	none	Feeling Pain in body.
GP 3 (16kg/kg)	4	4/4	Female	On 4 th & 5 th day	Uncomfortable, weak, shallow breathing, Coughing, hair loss from the side of ear and eye, itching.

GP: Groups; #: number of rats; E: expired rats; T: treated rats.

HISTOPATHOLOGICAL STUDY

After the 14 days of treatment, all the sacrificed rats were necropsies. Significant effects were noticed in liver and lungs at a dose of 16g/kg of body weight. Hemorrhagic lesions were observed at a time of necropsy, for example, blood was present under the skin and swelling was prominent at the time of necropsy on lungs, liver, heart, kidneys, and stomach. These organs were dark reddish in color. Hematoxylin and Eosin (H&E) staining methods were used for histopathological studies. All the prepared slides were observed at 10X resolution under the microscope.

LIVER

Important histopathological changes occurred in the liver after 14 days of the administration of the aqueous extract of *Carica papaya* leaves as cellular swelling dilated sinusoids with prominent kupffer cells were observed at 16g/kg of body weight in all the rats (Fig 5 & 6) but such changes occurred in only one rat the dose of 8g/Kg of body weight (Fig. 7). No histopathological changes occurred at the dose of 4g/kg of body weight.

LUNGS

Acute inflammatory exudates and increased lymphocytes in the alveolar wall of lungs were observed only in one rat at the dose of 8.0g/kg bodyweight (Figs. 8 & 9) while all the other rats remained normal. Fibrinous exudates filled in the alveolar spaces with acute inflammation were observed in all the rats at 16g/kg of body weights. (Figure 10). No change occurred at 4g/kg of body weight.

HEART

No significant result was observed in heart section in all the treated rats.

KIDNEYS

No important histopathological change occurred in all the rats.

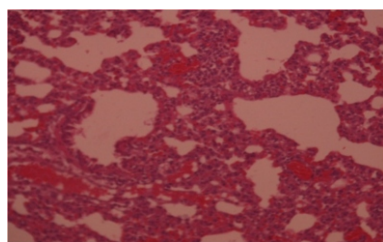
Photomicrograph of organs:

Figure 1. Lung Photomicrograph of control rat (H&E stain)

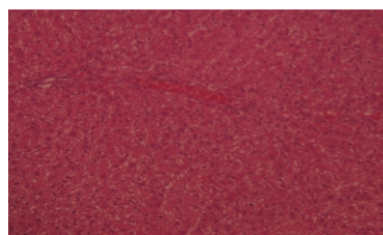


Figure 2: Liver Photomicrograph of control rat (H&E stain)

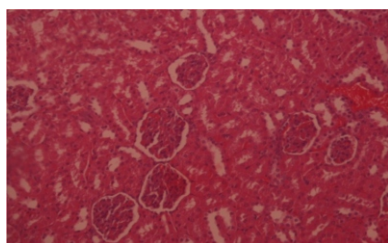


Figure 3: Kidneys Photomicrograph of control rat (H&E stain)

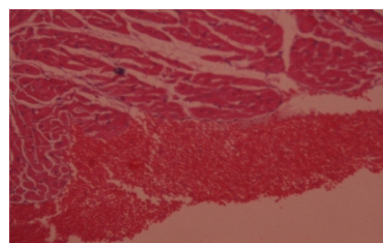


Figure 4: Heart Photomicrograph of control rat (H&E stain)

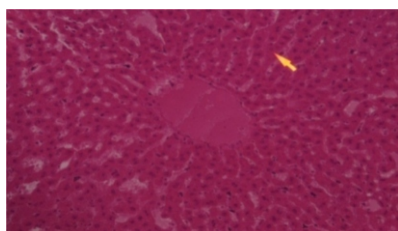


Figure 5: Liver photomicrograph of rat showing cellular swelling and congestion at 16g/kg of body weight (H&E stain)

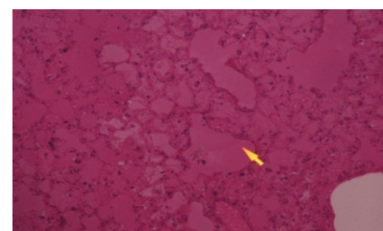


Figure 6: Liver photomicrograph of rat receiving 16g/kg of body weight showing cellular swelling dilated sinusoids. (H&E stain)

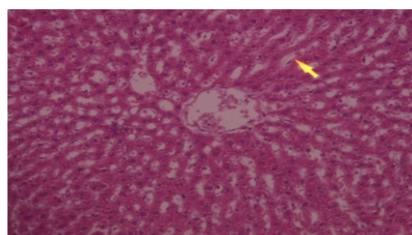


Figure 7: Liver photomicrograph of rat at 8g/kg of body weight showing sinusoids are mildly dilated with prominent kupffer cells (H&E stain)

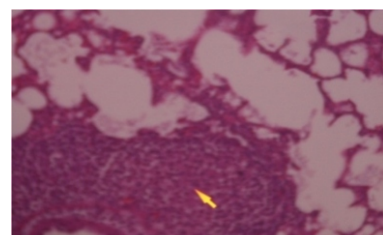


Figure 8: Photomicrograph of rat receiving 8g/kg of body weight showing increased lymphocytes in alveolar wall in lungs.(H&E stain)

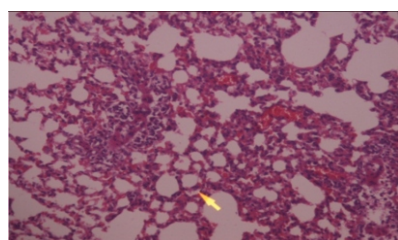


Figure 9: Lung photomicrograph of rat receiving 8g/kg of body weight showing fibrous material in the alveolar spaces. (H&E stain)

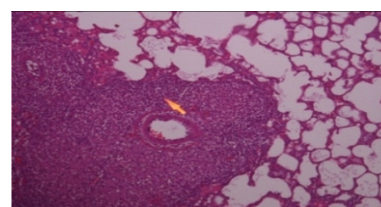


Figure 10: Lung photomicrograph of rat receiving 16g/kg of body weight of papaya extract showing acute inflammatory exudates in the alveoli at a place (H&E stain)

CONCLUSIONS

It is concluded that the oral administration of the aqueous extract of *Carica papaya* leaves at a dose of 16g/kg of body weight produces acute toxicity and mortality. Body weight of animals decreased gradually within 1-4 days of administration. Histopathology of the organs of animals at this dose shows that aqueous extract of *Carica papaya* affects mainly liver and lungs while no effects were observed on heart and kidneys. It shows lethal effect of *Carica papaya* aqueous extract of high dose (16g/kg of body weight). No mortality was recorded at a dose of 4-8g/kg of body weight. It can be concluded from this study that herbal medicines from *Carica papaya* leave extracts appear to be nontoxic as lethal dose is high (16g/kg of body weight) but phase I clinical trials on healthy volunteers are needed for safety testing of these medicines before their use for treatment of dengue fever patient

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