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RESEARCH ARTICLE
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A COMPARATIVE IN-VIVO STUDY TO EVALUATE CHRONIC BIOCHEMICAL EFFECTS OF SOME EDIBLE AND NON-EDIBLE PARTS OF *CARICA PAPAYA* PLANT REVEALED HEPATOTOXIC AND CARDIOTOXIC NATURE OF PAPAYA SEEDS

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Abstract

Objective: Prehistorically, the use of plants and plant-based natural products have often been considered for their medicinal properties in medicinal preparations. This is due to their low cost, accessibility, and minimal side effects. Globally, there is widespread assumption that such products are safe to use. Thus, awareness and reporting of adverse effects of plant-based products is very uncommon all over the world. Papaya plants, known for their nutritional and medicinal uses have often been consumed for numerous domestic and medical purpose. This study aims to investigate the impact of chronic dosing of the papaya's flesh, seeds, unripe fruit, and mature leaves on 3 main organs such as liver, kidneys and heart.

Method: The toxicity test was carried out on albino rabbits. Aqueous preparations of 4 parts of papaya i.e. ripe fruit, ripe seeds, unripe fruit and leaves were given for approximately 60 days and blood was withdrawn on day 61 for evaluation of biochemical effects.

Result: The study reveals that ripe and unripe papaya fruit and its leaves does not possess any adverse effect on the liver, kidney and heart during chronic daily use. However, ripe seeds elevates both liver and cardiac enzymes exceeding the normal acceptable limit.

Conclusion: Thus, our study suggests that among all parts; ripe fruit, unripe fruit and leaves are safe to be used for daily prolong use because of their ability to not affect the liver, kidney and cardiac enzymes.

Keywords: - Liver function test, serum creatinine, lactate dehydrogenase, papaya parts

INTRODUCTION

Consumption of plant-based products as a remedy predates recorded history[1]. Natural remedies plays a significant role in global health[2]. Plant based or derived remedies are often a popular choice in primary health care. They are considered to be low-cost, safe, and easy to access while having a wide variety of therapeutic and biological activities[3,4]. The tradition of consuming plant based remedies is very common all over the globe especially in various states of Pakistan, India, China, Sri Lanka, Thailand, Japan etc.[5,6]. Majority of the population prefers plant based remedies over conventional allopathic drugs to treat minor ailments and disorders[7]. The discovery of novel medicines is still heavily dependent on plant and herbal resources. Many of the existing conventional drugs which are currently used clinically have already been isolated and derived from plant sources[8].

Natural products offer a unique insight in pharmacological and therapeutic activities[9,10]. While numerous edible plant-based foods are commonly consumed every day, there are various parts of a plant known to provide medicinal potential to treat different health disorders[11,12,13]. *Carica papaya* Linn. Of the Caricaceae family is commonly known as pawpaw or papaya plant is widely cultivated in tropical countries of the world due to its health promoting benefits[11]. The pawpaw plant is a single stemmed large tree like plant approximately 16 – 33 ft. (5 – 10 meter) tall. Leaves are large and spiral shaped about 50 – 70 cm in diameter, palmately lobed with seven prominent lobes and are confined to the trunk. The flowers grow on the axils of the leaves which later mature into the fruit. The fruit is large with soft skin and brown to orange in color when fully riped[14].

Papaya plant is well recognized all over the world due to its unique nutritional value and biological activity. Different parts of this plant have been applied traditionally for diverse medicinal purposes such as fruit pulp for treating acidic urine and rheumatic disease, leaves for relieving asthma, flowers for treating high blood pressure and jaundice, fruit as immunity booster and as an antioxidant and anti-microbial[15].

Consumption and utilization of natural products as medicines is not uncommon all over the world. The perception of safety in natural remedies is often based on subjective evidence or tradition knowledge. The underreporting of adverse effects of natural products is a global issue. Papaya plant is famous all over the world due to its nutritional and medicinal uses. This study investigates the impact of chronic daily dosing of four parts of papaya plant that is fruit (ripe), seeds, unripe fruit and leaves on 3 main organs including liver, kidneys and heart in white albino rabbits.

MATERIALS AND METHODS

Study animals

This study was performed on rabbits. Albino rabbits of male gender weighing between 1.8 to 2.2 kg were purchased from local rabbit supplier and kept in the animal house of Pharmacology Department, University of Karachi for the purpose of conditioning and acclimatization for 11 to 15 days. The selected animals were kept under 12 hours light (08:45 a.m. to 08:45 p.m.) and dark (08:45 p.m. to 08:45 a.m.) cycle with maintained room temperature of 21 ± 4 ºC and humidity 54 to 64% with 24/7 availability of pure water and standard food. The guidelines of National Research Council (NRC) were followed for animal handling[16]. This study was performed after the approval from ASRB (Advanced Studies and Research Board), University of Karachi (ETHICAL APPROVAL: [BASR/No./02145/Pharm]).

Identification of plant

All parts of papaya which were used in this study were identified by Herbalist/Pharmacognocist Prof. Dr. Iqbal Azhar (Professor, Department of Pharmacognosy & Ex-Dean, Faculty of Pharmacy & Pharmaceutical Sciences)

Extract Preparation

1) Aqueous Extract of Ripe Fruit (AERF)

Ripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. All seeds were removed. AERF was prepared in 100mg/ml concentration for which 5 grams of ripe fruit was homogenized and blended with 50 milliliters of purified water using local mixer & grinder. The extract prepared was then stored in a glass bottle and denoted by "AERF"[17].

2) Aqueous Extract of Ripe Seed (AERS)

Ripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. Seeds were separated and washed with purified water and were dried at room temperature. The seeds were then manually crushed using domestic mortar & pestle. This mixture (5 gram) was then added to the grinder along with the purified water (50ml) for the purpose of blending and grinding to produce 100mg/ml concentration of AERS. The extract prepared was stored into a glass bottle and denoted by "AERS"[17].

3) Aqueous Extract of Unripe Fruit (AEURF)

Unripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. All seeds were removed. AEURF was prepared in 100mg/ml concentration for which 5 grams of unripe fruit was homogenized and blended with 50 milliliters of purified water using local mixer & grinder. The extract prepared was then stored in a glass bottle and denoted by "AEURF"[17]

4) Aqueous Extract of Mature Leaves (AEML)

Fresh mature leaves of papaya of size (8 to 9 inch) were plucked from papaya tree. Leaves were washed with tap water thoroughly and air dried. These air-dried leaves (50gm) were added to the local mixer/grinder along with purified water (50ml) where they were properly grinded and blended. AEML prepared was of 1000mg/ml concentration, which was stored in a glass bottle and denoted by "AEML"[18].

Grouping of study animals and their dosing protocol

5 groups of 10 animals were set for biochemical testing. Group I was control whereas the rest of the groups i.e. group II, III, IV and V were the treatment groups. Animal groups and their dosing is represented in Table "1"

The dosing was continued for 2 months (60 days) and all dosing was by oral route. The blood was withdrawn on day 61st for biochemical testing [19,20,21].

TABLE I

GROUP	DOSING MATERIAL	DOSE
Group I	Distilled water	2ml daily
Group II	AERF	250mg per kg daily
Group III	AERS	200mg per kg daily
Group IV	AEURF	250mg per kg daily
Group V	AEML	800mg per kg daily

Biochemical Investigation

For biochemical testing, blue capped siliconized glass tubes were used in which the blood samples were taken. After blood withdrawal, these tubes were centrifuged for 600 to 900 seconds at 3000 RPM to get the pure plasma which was then analyzed using Humalyzer- 3000 (Human-Germany) for the estimation of hepatic enzymes such as direct and total bilirubin, GGT, ALP, SGPT and SGOT, renal function including serum creatinine and cardiac enzymes including CPK and LDH. For estimation of these tests, standard kits were used which were purchased from the Human company[22,22,24,25,26,27,28,29,27,30,31].

Statistical Analysis

The data collected was expressed as Mean ± Std.Dev and analyzed using SPSS version-20. ANOVA (one-way) followed by post-hoc Tukey's test is used for evaluation of statistical significance. All P-values of less than 0.05 were considered significant. However P-values **p<0.05 *#!$, p<0.01 **##!!$$, p<0.001 ***###!!!$$$** represent level of significance i.e. significant, very significant and highly significant difference in comparison to control, AERF, AERS and AEURF respectively.

RESULTS

Table II, III, IV, V, VI, VII, VIII, IX and X represents the effect of different parts of papaya on total bilirubin, direct bilirubin, GGT, SGPT, ALP, SGOT, Serum Creatinine, CPK and LDH respectively. As shown in table II and III, All study groups i.e. AERF, AERS, AEURF and AEML showed no increasing or decreasing effect on total and direct bilirubin in comparison to control. There is no significant difference among the study groups.

According to table IV, AERF and AERS significantly increase serum GGT whereas AEURF and AEML showed no increasing or decreasing effect on serum GGT in comparison to control. In comparison to AERF, AERS significantly increased the serum GGT whereas the other two treatment groups i.e. AEURF and AEML significantly decreased the serum GGT. In comparison to AERS, both AEURF and AEML significantly decreased the serum GGT. There was no significant difference in serum GGT values among AEURF and AEML groups.

According to the results represented in table V, AERF and AERS significantly increase serum SGPT whereas AEURF and AEML showed no increasing or decreasing effect on serum SGPT in comparison to control. In comparison to AERF, AERS significantly increased the serum SGPT whereas the other two treatment groups i.e. AEURF and AEML significantly decreased the serum SGPT. In comparison to AERS, both AEURF and AEML significantly decreased the serum SGPT. There was no significant difference in serum SGPT values among AEURF and AEML groups.

According to the results represented in table VI, all treatment groups i.e. AERF, AEURF and AEML except AERS, significantly decreased serum ALP levels in comparison to control group and AERS group. Both AEURF and AEML group significantly decreased serum ALP in comparison to AERF group. However AEURF also significantly decreased serum ALP in comparison to AEML group as well.

According to the results represented in table VII, all treatment groups i.e. AERF, AEURF and AEML showed similar effects on serum SGOT as that of control group except AERS group which significantly increased the serum SGOT. All treatment groups i.e. AERF, AEURF and AEML significantly decreased serum SGOT in comparison to AERS. There was no significant difference in serum SGOT values among AERF, AEURF and AEML treatment groups.

According to the results represented in table VIII, AERF and AERS significantly raised serum creatinine whereas the other two groups i.e. AEURF and AEML showed no significant effect on

serum creatinine in comparison to control. In comparison to AERF group, AERS slightly raised whereas AEURF and AEML significantly decreased the serum creatinine levels. In comparison to AERS, both AEURF and AEML significantly decreased the serum creatinine levels. There was no significant difference in serum creatinine levels among AEURF and AEML groups.

According to the results represented in table IX, all treatment groups i.e. AERF, AEURF and AEML showed similar effects on serum CPK as that of control group except AERS group which significantly increased the serum CPK. In comparison to AERF group, AERS significantly raised whereas AEURF and AEML significantly decreased the serum CPK levels. In comparison to AERS, both AEURF and AEML significantly decreased the serum CPK levels. There was no significant difference in serum CPK levels among AEURF and AEML groups.

According to the results represented in table X, in comparison to control group, AERS significantly increased serum LDH whereas AEURF and AEML significantly decreased LDH. However AERF showed comparable effects as that of control. In comparison to AERF, AERS significantly increased serum LDH whereas AEURF and AEML significantly decreased LDH. In comparison to AERS both AEURF and AEML significantly decreased LDH. In comparison AEURF, AEML significantly raised LDH levels.

Table II Chronic effects of different parts of papaya on total bilirubin

GROUPS	Total bilirubin (mg/dL) (MEAN±S.D)
Control	0.05±0.02
AERF	0.08±0.04
AERS	0.05±0.02
AEURF	0.05±0.02
AEML	0.06±0.03

Table III Chronic effects of different parts of papaya on direct bilirubin

GROUPS	Direct bilirubin (mg/dL) (MEAN±S.D)
Control	0.03±0.01
AERF	0.02±0.01
AERS	0.04±0.02
AEURF	0.04±0.02
AEML	0.04±0.02

Table IV Chronic effects of different parts of papaya on gamma GT

GROUPS	Gamma GT (U/L) (MEAN±S.D)
Control	4.1±0.88
AERF	9.2±3.01***
AERS	12.8±1.81$^{***, \#\#}$
AEURF	3.9±2.13$^{\#\#\#, !!!}$
AEML	5.1±3.28$^{\#\#, !!!}$

Table V Chronic effects of different parts of papaya on SGPT

GROUPS	SGPT (U/L) (MEAN±S.D)
Control	46.9±7.4
AERF	60.8±5.94***
AERS	162.4±6.4$^{***, \#\#\#}$
AEURF	45±5.06$^{\#\#\#, !!!}$
AEML	44.7±3.02$^{\#\#\#, !!!}$

Table VI Chronic effects of different parts of papaya on ALP

GROUPS	ALP (U/L) (MEAN±S.D)
Control	43±6.88
AERF	27.9±2.38***
AERS	50.9±3.9###
AEURF	15.9±2.69***, ##, !!!
AEML	30.1±13.34**, !!!, $$$

Table VII Chronic effects of different parts of papaya on SGOT

GROUPS	SGOT (U/L) (MEAN±S.D)
Control	36±2.45
AERF	31.5±4.01
AERS	51.3±16.16**, ###
AEURF	33.8±4.08!!!
AEML	40.2±5.27!

Table VIII Chronic effects of different parts of papaya on creatinine

GROUPS	Creatinine (mg/dL) (MEAN±S.D)
Control	0.45±0.09
AERF	0.93±0.05***
AERS	1.08±0.06***, #
AEURF	0.45±0.07###, !!!
AEML	0.39±0.08###, !!!

Table IX Chronic effects of different parts of papaya on CPK

GROUPS	CPK (U/L) (MEAN±S.D)
Control	1146.1±105.12
AERF	752.4±50***
AERS	1604.2±73.61***, ###
AEURF	625.1±42.74***, ##, !!!
AEML	567.7±53.9***, ###, !!!

Table X Chronic effects of different parts of papaya on LDH

GROUPS	LDH (U/L) (MEAN±S.D)
Control	346.7±42.3
AERF	375.2±31.68
AERS	558.6±26.9***, ###
AEURF	240.4±17.11***, ###, !!!
AEML	307.6±23.99*, ###, !!!, $$$

DISCUSSION

World Health Organization in 2005 states that safety & efficacy evaluation of herbal remedies is worrisome and needs critical scientific methodologies and research. Safety is considered to be an essential aspect of any drug which is expected to cause no unwanted and harmful effects under the labelled use. The literature available on toxicity, adverse effects and safety of natural therapies is very confined and required more detailed screening which will aid in identifying the safety profile of medicinally active compounds in a plant [32,33,34,35].

To determine the safety of study parts of papaya plant after chronic daily dosing, biochemical tests were carried out to check their effects on hepatic, renal and cardiac enzymes. Effect on liver was evaluated by serum LFTs. Chronic dosing of all study parts i.e. AERF, AERS, AEURF and AEML have no effect on total and direct bilirubin. Bilirubin is produced from the heme part of the Hgb (hemoglobin) and is produced upon erythrocytes damage. High levels (the condition is known as hyperbilirubinemia) is a hallmark of jaundice. GGT (Gamma-glutamyl transferase) is an effective screening test for evaluating hepatic diseases. AEURF and AEML both didn't effect GGT levels but

AERF and AERS slightly raised GGT levels but not above the normal reported range. Serum glutamic pyruvate transaminase (SGPT) is a hepatic enzyme which is raised in various hepatic disease, inflammatory and infectious states. Serum levels of SGPT are widely used to diagnose hepatic diseases and for monitory of disease progression and effectiveness of treatment. Chronic dosing of AERF, AEURF and AEML showed no significant effects on SGPT whereas AERS significantly raised SGPT even above the normal acceptable range for which clinical correlation is required in future. ALP (Alkaline phosphatase) is a common screening test for the evaluation of bone and liver diseases. Chronic dosing of AERF, AEURF and AEML significantly decreased ALP levels whereas AERS showed no marked effects on ALP. SGOT (Serum glutamic oxaloacetate transaminase) is widely used to diagnose hepatic diseases and for monitory of disease progression and effectiveness of treatment. In numerous liver inflammatory and disease states, very high levels of SGOT are present in the blood. Except AERS, all treatment groups i.e. AERF, AEURF and AEML showed no significant effect on SGOT. AERS markedly elevated SGOT levels even above the normal reported range that in future requires further critical investigation. Thus the results of LFTs suggests that all extracts including AERF, AEURF and AEML can be chronically used since they do not have any negative effects on liver. Seeds should be avoided for chronic used because of its ability to derange the LFTs and for which future further research is required to discover the exact reason of its hepatotoxicity[33].

Serum creatinine was checked to evaluate the chronic effects of different parts of papaya on kidneys. Chronic dosing of AEURF and AEML showed no effect on serum creatinine whereas chronic dosing of AERF and AERS slightly raised serum creatinine but within the normal reported range. Serum creatinine production depends upon the alterations in muscle mass and physical activity. Chronic dosing of all parts does not negatively affect the renal function and hence is suggestive of its renal friendly nature. In future these parts can be evaluated in conditions of nephrotoxicity[34].

Serum CPK and LDH levels were checked to evaluate the chronic effect of daily dosing of different parts of papaya on heart. From clinical point of view, CPK (Creatine phosphokinase) is considered to be an essential screening test. High levels are suggestive of myocardial infarction. AERF, AEURF and AEML significantly decreased CPK whereas AERS markedly elevated CPK even above the normal range that in future needs more critical evaluation. Lactate dehydrogenase (LDH) is an important screening test for evaluating any cardiac injury. LDH is over-expressed in various body tissues including blood cells and heart muscle. It is present in high amount after tissue damage, and is a marker of common diseases and injuries such as cardiac failure. AERF has a non-significant effect on LDH. AEURF and AEML markedly decreased LDH whereas AERS elevated LDH even above the normal reported range and needs more detailed investigation. Thus results of serum CPK and LDH suggests that all parts except ripe seeds can be used safely for chronic use and possesses no cardio-toxic effects.

CONCLUSION

In the light of above discuss findings, it is concluded that daily and prolong consumption of ripe fruit, unripe fruit and papaya leaves is safe and has no hepato-toxic, renal-toxic and cardio-toxic effects. However ripe seeds should be very cautiously used in hepatic compromised and cardiac compromised patients due to its potential to raise liver and cardiac enzymes. In future these parts can be further evaluated to be used in different drug and disease induced organ toxicity states.

CONFLICT OF INTEREST

There is no conflict of interest.

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