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Toxicity evaluations of Papaya seed extract administration in Albino Wistar rats.

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Abstract

Background: Carica papaya seeds are regarded as waste or by-product of the fruit since it is the inedible part of the plant. However, it holds a diversified range of phytochemicals that anticipate their therapeutic role in different types of ailments. The seed extract has been proven for the presence of multiple health benefiting contents and some toxic agents. Studies evaluating the toxicity of the Papaya seed extract are still scarce to support its safe use as a therapeutic agent. Therefore, the undertaken study is to explore the safe dose of the aqueous extract of Papaya seeds.

Methodology: This study is designed to examine the toxic potential of Papaya seed aqueous extract on Wistar rats for 42 days by oral administration at dose regimens of 100, 200 and 400 mg/kg body weight in the liver. The groups (n=6) allotted include control untreated and group I (100 mg/kg), group II (200 mg/kg) and group III (400 mg/kg). The liver toxicity was assessed using liver enzymes as toxicity markers. The morphology of liver cells was observed by histology.

Results: Our results showed no mortality or signs of toxicity. The body weights were observed to be declining with increasing doses. However, other biochemical parameters displayed no significant alterations in treated groups as compared to the controls.

Conclusion: This study supports the evidence that chosen doses of aqueous extract of papaya seeds are safe for normal liver functioning. This evidence endorses the use of the extracts of the seeds as a therapeutic intervention.

Keywords

Carica Papaya, Toxicity Evaluation, Papaya Seed Aqueous Extract.

Introduction

Carica papaya is a tropical fruit that belongs to the Caricaceae family, different species cultivated widely throughout the year and have proven medicinal uses[1]. The whole fruit tree packages a variety of health benefits, including its bark, leaves, flowers, latex, roots and seeds[2]. The papaya seed's aqueous extract yields the highest number of bioactive phytochemicals, which anticipates the therapeutic role of these seeds[3]. The list of phytochemicals present in the seeds includes flavonoids, alkaloids, tannins, saponins, vanillic acid, vitamin C and cardiac glycoside[4-5]. Other than that, there are traces of chymopapain[6], papain[7], carpasemine and benzyl isothiocyanate[8,9] present in the seeds.

A study suggests the protective effect of Papaya seeds against diabetes linked disorders[10]. A similar study also anticipates the antihyperglycemic and hypolipidemic role of papaya seed[10]. Another study suggests the effective antibacterial role of the methanol and water extract of seeds[11]. Ethanolic extract of Papaya seeds exhibit antimicrobial attribute and heals wound effectively[12]. The Benzyl Isothiocyanate present in the seeds cast inhibitory effect on Candida albicans by generating reactive oxygen species and disintegrating mitochondrial membrane[13]. Papaya seeds also contain active substances that are regarded as toxic, such as carpine and papain. Carpine is considered as nerve and pulse rate depressing agent, and papain can cause asthma and rhinitis[14]. However, in vitro study has investigated papaya seed's role in fighting against cancer and claims papain in seeds to be involved in the anticancerous role[15]. Isothiocyanate and lycopene contained in seeds anticipate the role of papaya seed against cancer and inflammation-related disorders[15]. Use of papaya seed modulations as a vermifugal agent and an abortifacient is already evident for decades[16].

There are scarce research studies that provide evidence regarding papaya seed's toxicity or approval of its safe use as an anticancer treatment. The study aims to acknowledge the toxic potential of different OD of crude papaya seeds aqueous extract on the liver and its potential to cause oxidative stress by assessing biochemical parameters and examining histological feature.

Methodology

Papaya Seed Extract

The seeds of Papaya fruits obtained from the local market were separated, washed, dried and ground into powder. The grounded powder was processed in boiling water and filtered with Whatman filter paper. The aqueous extract was dried at 40°C and diluted to the required concentration before use[13].

Animal Grouping

Twenty-four matched Wistar rats weighing 150-220 g were housed in a clean, dry wire racked cage at 27-30°C, with 12 h light and dark. Animals were divided into four equal groups, each having three rats (n=6). Group I, control untreated, Group II was induced 100 mg/kg dose, Group III was given 200 mg/kg body weight, and Group IV was treated with 400 mg/kg body weight orally for 42 days. Observation of body weights was continued on regular specified intervals throughout the study, after weighing rats were sacrificed. Their blood was drawn, and tissues were isolated for histological examination.

Statistical Analysis

Results were analyzed by computing a t-test using SPSS version 16.0 and displayed as mean ± standard deviation. A p-value <0.05 was considered significant.

Ethical Guidelines

Ethical guidelines were followed strictly in handling animals and laboratory purposes in line with the instructions mentioned in the Health Research Extension Act of 1985 and the ethical guidelines of International ERB.

Biochemical Assessment of Liver Toxicity

Liver toxicity was assessed by calculation of liver enzymes; Aspartate Transaminase (AST)[17], Alanine Aminotransferase (ALT)[18], Alkaline Phosphatase (ALP)[19] and Bilirubin (BIL)[20] levels by colorimetric methods using Randox Kits.

Plasma Antioxidant Assessment

Levels of Malonaldehyde (MDA) were determined using methods proposed by Okhawa[21] and antioxidants; Catalase[22], Superoxide Dismutases (SOD)[23] and Glutathione (GSH)[24] were determined by colorimetric methods using Randox Kits.

Histopathology

A piece of liver was excised and, after embedding in paraffin, prepared for light microscopy. A section of about 6 µm was obtained, and H and E (hematoxylin and eosin) stained. The stained specimens were histologically evaluated and pictured by a light microscope.

Results

Comparison of Initial and Final Body weights

This study's results depict a decrease in body weights of rats treated with papaya seed extract (Table 1). Group, I treated rats showed no significant gain or loss of weight when matched to control ($p>0.05$). Group II shows a considerable reduction in body weight-matched to control ($p<0.001$) and the group I ($p<0.05$). Group III shows 400 mg/kg body weight papaya seed treatment considerably reduced the bodyweight of animals when matched with control ($p<0.001$) and group III ($p<0.01$). There is no considerable difference in body weights between group III and II ($p>0.05$).

Table 1: Comparison of body weights in control and groups I, II and III

	Control	Group I_1	Group $II_{1,2}$	Group $III_{1,2,3}$
Initial Body Weights (g)	174±12.17	180.5±13.63	172±11.93	183±6.14
Final Body Weights (g)	205.16±4.49	192.6±13.99 ^{n}	164.16±8.68 $^{c, a}$	180.83±2.99 $^{c, n, b}$

Above data is presented as mean ± SD.
Numerical are used to show comparison in following order;
1= control; 2= Group I; 3= Group II
a= $p<0.05$, b= $p<0.01$, c= $p<0.001$, n= $p>0.05$ (Non-significant)

Table 2: Comparison of Serum Liver Enzymes and Bilirubin

	Control	Group I_1	Group $II_{1,2}$	Group $III_{1,2,3}$
ALT (U/l)	67.5±20.43	59.9±37.58^{n}	93.36±4.01^{a,n}	84.43±6.12^{a,n,n}
AST (U/l)	18.5±4.67	21.83±2.46^{n}	14.08±3.78^{n,b}	13.34±8.32^{n,n,n}
ALP (U/l)	41.98±18.72	44.16±44.2^{n}	46.92±34.3^{n,n}	60.72±59.98^{n,n,n}
BIL (U/l)	0.13±0.11	0.557±0.53^{n}	0.25±0.18^{n,n}	0.244±0.12^{n,n,n}

Above data is presented as mean ± SD.
Numerical are used to show comparison in following order;
1= control; 2= Group I; 3= Group II
a= $p<0.05$, b= $p<0.01$, n= $p>0.05$ (Non-significant)
ALP-Alkaline Phosphatase, ALT-Alanine Transamine, AST-Aspartate Transaminase, BIL-Bilirubin

Table 3: Comparison of hepatic concentration of Antioxidant Enzymes:

	Control	Group I_1	Group $II_{1,2}$	Group $III_{1,2,3}$
Catalase (µmol/g tissue)	20.13±2.28	18.01±2.70 ^{n}	21.75±2.90 $^{n, n}$	22.14±4.34 $^{n, n, n}$
SOD (Unit/g tissue)	1.76±1.09	2.18±1.14 ^{n}	1.70±0.59 $^{n, n}$	1.88±0.41 $^{n, n, n}$
GSH (Unit/g tissue)	0.80±0.48	0.84±0.49 ^{n}	0.95±0.57 $^{n, n}$	0.93±0.54 $^{n, n, n}$
MDA (Unit/g tissue)	0.88±0.72	0.90±0.67 ^{n}	0.73±0.51 $^{n, n}$	0.79±0.67 $^{n, n, n}$

Above data is presented as mean ± SD.
Numerical are used to show comparison in following order;
1= control; 2= Group I; 3= Group II
n= $p>0.05$ (Non-significant)
SOD-Superoxide Dismutases, GSH-Glutathione, MDA-Malonaldehyde

Alanine Transaminase

The papaya seed aqueous extract given to rats orally 100 mg/kg, 200 mg/kg and 400 mg/kg did not produce any significant increase in serum concentration of ALP ($p>0.05$) when matched to control (Table 2). The concentration of ALT was not significantly different in control-treated rats.

Aspartate Transaminase

Different doses of Papaya seed aqueous extract in rats produced no considerable increase in AST serum concentration ($p>0.05$). No considerable alteration in AST levels was observed with respect to the control group (Table 2).

Alkaline Phosphatase

Serum concentration of ALP in rats administered with 100 mg/kg, 200 mg/kg and 400 mg/kg did not exhibit dose-related incremental changes in ALP levels. (Table 2) The concentration of ALP was not significantly different as compared to control ($p>0.05$).

Bilirubin

The plasma concentration of bilirubin in control rats was not significantly different from the rats treated with different dosages. No evidence of significantly increased bilirubin in rats ($p>0.05$) was reported in Group I, II and III (Table 2).

Catalase

The level of catalase enzyme did not significantly reduce in rats treated with these (100 mg/kg, 200 mg/kg, 400 mg/kg) doses of Papaya seed extract ($p>0.05$). In comparison, catalase levels in control-treated rats and Papaya seed treated rats were not significantly different (Table 3).

SOD

Group I, II and III papaya seed treated rats did not show any significant reductions in the level of SOD ($p>0.05$). The results show that dose-related papaya seed treated rats had similar levels of SOD reported as control-treated comparatively (Table 3).

GSH

The GSH levels were not significantly increased in papaya seed extract treated groups (I, II and III) ($p>0.05$) instead increased non-significantly in 200 mg/kg and 400 mg/kg (Group II and III) treated groups (Table 3).

MDA

The levels of MDA were not significantly increased in 100 mg/kg, 200 mg/kg, and 400 mg/kg papaya seed extract treated groups ($p>0.05$) rather reduced non-significantly in 200 mg/kg, and 400 mg/kg treated groups (Table 3).

Histopathology

Histological alterations were evaluated and reported in Control, Group I (Dose 100 mg/kg), Group II (Dose 200 mg/kg) and Group III (Dose 400 mg/kg). No evidence of significant alteration in histology was found in the hepatic architecture of tissues in the control group (Figure 1), Group I, II and III (Figure 2-4), respectively. Very mild histopathological changes were observed in Group I (Figure 2) and II (Figure 3).

These minor alterations include inflammation around the portal region. No signs of the portal and periportal fibrosis or bile duct proliferation and intracellular pigment deposition were found. There were no signs of degeneration in hepatocytes. Our experimental results showed no evidence of toxicity found in rats treated with the selected doses of Papaya seed extract. Hepatic architecture remained intact.

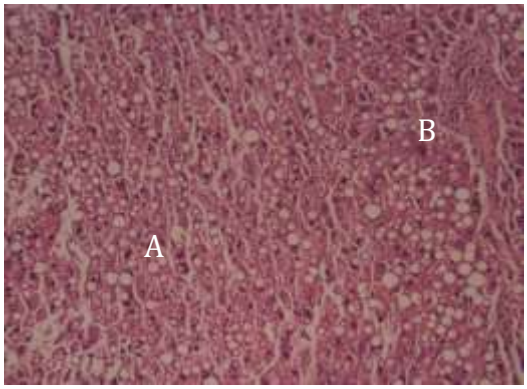

Figure 1: Histology of Control group of rats
A. Quiescent stellate cells
B. connective tissue around portal vein (20X)

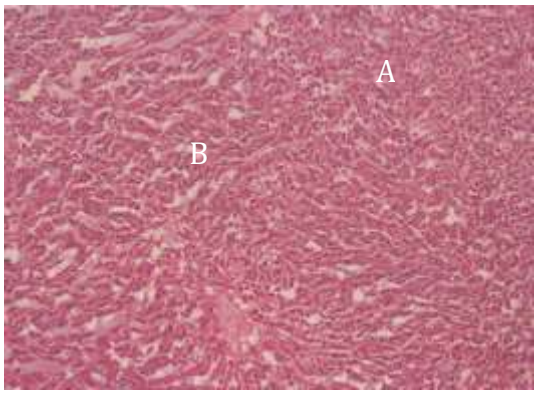

Figure 2: Histology of Group I (Dose 100 mg/kg)
A. Hepatocytes
B. Sinosoids (10X)

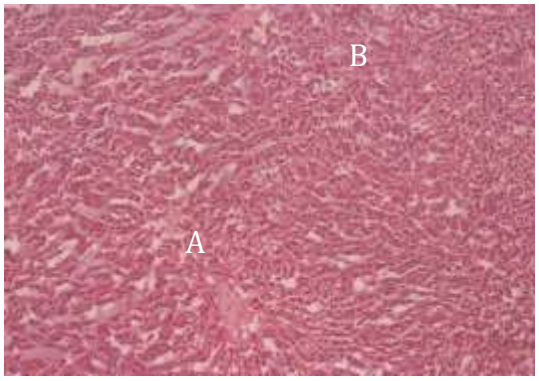

Figure 3: Histology of Group II (Dose 200 mg/kg)
A. Central vein
B. Hepatocytes (10X)

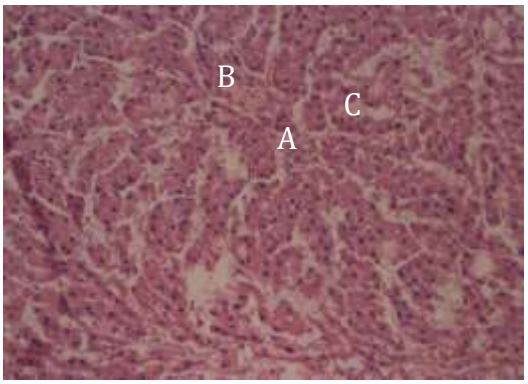

Figure 4: Histology of Group III (Dose 400 mg/kg)
A. Central vein
B. Portal area
C. Kuppfer cells (20X)

Discussion

The results of this study did not show any signs of mortality in treated rats. Administration of methanol extract of Papaya seed has increased liver enzymes indicating toxic effects of methanol extract in a previous study[25]. However, normal serum levels of ALP, ALT, AST and BIL in our study prove that hepatocytes function was not disturbed in rats who were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg aqueous extract of the Papaya seed (Table 2). This observation leads to the fact that using the aqueous seed extract of Papaya as herbal therapy with properly designed doses is non-toxic to liver health. The histological results of the rats' liver treated with these doses of papaya seed further confirmed this fact (Figure 1-4).

The results of our study are parallel with the previous study in which the LD50 for aqueous, methanol, ethyl-acetate, chloroform and n-hexane extracts of Papaya seeds were investigated. All the five seed extracts of Carica papaya were found to be non-toxic[3]. Antioxidants play a dominant role in killing oxidative stress, which is a leading cause of several ailments. Therefore, the substances which house plenty of antioxidants have been receiving exceptional insight into therapeutic studies. The papaya seeds are a reservoir of abundant antioxidants that anticipate their role in treatment against oxidative stress[26]. Seeds of Papaya are usually regarded as a waste or by-product even though these inedible parts of the plant are rich sources of antioxidant compounds[27].

Our study shows no signs of oxidative stress have been produced in rats treated with different doses of papaya seeds (Table 3). Another study reports that 250 and 500 mg dose of papaya seed has an increasing effect on quantitative hemoglobin concentration[28], which encourages the use of papaya seeds in treating deficiencies. The aqueous extract of papaya seeds proves a significant potential of decreasing total cholesterol and levels of Triglyceride and Low-density lipoprotein[29]. The phytochemicals of Saponins, flavonoids and tannins present in the seed extract contribute an active role in lipid metabolism[30]. In our study, the body weights were reduced significantly in rats treated with 400 mg/kg papaya seed extract compared to control rats (Table 1). This directs to the fact that the high dose of extract has prevented the rats from gaining weight. The decrease in body weights may indicate a sign of injury. An earlier study demonstrated that Benzyl isothiocyanate in the ethanol extract of seeds causes potential uterine tocolysis[31].

Conclusion

Papaya seeds are a reservoir of numerous beneficial contents that anticipate the seeds' extract's role in treating ailments. However, there is uncertainty regarding its safe oral dose because of a few toxic contents in the seeds. The study carried out fetches that the tested dosages of crude aqueous extract of Papaya seeds did not produce any liver toxicity or signs of oxidative stress in rats. This study provides evidence that the extract in proposed doses is safe and non-toxic; however, there is still a need to explore its effect on other organs and investigate the potential benefits.

Conflicts of Interest

None.

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