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Amelioration of ethanol-induced hepatotoxicity by aqueous seed extract of pawpaw (*Carica papaya* L.) in albino rats

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ABSTRACT: Plants being a reservoir of bioactive compounds with pharmaceutical potentials have been the source of raw materials for pharmaceutical industries. The present research investigated the phytochemical constituents and the ameliorating effects of aqueous seed extract of *Carica. papaya* on ethanol-induced hepatotoxicity in albino rats. Thirty rats weighing between 200g – 280g were randomly distributed into five groups of six rats each. Various doses of 5g/kg/12hr of 20% ethanol per day and (100, 200, 400) mg/kg/day oral doses of the extract were administered for 14 days, to groups C, D and E respectively. Group A took neither ethanol nor extract, while group B was given alcohol without the extract. The animals were sacrificed and biochemical parameters determined following standard protocols. Results showed that the significant increase ($p<0.05$) in serum activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and altered electrolytes concentration were reversed by the extract. The hepatic architecture was also preserved. It can be concluded that the extract has potential protective effects on the liver and kidney against ethanol-induced toxicity.

Keywords: Ethanol, Hepatotoxicity, *Carica papaya*, Phytochemicals.

Introduction

Alcohol and alcoholism are major worldwide health problems and alcoholic hepatotoxicity, a worldwide major cause of death since the ancient times and recorded as the fourth common cause of death (Sherlock, 1983; Rehm *et al*., 2009).

There has been a tremendous pressure on medical plants for their extensive utilization as sources of raw materials for the pharmaceuticals industries. Demands for medicinal plants are rapidly increasing not only in developing countries but also in developed ones. As far back as 2005, medicinal plants have been reported to have various effects on living systems; some of which are sedatives, analgesic, antipyretics, cardio-protective, anti-inflammatory, anti-oxidant, antispasmodic and Immunodulatory functions (Nunez-Selles, 2005). As such, herbs are becoming more relevant as synthetic drugs are known to be relatively unsafe and not good to the environment, while herbs are believed to be safe and secure for use leading to

herbal renaissance all over the globe (Roshan *et al*., 2014). All plant parts have medicinal values but the active components concentration varies from structure to structure (Lohidas *et al*., 2015). These medicinal values lie in some chemical substances which elicit physiological change (Srivastava and Singh, 2016)

The plant *Carica papaya* is a large tree-like herbaceous perennial plant with soft single stem growing up to 5-10m high, with sparcely arranged leaves at the top of the trunk, lower trunk is scarred where leaves and fruits are born, belonging to the family Caricaceae. (Sudhakar and Vidhia, 2014; Srivastava and Singh, 2016). This herbaceous plant is also known as paw paw, kapaya, lapaya, tapaya, papayao, papaya, papaia, papita, lechosa, fruta bomba, mamon, mamona, mamao and tree melon (Nordin 2010). It is one of the numerous gifts of nature to man for thousands of years where plant parts such as leaves, fruits, seeds, peel, roots and flowers are used as medicine (Lydia *et al*., 2016). It is a berry type fruit with parietal placentation, cultivated for its fruits, throughout the world's tropical and subtropical regions for its food and nutritional values (Agarwal *et al*., 2016; Radhakrishnan *et al*., 2017).

The specialized cells secrete latex involved in the defence system of the plants, which is a rich source of papain, chymopapain, caricain and glycolend peptidases in most of the tissues (Jaiswal *et al*., 2010; Dharmarathna *et al*., 2013). This plant has been used traditionally in cases of kidney failure, low sperm count, dental care, heart problems, natural memory enhancement, and remedy for fibroids in uterus (Uduak *et al*., 2013), hepatoprotection, and antioxidation in *in-vitro* and *in-vivo* studies (Adeneye et al.,2009). This study therefore was aimed at evaluating the ameliorating effect of aqueous seed extract of Pawpaw (*Carica papaya* L.) on ethanol-induced hepatotoxicity in albino rats

Materials and Methods

Plant Materials

Thirty (30) mature unripe *Carica papaya* fruits were collected from old barracks in Keffi local government area. The fruits were identified and authenticated at the Plant Science and Biotechnology Department of the Nasarawa State University, Keffi.

They were then cut into pieces, the seeds separated from the fruit pulp, washed gently but thoroughly with tap water, shade-dried at room temperature (28ºC) for two weeks. The dried *C. papaya* seeds were then pulverized into powder using electric blender. The powdered *C. papaya* seeds was then stored at room temperature in airtight containers for analysis.

Experimental Animals

Thirty (30) adult male albino rats weighing between 200g - 280g were bought from the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau state and transferred to Nasarawa State University. They were allowed to acclimatize two weeks in the Animal House of the Department of Biochemistry and Molecular Biology with adequate supply of feed and water.

Extraction of the Phytochemicals

Bioactive compounds were extracted by soaking 100 g of the powdered seed in 500 ml of distilled water (that is, ratio 1:5; weight to volume) heated to boiling point and maintained for 30 minutes. The extract was filtered using filter paper and then concentrated by heating at on the water bath at 40ºC to obtain a sweet smelling chocolate-colored sticky concentrate which was stored in an airtight container for analysis.

Preliminary Phytochemical Screening

The phytochemical component of the seed was determined using the methods described by Harborne, (1973), and Trease and Evans, (1989).

Administration of materials to the animals

After two weeks of acclimatization, the male albino rats were randomly divided into five (5) groups (A-E) with six (6) rats in each group.

Group A served as untreated control and were administered only feed and water.
Group B served as negative control and took feed, water and 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours.
Group C was administered with feed, water, 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours and 100g/kg/day of the extract
Group D was administered with feed, water, 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours and 200g/kg/day of the extract
Group E was administered with feed, water, 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours and 400g/kg/day of the extract

The animals were weighed and sacrificed using the standard ethically accepted protocol (Bamidele *et al.*, 2018), after 8-9 hours fasting. The blood sample of each rat was collected into an EDTA tube for liver and renal function tests. The livers of the rats were carefully excised and trimmed of extraneous tissues using sterile blades. They were immediately washed in physiologic saline solution and preserved in 10% neutral buffered formal saline and allowed to fix for 72 hours.

Biochemical Assays

Serum biochemical markers of hepatic and renal injury such as creatinine (CRE), urea (UR), Carbonate ion (HCO^-_3), sodium ion (Na^+), chloride ion (Cl^-), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (Alb), and Bilirubin (Bil) were estimated using Biolabo commercial kits (Biolabo S.A., Paris, France) according to the manufacturer's manual and URIT 8021Automated analyser (URIT Medical Electronic Group Co., Ltd).

Histopathological study

This was done according to the method of Bancroft *et al*, (2013) as described below.

Tissue Processing

The fixed tissues were dehydrated in ascending grades of isopropyl alcohol by immersing in 50%, 70%, 80%, 90%, 95%, and 100% for 1 hour each. The dehydrated tissues were cleared in two changes of xylene, 1 hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with rotary microtome at 5-micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides that had been previously smeared with equal parts of egg albumin and glycerol. The sections were then dried in an incubator at 60°C and after 5 min the sections were allowed to cool.

Tissue Staining (Haematoxylin and Eosin Staining)

The sections were deparaffinised by immersing in two changes of xylene for 10 mins each in horizontal staining jar. The deparaffinised sections were hydrated in descending grades of isopropyl alcohol (IPA) for 2 mins each and taken to water, after which it was stained in Ehrlich's hematoxylin for 10 min in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (1% HCl in 70% alcohol). The sections were then placed in running tap water for 10 min for blueing (slow alkalization). The sections were counter stained in 1% aqueous eosin (1 gm in 100 ml tap water) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was ensured by placing the sections in the incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mutant having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip). The tissue morphology was observed with low power objective under light microscope. The cell injury and other aspects were observed under high power dry objective (Bancroft *et al*, 2013).

Statistical Analysis

Statistical analysis was performed by the use of SPSS version l8 (Statistical Package for the Social Sciences). The differences between the groups were tested for significance by one-way ANOVA test. Data were expressed as the mean ± SD. P-values < 0.05 were considered statistically significant.

Results and Discussion

The results of the phytochemical screening of the powdered *C. papaya* seed showed the presence of saponins, alkaloids, tannins, flavonoids and steroids while tannins, phenols, glycosides, balsams, flavonoids, terpenoids and resins were found to be absent. It is made up of approximately ninety percent (90%) alkaloid (see Table 1).

Table 1: Qualitative and quantitative phytochemical composition of *Carica papaya* seed.

Phyto-constituents	Qualitative	Quantitative(%)
Tannins	+	0.578
Saponins	+	43.56
Flavanoids	+	0.184
Alkaloids	+	89.89
Phenols	–	
Glycosides	–	
Steroids	+	
Balsams	–	
Resins	–	
Terpenoids	–	

Although there were weight changes, neither the administration of the alcohol nor the treatment with the aqueous seed extract of *C. papaya* resulted in significant weight change of the animals.

Table 2: Effects of aqueous seed extract of *C. papaya* on the weight of the experimental albino rats

Group	**Before treatment**	**After treatment**
A (Control)	277.85±38.79	280.05±39.68
B (10g/kg/day of 20% ethanol)	268.10±73.85	243.90±58.82
C (10g/kg/day of 20% ethanol + 100mg)	271.68±54.43	227.60±75.81
D (10g/kg/day of 20% ethanol + 200mg)	211.00±63.66	186.90±51.79
E (10g/kg/day of 20% ethanol + 400mg)	231.43±89.05	209.00±78.51

Results are presented as mean ± standard deviation, (n=6).

The administration of 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours significantly ($p<0.05$) elevated the activities of AST, ALT and ALP and the concentrations of bilirubin (Group B), in comparison to the untreated group (Group A). The administration of the extract resulted in the reduction in the values of these parameters as seen in Table 3.

Table 3: Effect of aqueous seed extract of *C. papaya* on ALP, ALT, AST, BIL and ALB in ethanol-induced albino rats.

G.ROUPS	ALP(IU/L)	ALT (IU/L)	AST (IU/L)	BIL(IU/L)	ALB (IU/L)
A (Control)	157.50±3.59[a]	59.15±7.66[a]	26.88±3.01 [a]	3.53±1.50 [a]	3.63±0.29 [a]
B (Eth only)	304.25±3.45 [b]	121.63±11.76 [b]	42.00±2.70 [b]	13.45±7.6 [b]	2.85±0.70 [b]
C (Eth+ 100mg)	275.50±9.25[c]	21.85±3.99[c]	28.25±9.53[a]	4.45±3.90 [d]	4.15±0.91 [a]
D (Eth+ 200mg)	125.00±7.19 [d]	35.90±2.32[d]	36.75±6.77[c]	6.03±2.19 [d]	2.93±1.84
E (Eth+ 400mg)	291.25±9.16[c]	35.25±2.69 [d]	43.88±2.80 [c]	6.58±3.55 [d]	3.40±0.28 [a]

Results are presented as mean ± standard deviation, (n=6). Eth = Ethanol, = *C. papaya* extract. Values within the same column, with different superscripts are significantly different from one another

Whereas there was increase in the concentrations of creatinine and urea, the concentrations of carbonate ion, sodium ion and chloride ion were decreased in the alcohol-treated group when compared to the Control (untreated group). These values were however altered toward the normal with the administration of the extract in groups C, D and E (see Table 4).

Table 4: Effect of aqueous seed extract of *C. papaya* on kidney function in ethanol-treated albino rats

GROUPS	HCO_3 (mmol/l)	UREA (mg/dl)	CREATININE (µmol/l)	Na^+ (µmol/l)	Cl^- (µmol/l)
A (Control)	12.83±1.37 [a]	3.98±2.67[a]	40.80±4.08 [a]	87.44±3.48[a]	72.76±7.32 [a]
B (Eth only)	9.68±1.03 [b]	19.55±2.59[b]	63.63±4.61 [b]	43.66±4.66 [b]	68.55±1.95 [a]
C (Eth+ 100mg)	11.98±2.47[a]	5.47±0.82 [a]	51.85±3.36 [c]	87.33±2.45[a]	78.69±2.29 [b]
D (Eth+ 200mg)	10.15±6.40 [a]	14.48±1.41 [c]	43.78±2.37 [a]	52.30±2.08 [c]	80.80±2.21 [b]
E (Eth+ 400mg)	10.70±6.65 [a]	8.90±2.67 [d]	40.23±5.70 [a]	108.02±6.25[d]	72.28±3.58 [a]

Results are presented as mean ± standard deviation, (n=6). Eth = Ethanol, = *C. papaya* seed extract. Values within the same column, with different superscripts are significantly different from one another

In the present investigation, preliminary phytochemical screening of the *C. papaya* seed revealed alkaloids as the major bioactive component with little amounts of tannins, saponins and flavonoids. Alkaloids have been shown to have nephroprotective activity via its antioxidants and /or free radical scavenging activity (Miller and Rice- Evans, 1997; Adeneye and Benebo, 2008). It can therefore be adduced that the protective activity of the aqueous seed extract of *C. papaya* could be as a result of mainly the alkaloid content in synergy with the other minor bioactive components of the extract.

There was a marked increase in the weights of the animals in the 'Control' during the course of the study. This is attributable to adequate feeding. On the contrary, the administration of 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours led to tremendous loss of weight as seen all the animals in the test groups (untreated – B and treated – C to E). This loss of weight can be said to be as a result of excessive alcohol intake, which caused loss of appetite and consequently inadequate nutrient intake. However, these weight losses were not reversed by the intake of the extract. According to Da-Silva *et al*., (2000), ethanol produces 7 calories of energy which are utilized by the body and also, acetate produced by ethanol during its metabolism is used to produce lipids. This amount of energy is not sufficient for the maintenance of the animals. Therefore, there could be the depletion of the glycogen store and possibly the fat stores resulting in the observed weight loss. In addition, it was observed that the experimental animals during treatment, experienced watery stooling, bulging stomach, emaciation, weight loss and frequent urination.

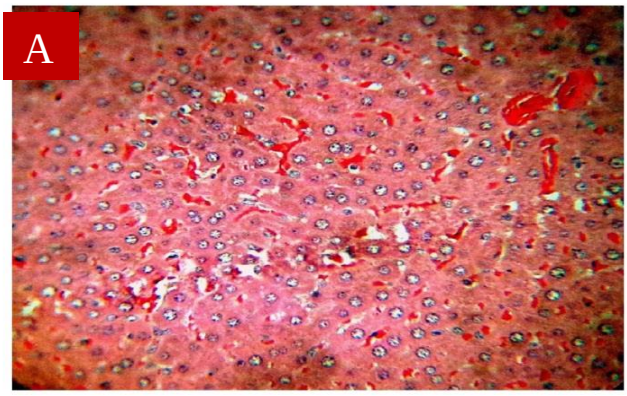

Plate A: Hepatic tissue shows a well preserved hepatic lobular architecture. Hepatocytes are generally seen to exhibit reactive nuclei and there is significant congestion of hepatic sinusoids with red blood cells. Islands of haemorrhage can also be seen. *H&E Stain*

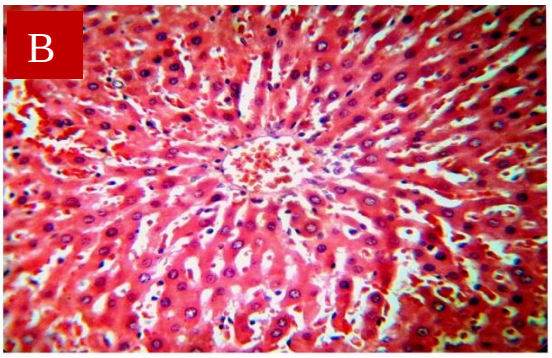

Plate B: Hepatic tissue showing a poorly preserved hepatic lobular architecture. Hepatocytes generally exhibit reactive nuclei with prominent nucleoli. The sinusoids are significantly dilated and congested. There is significant infiltration of tissue by chronic inflammatory cells in clusters. *H&E Stain*

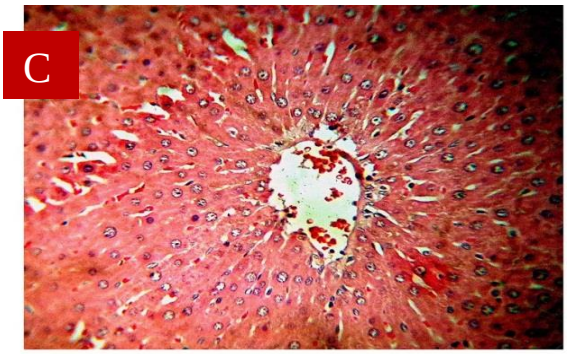

Plate C: Hepatic tissue showing moderately preserved hepatic lobular architecture. Hepatocytes generally exhibit reactive nuclei with prominent nucleoli and there is significant distention of hepatic sinusoids and some haemorrhage. Several karyopyknotic hepatocytes are also seen. *H&E Stain*

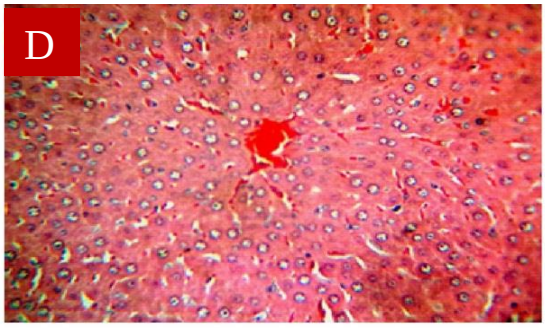

Plate D: Hepatic tissue showing moderately preserved hepatic lobular architecture. Hepatocyte anisokaryosis and reactive nuclei with prominent nucleoli are seen. There is mild distention and congestion of hepatic sinusoids as well as some haemorrhage. *H&E Stain*

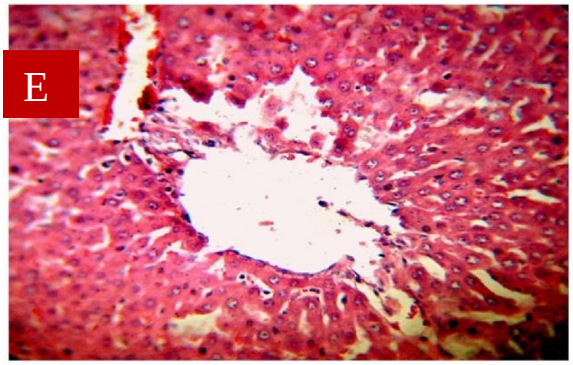

Plate E: Hepatic tissue showing well preserved hepatic lobular architecture. Hepatocytes generally exhibit reactive nuclei with prominent nucleoli. There is mild infiltration of tissue by chronic inflammatory cells. *H&E Stain*

Figure 1: Histological micrograph showing the effect of aqueous seed extract of Pawpaw (*C. papaya* L.) on ethanol treated Rats

The reduced appetite, watery stooling and bulging stomach as was observed in the animals during treatment could be attributed to the fact that alcohol (ethanol) causes intestinal injury that leads to impaired metabolism. Also, alcohol causes liver cirrhosis which is a disease associated with liver inflammation (Liber, 2004), while the emaciation and frequent urination as was observed with the animals could be as a result of ethanol-induced hemolysis (Tyulina *et al*., 2002), cytoplasmic changes in the RBCs, and this is accompanied with alteration in whole blood fluidity (Achagiotis *et al*., 1990; Maceira *et al*., 1997; Jesen *et al*., 2006). This could be responsible for the frequent urination that was also observed in the animals.

The administration of 10g/kg/day of 20% ethanol served as two equally divided gavages of 5g/kg/12 hours according to the result of this study, elicited both hepatic and nephron toxicity. This can be deduced form the significant increase in the serum activities of AST, ALT and ALP. The increased serum activities of AST, ALT and ALP have been reported in hepatotoxicity, caused by acetaminophen (Enemali and Udedi, 2018) and CCl_4 (Bamidele *et al.*, 2018). Other indices considered to arrive at this suggestion are the increase in the concentration of bilirubin, Urea and Creatinine and the decrease in the concentrations of albumin, Na^+, Cl^- and HCO^-_3. Igodaro and Omole, (2012) reported these signs as an indication of impaired liver and kidney functions (i.e. toxicity). Reduction in albumin concentration and a rise in the bilirubin concentration had been attributed to the impairment of the protein metabolizing activity of the liver and the inefficiency of the liver and the kidneys to conjugate and properly clear the metabolite from the blood stream (Enemali *et al.*, 2019). It was also observed that tissues of the animals administered with this dosage of alcohol was found to show poorly preserved hepatic lobular architecture, reactive nuclei with prominent nucleoli, dilated and congested sinusoids. They also had significant infiltration of tissue by chronic inflammatory cells in clusters which confirm the hepatotoxic effect of the administered dosage of the alcohol.

The simultaneous administration of aqueous seed extract with the alcohol intoxication, resulted in marked decrease in the activities of AST, ALT and ALP in the extract-treated groups in a dose-dependent order with the group administered with 100mg/kg/day (Group C), giving the highest rise. The lowering of the enzyme activities in the serum is a definite indication of hepatoprotective action of the drug (Zimmerman and Seeff, 1970; Igodaro and Omole, 2012). The concentrations of bilirubin, urea and creatinine were markedly decreased by the administration of the extract. While the concentrations of albumin, Na^+, Cl^- and HCO^-_3 were significantly increased ($p<0.05$) when compared to the group intoxicated without treating with the extract. The findings of this study is in consonance with Adeneye *et al*., (2009), where the administration of 100, 200 and 400mg/kg/day/oral route of ethanol leaf extract of *C. papaya* for 7 days to albino rats, treated with 20% CCl_4 (1.5ml/kg).

The co-administration of 100mg, 200mg and 400mg of aqueous seed extract of *C. papaya* with alcohol resulted in moderate preservation to proper preservation of the hepatic lobular architecture, in a dose-dependent manner. The ability of a drug/extract to ameliorate liver and/or kidney injurious effect or preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is an index of the protective effects.

Conclusion

It can be concluded from the results obtained from this study that treatment with aqueous seed extract of *C. papaya* decreases the ethanol-induced elevation of serum activities of ALP, AST and ALT and the concentrations of Bilirubin, Urea and Creatinine. It also increased the albumin concentration. There was also improvement of the preservation of the hepatic lobular architecture. The group administered with 100mg/kg/day of the seed extract returned the best overall result in the protection of the liver and kidneys. Suffix to say that increasing the concentration of the seed extract beyond 100mg/kg/day will be retrogressive instead of improving the results. Based on the results obtained here, aqueous seed extract of *C. papaya* has an important role in medicine as it helps in protecting the liver and kidney against ethanol-induced damage.

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