Hepatoprotective Effect Of Annona Muricata On Acetaminophen-induced Liver Toxicity

***[1]Usunobun U. and [2]Adegbegi A. J**.
[1]Department of Biochemistry, Faculty of Basic Medical Sciences,
Edo University Iyamho, Edo State, Nigeria
[2]Department of Science Laboratory Technology, Faculty of Food Technology,
Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria, muyithegreat@yahoo.com

***Corresponding author**
Email: usunobun.usunomena@edouniversity.edu.ng; usunsquare@yahoo.com
Tel.: +2348034174871

Abstract:
Background: *Annona muricata* commonly found in Africa possesses a variety of traditional medicinal uses in treatment of Kidney stones, liver problems, fever, nervousness, ulcers and wounds. This study investigated the hepatoprotective effect of *Annona muricata* leaf against acetaminophen-induced hepatic injury in male Wistar rats. **Materials and Methods:** Male Wistar rats divided into four groups were used. Group I served as control and received normal saline, group II received 300mg/kg *Annona muricata* aqueous leaf extract for seven consecutive days. Group III in addition to 1g/kg acetaminophen for seven days, received 300mg/kg *Annona muricata* extract for seven days while group IV received only 1g/kg acetaminophen for seven consecutive days. Following 24hrs of last treatment, the rats were sacrificed; serum collected, centrifuged and used for determination of aspartate aminotransferase(AST), alanine aminotransferase(ALT), alkaline phosphatase(ALP), total protein, albumin and bilirubin. **Results:** The results of this study indicated that rats treated with *Annona muricata* leaf showed significant decrease in ALT, AST, ALP and bilirubin levels which were all elevated in the acetaminophen alone treated rats ($p < 0.05$). Also a significant increase ($p \leq 0.05$) in total protein and albumin, was also observed in *Annona muricata* leaf treated groups compared to acetaminophen alone treated group. **Conclusion:** High dose of acetaminophen increased the markers of hepatotoxicity whereas co-administration with *Annona muricata* leaf decreased the markers of hepatotoxicity. This study provides evidence on the hepatoprotective effect of *Annona muricata* leaf against liver- induced damage which might be attributed to the presence of bioactive and antioxidative activity of *Annona muricata* leaf extract.

Key words: Acetaminophen, *Annona muricata*, Toxicity, Leaf-extract, Rat

Introduction

Drug-induced liver injury is a potential complication of nearly every prescribed medication and many fatal and near-fatal drug reactions occur each year (1). According to Pandit *et al.* (2), more than 900 drugs, toxins and herbs have been reported to cause liver injury, and drug-induced liver injury is responsible for 5 % of all hospital admissions and 50 % of all acute liver failures. Drug-induced liver injury is reported to be the most common reason for drug withdrawal from the market (1, 2).

Among the numerous drugs reputed to induce hepatotoxicity is acetaminophen . Acetaminophen also called paracetamol is a commonly used over-the counter antipyretic and analgesic which produces acute liver damage if overdoses are ingested (3). Acetaminophen toxicity accounts for most drug overdose (4-5). Prolonged daily use of acetaminophen increases risk of complications in upper gastrointestinal including stomach bleeding (6), and may cause damage to liver and kidney. The mechanism of acetaminophen toxicity involves the metabolic activation of the reactive toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, only a few percent of acetaminophen gets converted to NAPQI, which is further reduced by glutathione and subsequently excreted as glucuronidated and

sulfated (non-toxic) hydrophilic metabolites. In acetaminophen overdose, sulfate and glutathione supply get exhausted, thus more NAPQI is generated via CYP450 metabolism. This electrophilic intermediary then binds with available cellular proteins and initiate lipid peroxidation, mediated reactive oxygen species (ROS) and other free radical formation, thereby inducing oxidative stress and inflicting hepatic and renal tissue damage (7-8).

Annona muricata commonly called soursop has been reported to contain phytochemicals (such as flavonoids, saponins and alkaloid), mineral elements including calcium, sodium, potassium, magnesium iron etc. Also the *in vitro* antioxidant potential of *Annona muricata* leaves has also been reported (9-11). The leaves, bark and roots of *Annona muricata* posesses several properties including hypoglycemic, sedative, smooth muscle relaxant and antispasmodic (12). The aim of this study was therefore to show hepatoprotective effect of *Annona muricata* aqueous leaf extract against acetaminophen-induced liver toxicity.

Materials and Methods

Plant collection, Identification, Preparation and extraction

Fresh mature leaves of *Annona muricata* were collected from a tree in Upper Sakponba, Benin City, Edo state, Nigeria, identified and authenticated by a plant taxonomist at the Department of Basic Sciences, Benson Idahosa University, Benin City, Edo State. Aqueous extract was prepared by soaking 300g of the dry powdered leaves in 1.2 litres of distilled water at room temperature for two (2) days. At the end of the two (2) days, the soaked leaves was filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool, followed by concentration using a rotary evaporator set at 40^{o}C to one-tenth its original volume and then finally freeze dried. The dry residue (crude extract) was then stored at 4^{o}C until used for animal study.

Animal study

Male Wistar albino rats weighing between 190-200 g were obtained from the Anatomy Animal Unit facility of the University of Benin, Nigeria and housed in wooden cages. The rats maintained under controlled environmental conditions (temperature- 24 ± 2ºC; relative humidity- 50-70%; 12h light/dark cycle) were housed for one week for acclimatization. The rats were fed pelleted grower's mash (containing 18 % crude protein and 2600 Kcal/kg metabolizable energy, Guinea Feed, Nigeria PLC) and drinking water *ad libitum* until they were assigned to individual groups of five (5) rats each. The "Principles of laboratory animal care" (NIH) were followed as well as specific national laws where applicable.

Acetaminophen tablets, product of Emzor Pharmaceutical Company, Nigeria (before being turned into fine powder for easy dissolution) used in this study was obtained from Coka Pharmacy in Government Reservation Area (G.R.A), Benin City, Nigeria.

Male Wistar rats divided into four groups were used. Group I served as control and received normal saline, group II was given 300 mg/kg *Annona muricata* aqueous leaf extract for seven consecutive days. Group III in addition to 1 g/kg acetaminophen for seven days, also received 300 mg/kg *Annona muricata* extract for seven days while group IV received only 1 g/kg acetaminophen for seven consecutive days. Following 24hrs of last treatment, the rats were sacrificed; serum collected, centrifuged and used for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and bilirubin. All administration was orally and they were carried out in the morning between 8.30am and 10am. The acetaminophen was dissolved in physiological saline. The dosage of 300 mg/kg was used based on our previous non-toxic nature of *Annona muricata* leaf at 3200 mg/kg while a dose of 1g/kg of Acetaminophen was chosen based on previous report of Boyd and Bereczky (13) and Venkatesan *et al.* (14).

Biochemical Analysis

Serum aspartate aminotransaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total protein, albumin and bilirubin were determined using commercially available test kits, products of Randox laboratories, U.K.,

with the manufacturers instructions strictly adhered to.

Statistical analysis

Data were expressed as mean value ± standard deviation while differences between groups were determined by One-way ANOVA using SPSS. A probability level of less than 5% ($p < 0.05$) was considered significant.

Results

The result of the liver function enzymes (AST, ALT and ALP) and bilirubin shown in Table 1 and 2 showed significant increase ($p < 0.05$) in AST, ALT, ALP and Bilirubin levels in rats administered 1 g/kg acetaminophen only compared to control rats as well as compared to rats that received a combined dose of *Annona murica* (300 mg/kg) and acetaminophen (1 g/kg). Conversely, animals that received 1 g/kg acetaminophen only showed significant decrease in liver synthetic molecules (total protein and albumin) as shown in Table 2 compared to control animals and those that received combined treatment of *Annona muricata* (300 mg/kg) and acetaminophen (1g/kg).

Table 1: Effect of *Annona muricata* aqueous leaf extract on AST, ALT and ALP in Acetaminophen-induced liver toxicity.

Treatment groups	AST (U/l)	ALT (U/l)	ALP (U/l)
Control	24.00 ± 2.01^{a}	19.00 ± 2.11^{a}	31.00 ± 2.22^{a}
Annona muricata only (300mg/kg)	22.00 ± 2.30^{a}	17.00 ± 2.09^{a}	27.00 ± 2.05^{a}
Annona muricata (300mg/kg) + Acetaminophen (1g/kg)	47.00 ± 5.21^{b}	41.00 ± 4.84^{b}	64.00 ± 3.65^{b}
Acetaminophen (1g/kg)	101.00 ± 5.22^{c}	86.00 ± 5.13^{c}	103.00 ± 4.61^{c}

Values are expressed as Mean ± SD, (n=5), AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.
Mean values in each column having different superscript (a, b, c) are significantly different ($p \leq 0.05$)

Table 2: Effect of *Annona muricata* aqueous leaf extract on Total Bilirubin, Total Protein and Albumin in Acetaminophen-induced liver toxicity

Treatment groups	Total Bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Control	0.61±0.10[a]	7.96±0.09[a]	4.92±0.10[a]
Annona muricata only (300mg/kg)	0.68±0.13[a]	8.01±0.08[a]	4.89±0.07[a]
Annona muricata (300mg/kg) + Acetaminophen (1g/kg)	1.11±0.09[b]	6.16±0.10[b]	3.87±0.07[b]
Acetaminophen (1g/kg)	2.46±0.12[c]	5.01±0.06[c]	3.01±0.04[c]

Values are expressed as Mean ± SD, (n=5)
Mean values in each column having different superscript (a, b, c) are significantly different ($p \leq 0.05$)

Discussion

The liver, a vital organ plays several roles including detoxification, protein synthesis and production of biochemicals essential for the process of digestion (e.g. bile). Liver also plays a major role in metabolism, glycogen storage, red blood cell decomposition, hormone production (1). Liver damage may occur in the process of performing its essential role in metabolism and detoxification, especially as regards exogenous agents (1). Liver damage can be caused by xenobiotics, alcohol consumption, malnutrition, infection and medications (1, 15). Drug-related hepatotoxicity is the leading cause of acute liver failure, and liver problems are responsible for a significant number of liver transplantation and deaths worldwide. AST, ALT, ALP and bilirubin are sensitive markers employed in diagnosis of liver damage as they are normally located in cytoplasm and only liberated into circulation in response to hepatocellular damage (16-18). In the present study, acetaminophen treatment showed significant increase in the levels of AST, ALT, ALP and bilirubin and significant decrease in total protein and albumin levels indicating the hepatic toxicity of acetaminophen. This is in agreement with the results obtained in earlier investigations (19-20). The increased levels of the liver function enzymes (AST, ALT and ALP) are indications of cellular damage and loss of functional integrity of the liver cell membrane (21). The decrease in total protein may be as a result of alteration of protein and free amino acid metabolism and their synthesis in the liver. A low serum albumin indicates poor liver function and so albumin level reductions are generally suggestive of liver disease (22). An abnormal increase in the levels of bilirubin in serum indicates hepato-biliary disease and severe disturbance of hepatocellular function (23). However consecutive administration of *Annona muricata* leaf at a dose of 300 mg/kg greatly decreased hepatic damage marked with decrease in the serum activity of aminotransferases, bilirubin, ALP and also a significant increase in albumin and total protein levels. The ability of *Annona muricata* to modulate levels of these liver enzymes and synthetic molecules towards normal values reflects stabilization of plasma membrane, repair of acetaminophen-induced hepatic damage and improvement of the functional status and integrity of the liver cells.

Conclusion

The hepatoprotective effect of *Annona muricata* leaf may be due to the fact that it contains phyto-agents such as flavonoids, saponins and alkaloids which can scavenge free radicals, thus offering hepatoprotection. Attempts are currently being made in our laboratory to isolate and characterize the active principle to which the hepatoprotective activity can be attributed.

Conflicts of Interest

The authors declare no conflicts of interest.

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