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Assessment of Anti-inflammatory Potential Activity of Aqueous-Methanolic Extract of *Annona muricata* (soursop) Leaves on Liver Function Parameters in Formalin Induced Pain Model

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The present study was aimed to assess the anti-inflammatory activity of aqueous-methanolic extract of soursop (*Annona muricata*) leaves on liver function parameters in formalin-induced inflammation and oedema in male wistar rats. Inflammation and oxidative stress contribute significantly to liver damage. Soursop (*Annona muricata*) leaves have been traditionally used for their medicinal properties. Thirty male rats were divided into six groups: normal saline, formalin-induced, and two treatment groups receiving 100 and 300 mg/kg of soursop leaf extract while the other two groups receives Diclofenac and Aspirin (standard drugs) respectively. Liver function parameters Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Total protein were assessed. The result of the liver function test indicated that formalin induction into the right hind paw of the rats led to significant increase at ($p<0.001$) in serum levels of Aspartate aminotransferase (AST) 103.87 ± 1.66^{f}), Alanine aminotransferase (ALT) ($98.57 \pm 0.88^{e, f}$), and Alkaline phosphatase (ALP) (139.70 ± 0.91^{f}) in formalin –induced not treated rats compared to the normal control and treated group rats. These results therefore implied that formalin adversely affected liver function enzymes. Treatment with aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract at the doses of 100 mg/kg and 300 mg/kg body weight respectively ameliorated the ALT (93.50 ± 1.44^{e} at 100 mg/kg; 74.70 ± 2.66^{b} at 300 mg/kg) and AST (81.13 ± 1.13^{e} at 100 mg/kg; 71.63 ± 0.96^{b} at 300 mg/kg) levels associated with formalin-induced hepatotoxicity, with the effect being more pronounced at the higher dose of 300 mg/kg. Also, there was a highly significant reduction in serum AST (75.53 ± 0.23^{c}) and ALT (82.87 ± 2.17^{c}) concentrations ($p<0.001$) in diclofenac sodium treated rats when compared to the formalin-induced not treated rats. The highly significant dramastic reduction of ALP (68.90 ± 2.89^{b}) seen at 300 mg/kg of aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract treated rats showed that the plant extract has the potential to resuscitate the hepatocytes from damage, A relatively lower ALP level (85.30 ± 2.14^{c}) was also recorded for diclofenac sodium. This study demonstrated the anti-inflammatory activity of soursop leaf extract in protecting against formalin-induced liver damage, suggesting its potential as a therapeutic agent.

Key words: *Annona muricata*, anti-inflammatory, AST, ALT, ALP, formalin, inflammation.

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INTRODUCTION

The liver is described as a complex organ with interdependent metabolic, excretory, and defense functions. The detection of hepato-biliary abnormalities is improved by the use of several screening tests, which are also employed to differentiate the basis of clinically suspected disease and to determine the severity of liver damage (Eseoghene *et al*., 2022). For the initial assessment of liver disease, blood tests are commonly used, and levels of serum alanine and aspartate aminotransferases (ALT and AST), alkaline phosphatase, and others are measured (Eseoghene *et al*., 2022).

The pattern of abnormalities is generally used to distinguish hepatocellular from cholestatic liver disease and to determine whether the condition is acute or chronic, as well as whether cirrhosis or hepatic failure is present. Serum enzyme levels are known to fluctuate widely from normal to moderately abnormal, with high values rarely being recorded in the hundreds (Marghoob *et al*., 2013). A marked elevation of aminotransferases, when observed in the appropriate clinical context, is taken as an indication of acute cell necrosis caused by viral infections, drugs, toxins, alcohol, or ischemia (Marghoob *et al*., 2013)

Inflammation is recognized as a complex physiological response that is often accompanied by oedema, which is marked by the accumulation of excess fluid in tissues as a result of increased vascular permeability (Akande *et al*., 2021). Swelling and impaired function of affected organs can be caused by this condition. Inflammatory processes have been closely linked to liver dysfunction, given that the liver is centrally involved in detoxification and metabolic regulation (Akande *et al*., 2021; Nasiri *et al*., 2016). Oxidative stress, hepatocellular injury, and alterations in liver function parameters including elevated levels of liver enzymes and bilirubin can be induced by persistent inflammation (Nasiri *et al*., 2016). Therefore, inflammation must be effectively managed to ensure the maintenance of liver health.

Natural plant extracts, particularly those rich in bioactive compounds, have been increasingly recognized for their potential in the management of inflammation and the improvement of liver function (Cardia *et al*., 2022). *Annona muricata*, commonly referred to as soursop, has been traditionally used for its various therapeutic properties, including antioxidant, anti-inflammatory, and hepatoprotective effects (Okolie *et al.,* 2013). The efficacy of medicinal plants possessing antioxidant properties has been investigated in recent studies for their role in modulating liver function under inflammatory conditions, and promising results have been reported in the reduction of liver enzyme levels and the enhancement of overall liver health (Cardia *et al*., 2022).

Soursop (*Annona muricata*) is classified as a tropical plant species belonging to the family Annonaceae and is known for its numerous ethnomedicinal applications (Okolie *et al*., 2013). All parts of *Annona muricata* have been utilized in natural medicine throughout the tropics. It is regarded as a valuable source of natural antioxidants for the treatment of various diseases. Traditionally, the leaves have been used for the management of headaches, insomnia, cystitis, liver disorders, as well as for their antitumor and anti-inflammatory properties (Okolie *et al*., 2013). The health benefits associated with this plant have been attributed to its unique phytochemical composition (Okolie *et al*., 2013). In recent years, scientific interest has been stimulated by the biological activities of plants due to the recognized importance of plant-derived active compounds in both agricultural and medical applications (Cork-Tellez *et al*., 2019; Kumari *et al*., 2021).

Formalin is commonly used in experimental models to induce inflammation and to mimic pathological conditions such as liver damage (Akande *et al*., 2021). In male rats, significant increases in liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are observed following formalin exposure, indicating liver injury. In these models, the administration of medicinal plants with antioxidant properties has been associated with a reduction in these enzyme levels, suggesting that the liver is protected against formalin-induced toxicity and inflammation (Okolie *et al*., 2013).

Despite the documented medicinal benefits of *Annona muricata* leaves, particularly their antioxidant and anti-inflammatory properties, limited research has been conducted on the precise effects of aqueous-methanolic extracts on liver function during inflammation and oedema. Most studies have been focused on the broad-spectrum phytochemical benefits, while the specific mechanisms through which this extract influences liver biomarkers in formalin-induced models have been scarcely explored.

In addition, although synthetic drugs currently dominate the market, the potential toxicity associated with these drugs cannot be ruled out. Severe adverse effects have been reported with their prolonged use under chronic administration (Manjit *et al*., 2011), the most common of which include gastrointestinal bleeding and peptic ulcers (Manjit *et al*., 2011). Therefore, the development of new anti-inflammatory agents with minimal side effects is being prioritized. The search for safe and effective anti-inflammatory agents has been emphasized in scientific research within the field of herbal medicine. The leaves of soursop have been traditionally used in the southern regions of Brazil and Nigeria for the treatment of inflammatory and painful conditions.

The objective of this research is therefore to assess the anti-inflammatory activity of aqueous - methanolic extract of soursop (*Annona muricata*) leaves on liver function parameters in formalin-induced pain model in male wistar rats for scientific validation of the folklore claim of the plant.

MATERIALS AND METHODS

Plant collection and Extraction

Fresh leaves of soursop (*Annona muricata*) (1000 kg) were collected from Heipang, Barkin Ladi Local Government Area, Plateau State. The leaves were authenticated at Biology Unit, Science Department, Plateau State Polythenic, Barkin Ladi. The leaves of soursop (*Annona muricata*) were air dried at room temperature for 30 days. The dried leaves were later subjected to mechanical crushing using mortar and pestle and then to a blender to obtain a fine powdery homogenous mixture which was sieved using sieving machine. 1000 g of the powdered soursop was extracted with 700 ml of 99.8 % of analytical grade methanol and 300 ml of distilled water. The mixture was stirred for 72 hours and filtered using Whatman's No 1 filter paper. The filtrate was concentrated using water bath evaporator at 45^0C.

Drugs and Chemicals

Ibuprofen (Ranbaxy), formalin and other chemicals were of analytical grade.

Experimental Animals

Thirty (30) male wistar rats weighed (160–250 g body weight) were purchased at the College of Health Sciences, Animal House Unit of the College of Medicine, Benue State University, Makurdi, Nigeria. The rats were allowed to acclimatize to the laboratory for 14 days before the study. The animals were given vital feeds and tap water.

Experimental Design

Wistar albino rats weighing approximately (160–250 g body weight) were used. Animals were housed and used at least one week after their arrival. Five rats were housed per cage; animals were fed a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1°C with a 12 h light/dark cycle. The animals were used according to standards guidelines of the Committee on Care and Use of Experimental Animal Resources (Department of Health and Human Resources, DHIS, 1985).

Anti-inflammatory potentials of aqueous - methanolic extract of soursop (*Annona muricata*) leaves were measured in rat model of formalin-induced paw oedema. Albino rats fasted overnight were divided into 6 groups of five animals each, the dosage of the drugs administered to the different groups was as follows:

Group I - Normal Control (normal saline)
Group II - Formalin induced/ Test Control (0.1ml/kg b.wt.)
Group III - Formalin + lower dosage 100 mg/kg b.wt. of plant extract
Group IV - Formalin + higher dosage 300 mg/kg b.wt. of plant extract
Group V - Formalin + Standard drug (Diclofenac sodium, 10 mg/kg b.wt.)
Group VI - Formalin + Standard drug (Aspirin, 10 mg/kg b.wt.)

Induction of formalin in experimental animals

Thirty minutes pre oral treatment with extract/drug, following injection of formalin (0.1ml of 10% v/v) into the right hind paw of the tested rats. No injection of formalin into the normal control group animals. The paw thickness was measured before and after induction of inflammation by using vernier calliper. The increase in paw oedema was measured by vernier calliper according to method described by Akande *et al*. 2021; Taylor *et al*. (2000) and Joseph *et al.* (2005) with some modifications.

The difference in paw thickness after and before induction of inflammation was calculated and presented as mean increase in paw thickness (cm). The ability of aqueous - methanolic extract of soursop (*Annona muricata*) leaves and the standard drugs (ibuprofen and aspirin) used as anti-inflammatory drugs to suppress paw inflammation was expressed as a percentage of inhibition of paw oedema (Akande *et al*. 2021; Taylor *et al*., 2000 and Joseph *et al.*, 2005).

Collection and Preparation of Serum Sample for Liver Function Test

At the end of 3 days experimental period, rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture into plain serum tubes and allowed to stay for 1 hr. The clotted blood was centrifuged for 10 min at 3000 rpm. The serum was transferred into clean tubes and stored at 4°C until needed for use. Analysis of aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and protein concentration were carried out using methods in the recommended kits.

Statistical analysis

Data were presented as a mean ± standard error of mean of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Difference were statistically significant at $p<0.000$.

RESULTS

The anti-inflammatory activity of aqueous methanolic extract of soursop (*Annona muricata*) leaves on liver function parameters in formalin induced inflammation and oedema is presented in Table 1. There is a significant difference in the parameters in comparison with the normal and aqueous methanolic extract of soursop (*Annona muricata*) leaves treated groups.

Table 1: Anti-inflammatory Potential Activity of Aqueous - Methanolic Extract of Soursop (*Annona muricata)* Leaves on Liver Function Parameters in Formalin-Induced Pain Model

Groups (mg/kg)	ALT (IU/L) Mean ± SEM	AST (IU/L) Mean ± SEM	ALP (IU/L) Mean ± SEM	Total Protein (g/dL) Mean ± SEM
Control (Normal saline)	62.07 ± 1.92^{a}	55.20 ± 0.64^{a}	53.50 ± 2.60^{a}	76.87 ± 0.55^{a}
Induced/Formalin	98.57 ± 0.88^{e,f}	103.87 ± 1.66^{f}	139.70 ± 0.91^{f}	52.67 ± 1.29^{e}
I + *Annona muricata* (100 mg/kg)	93.50 ± 1.44^{e}	81.13 ± 1.13^{e}	123.75 ± 1.59^{e}	59.67 ± 0.83^{d}
I + *Annona muricata* (300 mg/kg)	74.70 ± 2.66^{b}	71.63 ± 0.96^{b}	68.90 ± 2.89^{b}	73.43 ± 0.84^{b}
I + Diclofenac sodium (10 mg/kg)	82.87 ± 2.17^{c}	75.53 ± 0.23^{c}	85.30 ± 2.14^{c}	71.37 ± 0.32^{b}
I +Aspirin (10 mg/kg)	89.65 ± 0.14^{c,d}	78.77 ± 0.23^{c,d}	108.50 ± 1.32^{d}	62.03 ± 0.94^{c}

Values are expressed as mean ± standard error of mean (n=5). Values with different superscript(s) in a column are significantly different ($p<0.001$)

Keywords:ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; T. Protein- Total Protein

DISCUSSION

Annona muricata is a medicinal plant that has high therapeutic efficacy index as an antimalarial, antipyretic, analgesic, anti-inflammatory and hypotensive properties. This study was carried out to investigate the anti-inflammatory potential activity of aqueous - methanolic extract of soursop (*Annona muricata*) leaves on the serum levels of ALP, AST, ALT and Total protein, in formalin-induced pain model in right hind paw of male wistar rats. Serum liver enzyme levels are considered as markers for monitoring the degree of chemically induced liver damage (Marghoob *et al*., 2013).

From the present study, significant decrease $p<0.001$ in serum concentration of total protein (52.67 ± 1.29^{e}) (Table 4.1) revealed increased proteinuria observed in the formalin-induced not treated rats compared with normal control (76.87 ± 0.55^{a}). Treatment with aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract at the doses of 100 mg/kg (59.67 ± 0.83^{d}) and 300 mg/kg body weight (73.43 ± 0.84^{b}) respectively protected the observed

alterations though 300 mg/kg was more effective than 100 mg/kg. Lower dosage of serum concentration of total protein (71.37 ± 0.32^{b}) was also recorded for diclofenac sodium.

The result of the liver function test presented in Table 1 indicates that formalin induction into the right hind paw of the rats led to significant increase at ($p<0.001$) in serum levels of aspartate aminotransferase (AST) 103.87 ± 1.66^{f}), alanine aminotransferase (ALT) ($98.57 \pm 0.88^{e, f}$), and alkaline phosphatase (ALP) (139.70 ± 0.91^{f}) in formalin –induced not treated rats compared to the normal control and treated group rats. These results therefore imply that formalin adversely affected liver function enzymes. The report of Nasiri (2016) indicates that rise in serum levels of AST, ALP but especially ALT is a valid indicator of liver damage. Liver enzymes ALT and AST are normally present in normal hepatocytes. These enzymes however, leak out into the circulation when hepatocytes or their cell membranes are damaged as reported by Charles (2012).

Treatment with aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract at 100 mg/kg and 300 mg/kg body weight respectively ameliorated the ALT (93.50 ± 1.44^{e} at 100 mg/kg; 74.70 ± 2.66^{b} at 300 mg/kg) and AST (81.13 ± 1.13^{e} at 100 mg/kg; 71.63 ± 0.96^{b} at 300 mg/kg) levels associated with formalin-induced hepatotoxicity, with the effect being more pronounced at the higher dose of 300 mg/kg. This result is in accordance with result reported by Eseoghene *et al*. (2022). Also, there was a highly significant reduction in serum AST (75.53 ± 0.23^{c}) and ALT (82.87 ± 2.17^{c}) concentrations ($p<0.001$) in diclofenac sodium treated rats when compared to the formalin-induced not treated rats.

Serum alkaline phosphatase (ALP) is a sensitive detector in diseases characterized by inflammation, regeneration, intrahepatic and extrahepatic bile obstruction (Marghoob *et al*., 2013). The highly significant dramastic reduction of ALP (68.90 ± 2.89^{b}) seen at 300 mg/kg dose aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract treated rats showed that the plant extract has the potential to resuscitate the hepatocytes from damage (Eseoghene *et al*., 2022). A relatively lower ALP level (85.30 ± 2.14^{c}) was also recorded for diclofenac sodium.

CONCLUSION

Results from the study support the fact that aqueous - methanolic extract of soursop (*Annona muricata*) leaves plant extract have excellent anti-inflammatory activity and compared favourably with the standard drug, diclofenac sodium in several instances.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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