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Full Length Research Article

Effects of Consumption of Methanol Extracts of *Ficus platyphylla* Del. Holl and *Ximenia americana* L. on Liver Function Indices in Albino Rats

*[1]S. Shaayau, [2]Y. Y. Salisu and [1]M. E. Sadiq
[1]Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto
[2]Department of Biochemistry, Al-Qalam University, Katsina
[* Corresponding author. Email address: elmafary@gmail.com]

ABSTRACT
Ficus platyphylla Del. Holl and *Ximenia americana* L. are medicinal plants used in the treatment of several diseases. This study was designed to investigate their effects on some markers of hepatotoxicity in albino rats. Twenty-four (24) adult Wistar rats of both sexes were divided into four (4) groups of six (6) rats each. Groups 1, 2, 3, and 4 were treated with distilled water, *F. platyphylla*, *X. americana,* and a mixture of *F. platyphylla* and *X. americana* extracts, respectively. LD_{50} showed that all three extracts are not toxic even at values as high as 5000 mg/kg body weight. A dose of 250 mg/kg body weight was administered orally daily for twenty-one (21) days, after which blood samples were collected in a clean container and centrifuged for 10 minutes at 3000 rpm. The serum obtained was used for biochemical analysis. Markers of hepatocellular injury specifically, ALT, AST, albumin, total bilirubin, and protein, were assayed accordingly. The activity of ALT showed no significant ($p>0.05$) difference in group 4 with the control. In AST, there was a significant difference ($p<0.05$) between group 4 and the control group. There was a significant difference ($p<0.05$) in total bilirubin across the groups when compared to the control, there is no significant difference in total protein between group 3 and the control group. For albumin, group 4 was not significantly ($p>0.05$) different from the control. These findings illustrated that aqueous extracts of *X. americana* and *F. platyphylla* are not hepatotoxic and may, therefore be considered safe for consumption as medicinal plants.
Keywords: *Ficus platyphylla, Ximenia americana, H*epatocellular injury, Medicinal plants.

INTRODUCTION
Medicinal plants have been utilized extensively as a hot spot for various bioactive compounds for treating several human diseases (Nostro *et al*., 2000). It has been reported that herbal medicine is regarded as a primary healthcare need by about 60% of the world's population and 80% of the population of developing countries (Ahmad Khan and Ahmad, 2019). This practice is prevalent in African countries because of inadequate modern healthcare facilities, cultural and traditional acceptance, socio-economic factors, poor government policies on healthcare delivery (Njan *et al*., 2019), and the efficacy of medicinal plants for treating certain diseases not treated with modern drugs (Saklani and Kothiyal, 2011). It is not surprising that many people are venturing into herbal medicine as can be seen in various television channels, radio stations, and social media handles. It was also reported that most Nigerian citizens today use medicinal plants and consult traditional medicine practitioners for their healthcare needs (Balogun, 2021). Despite not having scientific knowledge, traditional herbalists have successfully established treatments of several diseases using different medicinal plants. This study was designed to investigate the effects of two commonly used medicinal plants *Ficus platyphylla* and *Ximenia americana* on some markers of hepatotoxicity in albino rats.

Ficus platyphylla, is a deciduous plant that belongs to the family Moraceae. It is known in the Hausa language as "Gamji" and is widely distributed throughout the savannah region of West Africa (Chindo *et al*., 2003). As far back as 1989, Audu reported that *F. platyphylla* preparations have been used by Nigerian herbalists for the treatment of insomnia, epilepsy, psychosis, depression, pain, inflammation, and central nervous disorders for decades. The cold water extract, decoction or powder of the stem or root bark are usually taken orally, while the powder is often mixed with food and eaten, or placed in burning charcoal and inhaled. Another study reveals that the bark aqueous extracts of *F. platyphylla* are rich in phytochemicals and all doses of the extracts could possess a protective potency against various human disorders. The study also concluded that all doses of the extract are toxic-free and may be promising in the management of diseases as acclaimed by traditional herbalists (Ndatsu and Abdullahi, 2018).

Ximenia americana is also a very important medicinal plant that belongs to the family Olacaceae. It is indigenous to West African countries like Nigeria, especially in rural areas where the plant parts are prepared in different forms targeting different ailments in ethnomedicine (Ali *et al*., 2016). The extract of the plant was found to be effective in the treatment of parasitic diseases like malaria and trypanosomiasis infection in mice (Length, 2008). Leaves, barks, and roots are used in different African countries for treating toothaches, mumps, and conjunctivitis in frontal applications (Sumarah *et al*., 2008). A formulation from the plant is also shown to be promising in the treatment of oral infections (Almeida *et al*., 2019). The leaves are also used to treat infections in wounds and promote healing effects whilst its bark tea is used to combat hepatitis and malaria. (Da Silva Pantoja *et al*., 2018). The fruit of *X. americana* is blessed with a considerable amount of total polyphenol, vitamin C, and free radicals scavenging activity. The seed was also found to possess high polyphenols and antioxidant activity which makes it a potential therapeutic

agent (Jose *et al*., 2015). A combination of the aqueous leaf extracts of *X. americana* and *Pappea capensis* was found to confer cardiocurative activities in rats induced with myocardial infarction (Gaichu *et al*., 2023).

Despite the ubiquitous nature and effectiveness of medicinal plants, they can still possess some adverse effects. It is noteworthy that they can contain some potentially harmful substances, especially to the liver which is the organ tasked by nature to take care of the metabolism and detoxification of drugs and other xenobiotics (Alrashood *et al*., 2020). Apart from drugs and xenobiotics, the liver also plays major roles in diverse metabolic pathways, breakdown of red blood cells, and in the synthesis of proteins and hormones, as well as storage functions (Perry, 2012). Due to its diverse metabolic functions, the liver is susceptible to injuries from infections and metabolic insults which manifest as liver damage (Jiang *et al*., 2020). Hence, there is a need to investigate the effects of medicinal plants on liver injury and hepatic failure to ascertain their safety.

EXPERIMENTAL METHODOLOGY

Sample Collection and Preparation

The parts of the plants; *F. platyphylla* and *X. americana* were obtained from Wamakko town, in Wamakko Local Government Area of Sokoto State. The plants were identified by a Botanist at the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where specimens were deposited for reference, with Voucher numbers UDUH/ANS/0545 and UDUH/ANS/0548 respectively. The stem bark of both *F. platyphylla* and *X. americana* were air dried (under shade) for ten (10) days to a constant weight and subsequently ground into a fine powder suitable for analysis using mortar and pestle. Thirty (30) grams of each powder were macerated separately in 800 mL absolute methanol and left in an airtight aspirator container for 48 hours. The preparation was filtered using muslin cloth and Whatman's number one filter paper. The filtrates were concentrated with the aid of a rotary evaporator and drying cabinet at a temperature of 45°C. The extracts were weighed, labelled, and stored in an airtight container.

Experimental Animals

The sample size was calculated using the relation

$$n = \frac{N \times (t^2)}{N + t^2 - 1}$$

where:
n= is the sample size per group.
N= is the total sample size across all groups.
t = is the critical value from the t-distribution corresponding to the desired level of significance.

A total of twenty-four (24) Wister albino rats weighing between 110-150g were used for this study. The animals were purchased from Abraham farm, Kaduna and were transported to Usmanu Danfodiyo University Sokoto, overnight to avoid the scorching heat normally experienced during the daytime. The rats were housed in the animal house of the Department of Biochemistry, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto. The animals were kept in cages under normal environmental temperatures and were fed with plated feed growers (Vital feeds, UAC foods) and tap water *ad libitum*. The rats were allowed to acclimatize for two (2) weeks before the experiment commenced. All animals received humane care according to the Guideline of the Organization for Economic Cooperation and Development (OECD) as revised by Bayne (1996).

Acute Toxicity Studies (LD_{50})

LD_{50} was determined orally using the method of Lorke (1983) in rats. The study was carried out in two phases:
In the first phase, nine rats were randomized into three groups, each consisting of three rats and were given 10, 100, and 1000 mg/kg body weight of the extract orally. The rats were kept under the same conditions and observed for signs of toxicity which included but was not limited to paw-licking, stretching, respiratory distress, and mortality for the first 4 hours and then thereafter for 24 hours.

In the second phase of the study, three rats were placed into 3 groups of one rat each. The animals were orally administered 1600, 2900, and 5000 mg/kg of the extract, respectively based on the result obtained from the first phase. The rats were also observed for signs of toxicity and mortality for the first critical 4 hours and thereafter for 24 hours. The oral median lethal dose was calculated as the square root of the product of the highest non-lethal dose and the lowest lethal dose, that is, the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1). The unused extract of 250 mg/kg was used to test the effects on liver enzymes.

Experimental Design

The animals were weighed and grouped according to their body weights. The animals were grouped into 4 groups of six rats each and were labeled as follows:
Group 1: Control group fed with normal chow and water
Group 2: Treated with *F. platyphylla* extract (250 mg/kg body weight)
Group 3: Treated with *X. americana* extract (250 mg/kg body weight)
Group 4: Treated with mixture of *F. platyphylla* and *X. americana* extracts 1:1 weight by weight (250 mg/kg body weight).

For the treatment, a concentration of 50 mg/mL of the methanolic extracts of *F. platyphylla* and *X. americana* was prepared by dissolving 1g in 20 mL of distilled water. A dose of 250 mg/kg body weight was administered orally for 21 days. After which the animals were sacrificed and the blood samples were collected in a clean container, centrifuged for 10 minutes at 3000 rpm and the serum obtained was used for biochemical analysis.

Biochemical Analysis

Biochemical analysis was carried out to determine the concentration of some markers of liver functions in the serum. Alanine and Aspartate aminotransferases (ALT and AST) were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957). Total protein was determined by the Biuret method (Peters, 1968). Serum albumin was by the Bromocresol green method (Doumas *et al*., 1971) and bilirubin was estimated by the method described by Garber (1981).

Statistical Analysis

All data were subjected to statistical analyses. The values were expressed as Mean ± Standard deviation. All values were normally distributed. One-way Analysis of Variance (ANOVA) was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test using the statistical software; Instat 3 version (San Diego, USA). $P<0.05$ was considered a significant level.

RESULTS AND DISCUSSION

Oral median lethal dose (LD_{50}) values of extracts of *Ximenia americana*, *Ficus platyphylla,* and mixture of the two extracts are shown in Table 1. The result suggested that all three extracts are not toxic even at values as high as 5000 mg/kg. The levels of liver function parameters of the control and test rats are presented in Table 2. As shown in the Table, the activity of serum ALT is between 5.42±1.36 U/L in the control group to 6.17±1.17 U/L for the *F. platyphylla* treated group with all groups falling within a similar range of values. AST values are between 7.67±1.23 U/L in the mixed group to 8.61±1.21 U/L in the *F. platyphylla*-treated group. Animals treated with *F. platyphylla* also had the highest total bilirubin mg/dL with all the values falling within a similar range. Control groups were also found to have a similar total protein; 59.25±3.71 g/dL with the animals treated with *F. platyphylla* 59.33±8.85 g/dL while the animals treated with *X. americana* had the highest amount 61.16±4.71 g/dL. Total serum albumin was also found to follow a similar pattern.

Table 1: Oral median lethal dose (LD_{50}) values of methanolic extracts of *X. americana*, *F. platyphylla* and Mixture of the two extracts.

EXTRACT	MEDIAN LETHAL DOSE (LD_{50}) (mg/dl)
XA	>5000
FP	>5000
M	>5000

XA= *Ximenia americana* FP= *Ficus platyphylla* M=Mixture

To ascertain the safety of the plant extracts, LD_{50} was conducted which showed that all fractions of the extracts are safe even at high concentrations which is not surprising considering how frequently the plants are being used. To investigate any kind of liver injury or function, specific biochemical tests were developed to assess some markers in the plasma or serum. These markers include AST and ALT for hepatocellular injury. Bilirubin is for excretory functions while total proteins and albumin are for synthetic functions. We assessed the levels of AST and ALT in the serum of the animals treated with *F. platyphylla* and *X. americana* extracts (250 mg/kg body weight), as well as the mixture of the two extracts in the ratio 1:1 weight by weight.

It is known that these enzymes although not very specific to the liver, are present in the liver in large amounts and tend to leak in any kind of hepatic injury. The findings of the study showed that the treated animals have ALT and AST levels that do not deviate from the control group suggesting that the extracts have no toxic effect on hepatocytes. Previous studies suggested that methanolic extract of *F. platyphylla* has a hepatoprotective role against CCL4-induced liver damage (Sheidu *et al*., 2020; Ugwah-Oguejiofor *et al*., 2022). The results obtained are also in line with another study which reported an increase in levels of AST and ALT in rats induced with liver injury by acetaminophen, suggesting a significant hepatoprotective activity of aqueous extracts of *X. americana* when compared to Silymarin (Venkateswararao *et al*., 2011). It is also common knowledge that liver cell damage is usually associated with an increase in serum level of bilirubin.

Table 2: Serum biochemical markers of liver injury

GROUP	ALT (U/L)	AST (U/L)	TB (mg/dL)	TP (g/dL)	ALBUMIN (g/dL)
NC	5.42±1.36[a]	8.00±1.26	0.50±0.06	59.25±3.71[b]	35.67±0.82[c]
XA (250 mg/kg	5.67±0.82	8.43±1.17[b]	0.60 ±0.21	61.16±4.71	36.00±4.15
FP (250 mg/kg	6.17±1.17	8.61±1.21[b]	0.65 ± 0.1	59.33±8.85[b]	37.17±2.32
M (250 mg/kg	5.44±1.17[a]	7.67±1.23	0.62± 0.15	60.83±2.79	35.50±1.76[c]

Values are expressed as mean ± SD of three replicates. One-way analysis of variance was used to compare the means followed by the Tukey-Kramer Multiple Comparisons Test. Mean values in the same column superscripted by the same letters are not significantly different from each other (p>0.05), NC= Normal control, XA= *Ximenia americana*, FP= *Ficus platyphylla*, M= Mixture.

The study also showed that the interventions had serum bilirubin levels similar to control groups suggesting the safety of the extracts. Albumin is known to be produced entirely in the liver and constitutes about 60% of total serum protein. Therefore, the measurement of serum albumin serves as an important assay to assess the synthetic functions of the liver. As seen in the range of values obtained in this study, the extracts also showed a promising effect on serum levels of albumin and total protein contents suggesting the safety of the extracts as far as liver injury is concerned. It should be noted that decreased levels of serum proteins are found in renal disease, malnutrition, albuminuria, and terminal liver failure. Although the concentration of the serum albumin is reduced in severe liver diseases, that of the globulins is usually increased so that the total protein concentration is rarely low. These findings supported the claim that *X. americana* could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can be made via a simple process of maceration or infusion (Sharief *et al*., 2022). Despite that some of the results in the *X. americana* group are better than that of the mixture, the overall result suggests that the mixture of the extracts is a better option than individual extracts which may be due to synergistic effects of the individual phytochemical constituents in the extracts.

CONCLUSION

In conclusion, the findings of this study illustrated that extracts of *Ficus platyphylla* and *Ximenia americana* are not hepatotoxic, at the tested dose and may therefore, be considered safe for consumption in the treatment of diseases.

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