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(Kodavanti *et al*., 1989). The absence of reliable liver protection drugs in modern medicine has led to an increase in the application of traditional medical remedies, including herbal preparations with various scientifically unauthenticated therapeutic claims (Jyothi Reddy *et al*., 2013).

Acetaminophen hepatotoxicity is now known as the most common cause of the potentially devastating clinical syndrome of acute liver failure and transplantation in many Western countries (Ostapowicz *et al*., 2002). Acetaminophen is widely used as analgesic and antipyretic. It is however reported to be responsible for a wide range of toxicities especially when taken in large single dose with or without an equally large amount of alcohol (Tenebein, 2004). These toxicities often involve virtually all organs and systems of the body. In the liver, for example, acetaminophen overdosing causes a potentially fatal condition known as acetaminophen-induced hepatotoxicity to both man and experimental animals (Kaplowitz, 2005; Ghanem *et al*., 2009). Because of its wide availability as an over-the-counter drug, acetaminophen is liable to abuse and consequent hepatotoxicity. However, the toxic dose for acetaminophen is highly variable. The lowest dose of acetaminophen to cause hepatotoxicity is accepted to be between 125 and 150mg/kg body weight (Lee, 2004). The threshold dose of acetaminophen to induce hepatotoxicity for adult is 10 to 15g or chronic ingestion of doses as low as 4g/day (Dart *et al*., 2006). In children, acetaminophen acute doses of above 200mg/kg would be required to induce same degree of hepatotoxicity as seen in adults (Rumack and Matthew, 1975). This higher threshold is due to the relatively larger liver size to body ratio of children when compared to adults, thus being more tolerant to acetaminophen overdose (Tenebein, 2004). The hepatotoxic effect of acetaminophen is reported to be mediated by inducing lipid peroxidation which is principally by a highly reactive intermediate metabolite of acetaminophen, N acetyl-*P*-benzoquinoneimine (NAPQI) (Murriel *et al*., 1992). This highly reactive electrophilic molecule covalently binds to hepatocyte intracellular and membranal macromolecules with a consequent modification of their structure and function. This cellular disturbance leads to a decrease in calcium ATPase activities and increase in cytosolic calcium levels (Jaeschke and Bajt, 2006). Abnormal calcium homeostasis can alter the permeability of the cell, causing the formation of blebs in the cell membrane and loss of cell membrane integrity (Lee, 1995) to cause cell death and consequent liberation of cellular contents including cytosolic enzymes of liver origin. Hence, acetaminophen hepatotoxicity is often associated with significant elevation in the circulatory levels of liver enzymes particularly the aminotransferases (ALT and AST). These marker enzymes are often required to act as indicators of hepatocellular injury (Olagunju *et al*., 2004).

Ficus exasperata vahl is a terrestrial plant that grows 20m high and inhabits the evergreen and secondary rainforest of West Africa. It belongs to the family Moraceae with more than 800 species occurring in the warmer parts of the world. The plant is commonly known as sand paper tree, it is also known locally as "anwerinwa" (Ijeh and Ukweni, 2007). The plant is popular in Africa for its use in the treatment of various ailments. In Ivory Coast, the viscid non-milky sap is used for treating sores eye trouble and stomach pains (Burkill, 1997). The sap is used to arrest bleeding in Ghana (Abbiw, 1990). The aqueous decoction of the stem bark is used in Congo to ease childbirth (Bouquet, 1969). In Zaire, a leaf poultice is used in medication for ring worm (Burkill, 1997). The leaves are also used as medication for a number of life stock diseases (Abbiw, 1990). The leaves extract from *F. exasperata* is also reported for its use in treating hypotensive patients (Buniyamin *et al*., 2007), cough and haemorrhoid (Odunbaku *et al*., 2008). The young leaves of the plant are used in Nigeria as an anti-ulcer remedy. However, despite the various therapeutic uses of *F. exasperata*, few studies have been carried out to scientifically validate the folkloric medicinal claims. The present study was therefore carried out to investigate the hepatoprotective activity of ethanol extract of Ficus *exasperata* leaves on acetaminophen-induced liver damage in rats.

MATERIALS AND METHODS

Plant Extract Preparation

Ficus exasperata leaves were collected from Ikeji-Arakeji forest and identified by a botanist in the Department of Plant Science, Joseph Ayo Babalola University, Ikeji-Arakeji. Osun State. The plant material of *Ficus exasperata* leaves were air-dried under shade for three weeks and blended into powder using an industrial blender. About 250g of the powder was macerated in 700ml of ethanol at room temperature for 48hour. It was then filtered using Whatmann filter paper (number 1). The filtrate was concentrated using a rotary evaporator and the concentrated extract was allowed to dry under a fast moving ceiling fan at room temperature. The residue was weighed and used in the preparation of stock extract for administration.

Proximate composition and phytochemical screening

The proximate analysis of *F. exasperata* leaf powder was carried out using standard procedures described by AOAC (2001). Phytochemical screening of the ethanol extract of *F. exasperata* leaves was carried out using standard methods described by Evans (1989) and Harbone and Baxter (1993).

Experimental animals and design:

Thirty healthy albino rats weighing between 150g-160g were obtained from the animal house of the Biochemistry section, Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji. The animals were housed in plastic cages at 25-30^{0}C with constant 12hours light/darkness condition. Maintenance and treatment of animals were in accordance with the principles of the "Guide for care and use of laboratory animals in research and teaching" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH) publication 86-23 revised in 1985. The animals were fed with standard commercial rat pellets (Pfizer Feeds Plc, Nigeria) and water *ad libitum* for two weeks of acclimatization and for one week experimental period. The rat were randomly divided into 5 study groups of 6 rats each with weight differences within and between groups not exceeding ± 20% of the average weight of the total rats. Group I served as the normal control, group II served as the hepatotoxic control, group III, IV and V served as single daily oral varied dose of *Ficus exasperata* extract.

Experimental induction of hepatotoxicity and acute toxicity study

Group I rats were administered single daily oral and intraperitoneal dose of 10ml/kg of distilled water. Group II rats were administered single daily dose of 200 mg/kg of acetaminophen, single dose of 10ml/kg of distilled water via oral and intraperitoneal routes for another 7 days. Groups III, IV and V rats were administered single daily oral varied doses; 125mg, 250mg and 500mg/kg of *F. exasperata* extract for 7days proceeded by 200 mg/kg of acetaminophen via intraperitoneal route (Emzor Paracetamol®, Emzor Pharmaceuticals, Isolo, Lagos State, Nigeria) for another 7days. Eighteen rats were randomly selected and used to evaluate the acute toxicity of the extract. The rats were divided into 3 groups (n=6) and administered 500, 1000 and 1500mg/kg body weight ethanol extract of *Ficus exasperata* leaf orally. The rats were then observed for 48hours for signs of toxicity and death.

Collection of blood sample and tissue homogenate preparation

At the end of the treatment period, the animals were fasted overnight but allowed access to water *ad libitum*, weighed, and sacrificed by cervical dislocation while under mild anaesthesia. Blood was collected by cardiac puncture and centrifuged at 3000 rpm (Beckman GS-6R, Germany) for 5 min at 4^{0}C. Serum was obtained and the supernatant for measuring enzyme activity and bilirubin level. Liver and kidneys were quickly dissected out, rinsed in isotonic sterile saline, blotted dry on a filter paper and weighed. Each tissue was placed in a separate plastic vial containing ice-sterile saline and stored at -8^{0}C until required for further analysis. A weighed portion of liver and kidneys was cut out and chopped into small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenate (5%) in buffer solution (50mM Tris-HCl, 0.25M sucrose, pH 7.4) was prepared and stored at 4^{0}C until further analysis was carried out. Biochemical assays for aspartate aminotransferase(AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and total bilirubin (TB) concentration were determined using commercially available enzymatic test kits (Randox Laboratory Ltd, UK) methods following the manufacturer's instructions.

RESULTS AND DISCUSSIONS

Table 1 show that the ether extracts is approximately 18.0 percent of *F. exasperata*. The ether extract contains the phytochemicals (table 2) of all the phytochemicals screened, only anthraquinones were absent.

The liver is a versatile organ in the body concerned with the regulation of the internal chemical environment. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds; and it is functionally interposed between the site of absorption and the systemic circulation. These features, unfortunately, predisposes the liver as the preferred target for drug toxicity. Any injury or impairment of liver function poses several implications which may have damaging consequences on one's health (Ostapowicz *et al*, 2002).

Phytochemical study of the plant extract revealed the presence of alkaloids, steroid, tannins, flavonoids, saponins and glycosides. The presence of high amounts of flavonoid and alkaloid contents of the plant has been documented (Ijeh and Ukweni, 2009). These phytochemicals have been noted for their inherent antioxidant effect and the ability to scavenge free radicals liberated during lipid peroxidation (Lanhers *et al*., 1991); this may be the reason for the hepatoprotective effect of the extract. The ethanol extract of *Ficus exasperata* groups (III-V) resisted the acetaminophen-induced serum enzyme activity that indicates the protection of the structural integrity of hepatic cell membrane or the regeneration of damaged liver cells.

The acute toxicity study revealed the absence of lethality among the tested animals when the ethanol extract of *F. exasperata* leaves was administered as a single dose (500, 1000 and 1500 mg/kg). There were no signs of any gross behavioural changes, except for an increase in urination, indicating the safe usage of the extract at a dose of 1500 mg/kg. The results of the current study indicated that intraperitoneal injection of

Table 1. Proximate composition analysis of *F. exasperata* leaf powder

S.No	Nutrient composition	Weight (%)
1	Total ash	12.8±.
2	Crude protein	13.5±
3	Crude fibre	10.2±
4	Ether extract	18.2±
5	Nitrogen free extract	45.3±

Values are the Means of three determinations ± SD.

Table 2. Preliminary phytochemical screening

S.No	Phytochemical constituents	Ficus exasperata
1	Alkaloids	+ve
2	Anthraquinones	-ve
3	Cardiac glycosides	+ve
4	Flavonoids	+ve
5	Saponins	+ve
6	Steroids	+ve
7	Tannins	+ve

+ve = present; -ve = absent

Table 3. Effect of graded oral dose of ethanol extract of *Ficus exasperata* leaves on serum AST, ALT, ALP activities and TB concentration.

I	18.75 ± 0.45^{b}	10.77 ± 0.68^{b}	196.63 ± 0.86^{b}	1.34 ± 0.05^{b}
II	59.76 ± 0.62^{a}	36.87 ± 0.62^{a}	157.05 ± 0.22^{a}	5.30 ± 0.02^{a}
III	$21.14\pm0.41^{a,b}$	$13.60\pm0.46^{a,b}$	$154.96\pm0.26^{a,b}$	$2.42\pm0.02^{a,b}$
IV	$19.20\pm0.70^{b,c}$	$19.16\pm0.64^{a,b,c}$	$185.73\pm0.83^{a,b,c}$	$2.47\pm0.02^{a,b}$
V	$20.76\pm0.26^{a,b}$	$13.01\pm0.20^{a,b}$	$127.50\pm0.70^{a,b,c}$	$2.40\pm0.06^{a,b}$

Values are Mean± SD of three determinations.
[a] Significantly different from normal control at $P<0.05$.
[b] Significantly different from hepatotoxic control at $P<0.05$. [c] Significantly different from Group III at $P<0.05$.
Group I (normal control): 10mL/kg of distilled water via the intraperitoneal and oral routes respectively.
Group II (hepatotoxic control): 10mg/kg b.w of F. exasperata + 200mg/kg intraperitoneal acetaminophen.
Group III: 125mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen
Group IV: 250mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen
Group V: 500 mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen.

Table 4. Effect of graded oral dose of ethanol extract of *Ficus exasperata* leaves on liver AST, ALT, ALP, and total bilirubin (TB) concentration

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)
I	34.57 ± 0.25^{b}	33.83 ± 0.40^{b}	96.75 ± 1.04^{b}	2.80 ± 0.15^{b}
II	26.18 ± 0.85^{a}	23.78 ± 0.63^{a}	245.01 ± 1.65^{a}	3.60 ± 0.09^{a}
III	$31.44\pm0.43^{a,b}$	$35.00\pm0.23^{a,b}$	$148.76\pm0.40^{a,b}$	$3.16\pm0.14^{a,b}$
IV	$30.93\pm0.89^{a,b,c}$	$30.90\pm0.29^{a,b,c}$	$175.63\pm0.57^{a,b,c}$	$3.33\pm0.13^{a,b}$
V	34.16 ± 0.50^{b}	$31.00\pm0.70^{a,b}$	$154.08\pm0.65^{a,b,c}$	$3.04\pm0.05^{a,b}$

Values are expressed Mean± SD of three determinations.
[a] Significantly different from normal control at $P<0.05$.

[b]Significantly different from hepatotoxic control at $P<0.05$. [c] Significantly different from Group III at $P<0.05$.
Group I (normal control): 10mL/kg of distilled water via the intraperitoneal and oral routes respectively.
Group II (hepatotoxic control): 10mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen.
Group III: 125mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen
Group IV: 250mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen
Group V: 500 mg/kg b.w. of F. exasperata + 200mg/kg intraperitoneal acetaminophen.

200mg/kg/day of acetaminophen for 7 days reliably induced hepatotoxicity. This was demonstrated in the hepatotoxic control rats by the significant ($P>0.05$) rise in the serum levels of marker enzymes of hepatocellular injury; alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are reliable markers of hepatocellular damage (Johnson, 1995). Data from the present study showed the effect of repeated high dose of acetaminophen treatment preceded by a 7-day pre-administration with graded oral doses of ethanol extract of *Ficus exasperata* leaves on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and total bilirubin concentration. As shown in the table, repeated high doses of intraperitoneal injection of acetaminophen induced significant ($P<0.05$) increase in the serum AST, ALT activities and TB level when compared to normal control rats. However, this elevation was prevented in the treated rats (Groups III-V) compared to the hepatotoxic control (Group II) in a non-dose dependent pattern. On the other hand, the table also revealed a significant ($P<0.05$) fall in ALP activity in Groups III-V when compared to normal and hepatotoxic controls despite oral pretreatment with varying doses of the plant extract. The aminotransferases constitute a group of enzymes that catalyse the inter-conversion of amino acids and α-keto acids by the transfer of amino groups. Groups III to V that were pre-treated with graded doses of the extract and acetaminophen for 7 days showed that serum activities of ALT and AST were significantly ($P<0.05$) reduced when compared to the hepatotoxic control (Group II) which indicated the hepatoprotective action of the plant extract.

The enzyme alkaline phosphatase (ALP) reaches the liver mainly from bone. In addition to the markers of hepatocellular injury (ALT and AST), measurements of serum levels of ALP and total bilirubin concentration (TB) are considered adjunct markers of acute and chronic hepatic damage. Serum ALP and bilirubin are reflective of hepatobiliary damage or obstruction. A sharp rise in ALP activity is a useful index of bile duct damage, while elevated serum levels of bile salts and bilirubin are also seen in drug induced hepatobiliary injury (Kaplan, 1986; Fredman *et al*., 1996). In the present study, there was a profound increase in the total bilirubin (TB) concentration, an effect which was attenuated on pre-administration of graded doses of the extract as seen in Groups III-V. The fall in serum ALP activity of the hepatotoxic control compared to the normal control suggests that the toxin may not have induced acute hepatobiliary damage or obstruction. Also, the short duration over which toxins were administered ruled out the possibility of chronic hepatobiliary injury. Thus, the opposing results of ALP activity and TB concentration can only indicate that findings of the plant extract on hepatobiliary status is inconclusive. The results obtained for the activities of AST, ALT, ALP and TB concentration was in direct concordance with that obtained in the serum. This further echoed the possibility of ethanol extract of *F. exasperata* leaves in preventing drug-induced hepatocellular assault. The above study thus suggested that intraperitoneal administration of ethanol extract of *Ficus exasperata* leaves have good hepatoprotective properties. The hepatoprotective role of the extract is due to the antioxidant potential mechanism. These results indicate that it is worth undertaking further studies on possible usefulness of the ethanol extracts of *Ficus exasperata* on hepatotoxicity.

CONCLUSION

Thus the present study indicates that the ethanol extract of *Ficus exasperata* leaves may be used as an effective hepatoprotective agent. Further studies on isolation and structural determination of the active principles might be worthy.

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