Biokemistri
Vol. 32, No. 1, March 31, 2020, pages 47 - 53
Printed in Nigeria

0795-8080/2020 $10.00 + 0.00

http://www.niseb.org.ng/journals
Also available online at http://www.bioline.org.br/bk

BKR 20200010/32105

Acute toxicity study of crude methanol leaf extract of *Ficus exasperata* Vahl on male Wistar albino rats

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(Received January 18, 2020; Accepted March 9, 2020)

ABSTRACT: There is an increase in demand for the use of traditional folk medicine globally due to their low cost, efficacy and easy accessibility especially for people living in developing countries. This study was carried out to assess the acute toxicity as well as the LD_{50} of *Ficus exasperata* Vahl in male Wistar albino rats. Lorke's method was adopted for the determination of the LD_{50} of the crude methanol leaf extract of plant. The extract administration was in two phases. In phase one, doses of 10, 100 and 1000 mg/kg body weight of rats was administered while in phase two, 1500, 3000 and 5000mg/kg body weight of rats was orally administered. Behavioral changes, signs of toxicity and mortality were observed from the point of administration to 24 hours in the first phase only and 14 days in the second phase included. The qualitative phytochemicals screening revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, saponins and steroids. Signs of toxicity observed in the rats administered with 1000mg/kg body weight of rats were uneasiness, sluggishness and dizziness after 24 hours of observation. However death was not recorded in all the groups after 14 days of administration. There was dose dependent increase in the level of the selected liver enzymes assayed (AST and ALT) across all the groups. Liver malondialdehyde concentration in the rats administered higher doses differed significantly ($p < 0.05$) when compared with the control group. From our study, crude methanol leaf extract of *Ficus exasperata* vahl possess medicinally important phytochemicals and the acute toxicity studies revealed that the extracts has LD_{50} above 5000mg/kg because no death was recorded. However, the results of the liver enzymes and lipid peroxidation suggested that prolonged use of the extract may cause damage to the liver and some vital organs. It is recommended that a long-term study such as sub-chronic toxicity studies should be conducted to know the long term effect of the leaf extract of *Ficus exasperata* Vahl.

Keywords: Phytochemicals screening, Acute Toxicity Studies, *Ficus Exasperata*, Malondialdehyde, Liver enzymes

Introduction

Ficus exasperata Vahl (Moraceae), with its various species, is widely used in traditional medicine. In tropical African countries like Nigeria, the plant is used locally for the treatment of a variety of diseases/disorders such as coughs, hemorrhoids anxiety disorders, epilepsy, high blood pressure, rheumatism, arthritis, cancer, intestinal pains, colics, bleeding and

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wounds (Cousins *et al*., 2002). The presence of important phytochemicals with antioxidant and antimicrobial activities has been well reported (Awala *et al*., 2017; Pallav *et al.,* 2014).

Recent toxicity studies in rats involving crude aqueous and ethanol extracts of *Ficus exasperata* roots have shown potential hepatic and renal toxicity as reflected by significantly increased serum transaminases and bilirubin (Ahmed *et al.*, 2012). The screening of plant extracts for their activities against diseases should be combined with evaluation of the toxicities of such plant extracts with traditionally acclaimed therapeutic properties (Efosa and Ngozi 2018). This study was carried out to determine the phytochemical constituents and acute toxicity studies of crude methanol leaf extract of *Ficus exasperata* Vahl in male Wistar rats.

Materials and Methods

Sample Collection

The *Ficus exasperata* plant leaf sample was collected in March, 2019 at Unguwar Jeji Village, Kalgo Local Government, Kebbi State, Nigeria. They were identified by a plant taxonomist in the Department of Biology, Federal University Birnin Kebbi were a herbarium specimen with voucher number BIOHB/0034 was deposited. The leaves were washed with clean water and air dried at room temperature. After drying, the leaves were ground with an electric blender and the obtained powder form were stored in air tight containers till needed for further analysis.

Sample Extraction

An amount of 1000g powder of *Ficus exasperata* Vahl leaf was soaked into 2000ml of 99.8% methanol for 72 hours (3 days). The sample was then filtered using muslin cloth after three days soaking. The filtrates were concentrated in a vacuum rotary evaporator at 50℃, after which the concentrated crude extracts were exposed to allow the remaining methanol to evaporate. The percentage yield was calculated after which the solid extract was stored in the refrigerator until further use

Experimental Animals

Adult male Wistar rats weighing 150- 250g, bred in Biology Department Animal house, Federal University Birnin Kebbi, were used in this study. They were kept in clean plastic cages, fed with rat pellets and watered *ad libitum* and left to acclimatize for two weeks. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998).

Phytochemical Screening

Preliminary phytochemical screening was carried out using standard procedure as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Acute Toxicity Studies

Standard method was used as described by Lorke (1983). The experiment was conducted in two phases. In the initial phase, rats randomly divided into 4 groups of 3 animals each. Groups 1, 2 and 3 were orally administered 10, 100 and 1000 mg/kg body weight crude methanol leaf extract respectively. The fourth group, which was the control, was administered distilled water orally. Behavioral observation and body weight changes were monitored during the 14 days period of study. Prior to sacrifice all animals fasted for 12 hours and were weighed and euthanize in a chloroform chamber.

In the second phase, twelve (12) Male Wistar were divided into 4 groups of three animals per group. Groups 1, 2 and 3 were orally administered 1500, 3000 and 5000 mg/kg body weight crude methanol leaf extract. The fourth group which was the control was administered distilled water. Behavioral observation, body weight changes were carried out during the 14 days period of study. Death was monitored over a period of 24 hours and the

serum liver function enzymes as well as lipid peroxidation parameters in the liver was analysed at the end of 14 days period.

Liver Enzymes Level Estimation

Serum ALT Estimations: Serum alanine aminotransferase was assayed by the method described by Raitman *et al*. (1975) and Schmidt *et al*. (1963). The assay is based on the reaction between α-ketoglutarate and L-alanine catalyse by alanine aminotransferase to give L-glutamate and pyruvate, the pyruvate hydrazone complex formed between pyruvate and 2, 4-dinitrophenylhydrazine was measured at 546nm.

Serum AST Estimation: Serum aspartate aminotransferase was measured according to the methods of Raitman *et al*. (1975) and Schmidt *et al.*(1963). The assay is based on the reaction between α-ketoglutarate and L-aspartate catalysed by aspartate aminotransferase to give L-glutamate and oxaloacetate, the oxaloacetate-hydrazone complex formed between oxaloacetate and 2,4-dinitrophenylhydrazine was measured at 546nm.

Lipid Peroxidation Estimation

Thiobarbituric acid assay (TBARS) method was used for malondialdehyde concentration determination as described by Janero (1990). The assay is based on the reaction between the malondialdehyde in the sample and thiobarbituric acid in the reagent to give a pink coloured complex measured at 535nm.

After dissection, the liver was isolated, weighed and chilled in ice-cold 0.9% NaCl. After washing the liver homogenate was prepared in a ratio of 1g of wet liver tissue to 9ml of 1.15% KCl using Teflon homogenizer.

Calculation:

The malondialdehyde concentration of sample can be calculated using extinction co-efficient of $1.56 \times 10^5 m^{-1}cm^{-1}$

$$\text{TBARS activity} = \frac{O.D \times V \times 1000}{A \times v \times 1 \times Y}$$

Where O.D = absorbance of sample test at 535nm

V=Total volume of the reaction = 3ml

A = molar estimation co-efficient of product = $1.56 \times 10^5 m^{-1}cm^{-1}$

1 = Light path = 1cm

v = volume of tissue extract used = 1ml

Y = mg of tissue in the volume of sample used.

Statistical Analysis

All analyses were carried out in triplicate and, where applicable, results are expressed as mean ± SEM. The data were subjected to one-way analysis of variance (ANOVA) and Tukey Post-hoc test was used for the multiple comparison. P values less than 0.05 ($p < 0.05$) were regarded as statistically significant.

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Results

The extraction process yielded 12.64 % (w/w) at room temperature. The obtained results of the qualitative phytochemicals screening of crude methanol extract of *Ficus exasperata* leaf extract revealed the presence of alkaloid, tannin, flavonoids,, saponin and steroids. However, cardiac glycoside was not detected (Table 1). Signs of toxicity observed were unease, sluggishness and dizziness three hours after administration of extract as from the dosage of 1000mg/kg body and above. Changes in the weight in both treated groups and control groups were also observed (Table 2).

The results revealed variations in the level of both ALT and AST at lower doses but slight increase was recorded at higher doses. The results also revealed that malondialdehyde concentration obtained from those with lower doses (10mg-1500mg) did not show significant difference from control. However, those groups that were administered 3000 mg/kg and 5000 mg/kg of the extract showed significant difference from control (Table 5).

Table 1: Phytochemical screening of crude methanol extract of *Ficus exasperata* vahl leaf

Phytochemicals	Inference
Alkaloids	+
Cardiac glycoside	-
Flavonoid	+++
Phenol	+++
Saponin	+
Steroid	+
Tannin	++
Terpenoid	++

Keys: +: Present in a trace concentration; ++: Present in a medium concentration; +++: Present in a high concentration; -: Absent or in negligible amount.

Table 2: Effect of administering different doses of crude methanol extract of *Ficus exasperata* leaf on body weight of albino rat over a period of two weeks

Dose (mg/kg b.wt)	Day 0	Day 7	Day 14
Control	203.86^{d}±2.40	207.69^{d}±1.56	212.42^{d}±1.99
10	206.48^{a}±7.96	203.84^{a}±7.05	198.56^{a}±9.40
100	218.76^{b}±2.06	214.16^{b}±3.63	209.43^{b}±1.99
1000	232.09^{c}±9.46	227.53^{c}±6.75	223.68^{c}±5.75
1500	201.26^{e} ± 2.35	203.62^{e} ± 3.66	202.70^{e} ± 4.32
3000	224.81^{f} ± 4.21	220.82^{f} ± 6.32	217.62^{f} ± 5.78
5000	238.10^{g} ± 4.32	236.17^{g} ± 6.67	231.28^{g} ± 3.29

Data expressed as mean ± standard deviation; mg/kgbw: Milligram Per Kilogram Body Weight. Same super script means there is no significance difference while different super script means there is significance difference ($p<0.05$). ANOVA was used for the multiple comparism (Turkey).

Table 3: Effect of administering different doses of crude methanol extract of *Ficus exasperata* leaf on the organ weight of male Wistar rats

Dose(mg/kg	Liver	Kidney	Lungs	Heart	Spleen
Control	6.49^{a}±1.30	1.26^{b}±0.10	1.15^{a}±0.10	0.65^{a}±0.08	0.48^{a}±0.55
10mg	5.88^{a}±0.41	1.39^{b}±0.02	1.45^{b}±0.49	0.75^{b}±0.10	0.63^{b}±0.14
100mg	9.05^{c}±1.27	1.85^{c}±0.22	1.84^{c}±0.09	0.87^{c}±0.09	0.77^{c}±0.15
1000mg	8.52^{b}±1.11	2.15^{d}±0.19	1.94±0.22	1.13^{d}±0.17	0.76^{c}±0.12
1500mg	8.72^{b} ± 1.02	1.14^{a} ± 0.04	1.39^{b} ± 0.04	0.80^{c} ± 0.21	0.72^{c} ± 0.06
3000mg	9.34^{c} ± 0.93	2.16^{d} ± 0.23	1.98^{d} ± 0.06	1.20^{e} ± 0.01	0.84^{d} ± 0.03
5000mg	10.04^{d} ±1.11	2.18^{d} ± 0.21	1.48^{b} ± 0.03	1.36^{f} ± 0.06	0.87^{d} ± 0.02

Data expressed as mean ± standard deviation; mg/kg bw: Milligram Per Kilogram Body Weight. Same super script means there is no significance difference while different super script means there is significance difference ($p<0.05$). ANOVA was used for the multiple comparison (Tukey).

Table 4: Effect of administering different doses of crude methanol extract *Ficus exasperata* leaf on the major serum liver marker enzymes (ALT and AST) of male Wistar rats

Experiments	Dose(mg/kg b.wt)	ALT (U/L)	AST (U/L)
Control	0	5.60^{a}±0.80	7.93^{a}±0.53
Phase 1	10	9.60^{b}±1.60	8.87^{a}±0.81
	100	12.27^{b}±0.92	16.80^{b}±2.45
	1000	15.57^{c}±1.76	22.05^{c}±0.35
Phase 2	1500	19.68^{d}±0.08	27.30^{d}±0.15
	3000	21.76^{d}±0.06	47.25^{e}±0.25
	5000	24.00^{e}±0.05	53.90^{f}±1.10

Data expressed as mean ± standard deviation; mg/kgbw: Milligram Per Kilogram Body Weight. Same super script means there is no significance difference while different super script means there is significance difference ($p <0.05$).

Table 5: Effect of administering different doses of *Ficus exasperata* methanol leaf extract on the lipid peroxidation using liver tissue of male Wistar rats

Experiment	Dose(mg/kg b.wt)	MDA conc.in (moleMDA/g wet tissue × 10^{-5})
Control		0.865^{a}±0.002
Phase 1	10	0.764^{b}±0.139
	100	0.866^{a}±0.051
	1000	0.942^{c}±0.019
Phase 2	1500	1.250^{d} ± 0.12
	3000	1.370^{d} ± 0.04
	5000	1.670^{e} ± 0.06

Data expressed as mean ± standard deviation; mg/kg bw: Milligram Per Kilogram Body Weight. Same superscript means there is no significant difference while different super script means there is significance difference ($p<0.05$).Student T-test was used for single comparison. MDA=Malondialdehyde

Discussion

Studies have demonstrated that phytochemicals possessed various pharmacological properties. It is obvious that most therapeutic effects of medicinal plants are a function of their phytochemical constituents and, in recent years, secondary plant metabolites

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(phytochemicals) have been investigated extensively as sources of medicinal agents (Krishnaraju, 2005). The presence of alkaloid, tannin, flavonoids, cardiac glycosides, saponin and steroids in *Ficus exasperata* crude leaf extract detected in this study was also reported by Awala *et al*. (2017), who worked on the same crude methanol extract of *Ficus exasperata* vahl leaf. More so, Ugwah-Oguejiofor *et al*. (2011), also reported the present of tannin, saponin, flavonoid, volatile oils, steroids and glycosides at various concentrations. Phytochemicals are thought to have a positive and negative effect on animals upon administration. For instance tannins and anthraquinones are thought to have both proxidant and antioxidant effects on the body. While the antioxidant protects the tissues and organs, the proxidant damages the tissues and organs.

The weight changes of the animals during the period of observation which was more visible at higher doses, suggest the presence of tannins and other phenolics which are thought to interfere with absorption of nutrients making them unavailable and thereby reducing feed intake (Kumar and Singh, 1984). The organ weights were not significantly different form the control. Generally, the reduction in body weight is a simple and sensitive index of toxicity after exposure to toxic substances. Body weight changes are indicators of the adverse effects of drugs and chemicals and it would be regarded as significant if the body weight loss is more than 10% from the initial body weight (Teo, 2002).

From the results of ALT and AST, it can be suggested that since ALT and AST are mostly released as a result of liver injury. As such elevation of the level of these enzymes can be an indication of cellular damage, leakage and loss of functional integrity of hepatic cell membrane due effects of the plant extract. But this is not always true as other organs and physiological conditions can result to the release AST in the serum. The results obtained in these studies revealed that malondialdehyde concentration obtained of those with lower doses (10mg-1500mg) did not showed significant difference with control. More so, the groups that were administered 3000mg/kg and 500mgkg of the extract showed (MDA concentration) of more than 1.00 mole MDA/g tissue wet $\times 10^{-5.}$ It can suggested that increased in doses might be a reason for the increase of malondialdehyde which serves as a primary index of lipid peroxidation.

Conclusion

This study has established that crude methanol extract of *Ficus exasperata* leaf obtained from the northern part of Nigeria, particularly (Kebbi State), is rich in phytochemicals. The LD_{50} appears to be more than 5000mg/kg body weight because no death of the rats was recorded throughout the period of the study. However, the methanol extract of *Ficus exasperata leaf* manifested slight signs of toxicity at higher doses as the level of the liver enzymes increased as well as malondialdehyde concentration (MDA). In a nutshell, prolonged use of specific plant extract may lead to toxicity irrespective of its medicinal importance.

We suggest future research on the effect of the leaf extracts of this plant on histopathological and hematological parameters. Furthermore, it is recommended that a long-term study such as sub-chronic toxicity and chronic studies to be conducted to know the actual LD_{50} of the leaf extract of *Ficus exasperata* Vahl. Finally, it is suggested that further studies should focus on the isolation, purification and characterization of the active constituents of crude methanol extract of *Ficus exasperata* leaf in order to add to our knowledge of its medicinal profile.

Conflicts of Interest: No Conflicts of Interest

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