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Serum total protein concentration and liver enzymes activities in albino rats model administered with ethanolic leaf extract of *Ficus capensis*

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There is an increased use of the *Ficus capensis* plant and concerns about safety alongside efficacy have been raised. The aim of this work is to study the total protein, bilirubin and serum liver enzyme activities in albino rats administered graded concentrations of ethanol extract of *F. capensis* leaf. Twenty-four albino rats were shared into four groups and different concentrations of the leaf extract were administered with respect to body weight (250, 150 and 100 mg/kg) respectively while the fourth group (Control) was administered distilled water only. The serum liver enzymes activities were determined using the Randox Laboratory Kit while the total protein and bilirubin concentrations were determined by Spectrophotometric methods. The results showed p≤0.05 decrease in total protein and globulin concentrations, with an increase in bilirubin and albumin/globulin concentration and a slight decrease in the albumin concentration in 250 and 150 mg/kg. Furthermore, the administration of the extract caused p≤0.05 increase in the enzyme activity of alkaline phosphatase and alanine transaminase at 250 and 150 mg/kg. For aspartate transaminase, there was p≤0.05 increase in 250 mg/kg. From the study, the liver is implicated in the administration of high doses of ethanol extract of *F. capensis* hence lower doses are recommended.

Key words: *Ficus capensis*, liver enzymes, total protein, bilirubin, spectrophotometer.

INTRODUCTION

The use of medicinal plants in West Africa is probably as old as the duration of human settlement in the region (Bakkali et al., 2008). Medicinal plants are important sources of pharmaceutical manufacturing. Medicinal plants and herbal medicines account for a significant percentage of the pharmaceutical market (WHO, 2014). The therapeutic properties of medicinal plants are conditioned by the presence of active substances which include alkanoids, flavonoids, glycosides, vitamins, tannins and comarin compounds in their organs which physiologically affect the bodies of humans and animals (Thomas, 2001). Most researchers have focused on plants that have been traditionally used for various therapeutic reasons as these plants are more abundantly

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or readily available; thus, they are found to be less expensive and seemingly pose lesser side effects than synthetic drugs (Yakubu et al., 2007).

Ficus capensis which belongs to the Moraceae family is a tropical or subtropical plant that contains some phytochemicals traditionally used for various therapeutic benefits. Many of these photochemicals have beneficial effects on long term health when consumed by humans and can be used to effectively treat human diseases (Darshan, 1996). The leaves of *F. capensis* are found to contain flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids and alkaloids, and these phytochemicals have shown effectiveness in antimicrobial, anti-diarrheal, antiallergic and anticancer treatments as well as treatment of cardiac problems (Uzoekwe and Mohammed, 2015). Serum liver enzymes are predominantly contained within the liver cells. If the liver is injured or damaged the liver cells spill these enzymes into the blood, raising their levels in the blood and signaling liver disease (Daniel and Marshall, 1999). Total protein and bilirubin could be implicated in liver function in humans and animals. In this research, the ability of well directed ethanol extracts of *F. capensis* to cause changes to the serum liver enzymes and total protein, and bilirubin levels in albino rats *in vivo* hence determining its safety and possible hepatotoxicity at different concentrations will be studied.

MATERIALS AND METHODS

Sample collection and identification

Fresh leaves of *F. capensis* were randomly harvested from Okporo Community, Orlu Local Government Area of Imo State, Nigeria. It was identified by the School of Agriculture and Agricultural Sciences (SAAT), Federal University of Technology, Owerri, Imo State with voucher identification number 001/FWT/FUTO/2015. The leaves were washed and dried at room temperature, crushed, allowed to cool, then bottled and deposited at the university herbarium.

Sample preparation

The crude extract of *F. capensis* was obtained by soaking crushed leaves in 98% ethanol for 72 h, thoroughly shaken, filtered and filtrate was put in the Soxhlet extraction unit. The final leaf extract obtained after Soxhlet extraction was labeled Extract F.

Experimental animals

Twenty-four albino rats weighing between 90-120 g were obtained from Department of Biochemistry FUTO and housed in a well ventilated metal cage. They were fed with rat feed twice daily and left to acclimatize for 14 days and their average weight was taken note of preceding commencement of administration.

Experimental design

The randomized complete block design was used. Rats were grouped into four (4) groups of six (6) rats each; Groups A-C served as groups administered various fractions of *F. capensis* leaf extract while Group D served as the control.

The grouped albino rats were administered doses of the plant extract with respect to their body weight (250, 150 and 100 mg/kg). The administration of the extract was done on a daily basis for 14 days after which the blood samples were collected in triplicates. The statistical analysis of data obtained from test groups and control groups were evaluated by ANOVA, applying the level of significance ($P \leq 0.05$) using SPSS 8.1.

Preparation of serum

The method of Yakubu et al. (2005) was adopted for the preparation of serum. Twenty-four hours after the last extract administration, briefly under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being displaced (to avoid contamination with interstitial fluid) were cut with sterile scapel blade and an aliquot of the blood was collected into labeled vacutainer sample tubes.

A table top centrifuge was used to centrifuge the blood samples at 700 rpm for 15 min after which the serum was transferred from the clotted blood into plain tubes with the aid of a pasture pipette. The collected serum was stored at 4°C till the next day for analysis.

Determination of serum total protein

The commonly used method for measuring serum protein is the biuret reaction (Gornall et al., 1949). The principle of the reaction is that serum proteins react with Cu^{2+} (Copper Sulphate) in sodium hydroxide to form a purple/violet complex. The intensity of the purple/violet colour is proportional to the total protein present. The absorbance of sample and standard were read against reagent blank at 540 nm using a DRE 3000 HACH Spectrophotometer.

Determination of serum albumin

The measurement of albumin is generally by a dye binding technique that utilizes the ability of albumin to form a stable blue coloured complex with bromocresol green dye (Tietz et al., 1994). The intensity of the colour is proportional to the amount of albumin present. The absorbance of sample and standard were read against reagent blank at 630 nm using a DRE 3000 HACH spectrophotometer.

Determination of serum globulin

Owing to the fact that bromocresol green-albumin complex absorbs light at different wavelengths from the unbound dye, the method may overestimate albumin by binding to other proteins (Willacy, 2019). As such, the total globulin fraction is generally determined by subtracting the ALBUMIN fraction from the total protein fraction.

Determination of serum bilirubin

The dimethylsulphoxide method as described by Tietz et al. (1994) was used to determine serum bilirubin concentration. The principle of the reaction is that bilirubin reacts with diazotized sulphanic acid in the presence of dimethylsulphoxide to form a coloured complex whose colour intensity is proportional to the amount of bilirubin present. The absorbance of the sample and standard were read against the blank at 550 nm using a DRE 3000 HACH

Table 1. Serum total protein and bilirubin concentration of ethanolic leaf extract of *Ficus capensis.*

Group	Total protein concentration (umol/L)	Albumin concentration (umol/L)	Globulin concentration (umol/L)	Bilirubin concentration (umol/L)	Albumin/Globulin ratio concentration (umol/L)
A (250 mg/kg)	52.81±2.61[a]	31.57±1.14[a]	21.24±2.50[a]	36.23±3.46[a]	1.50±0.20[a]
B (150 mg/kg)	62.20±1.93[b]	32.52±1.11[a]	29.68±2.83[b]	32.31±3.46[b]	1.11±0.14[b]
C (100 mg/kg)	72.41±2.22[c]	33.46±1.13[a]	38.95±1.52[c]	17.08±0.78[c]	0.86±0.03[b]
D (Control Distilled water)	74.88±1.62[c]	34.18±1.33[a]	40.70±1.11[c]	15.81±0.67[c]	0.84±0.04[b]

Values are Means + standard deviation of triplicate determination. Superscripts with different alphabets = p≤0.05; Superscripts with same alphabets = p>0.05.

Table 2. Serum liver enzymes activities of ethanolic leaf extract of *Ficus capensis*.

Group	A (250 mg/kg)	B (150 mg/kg)	C (100 mg/kg)	D (Distilled water)
ALP activity(IU/L)	49.32±2.19[a]	38.53±4.26[b]	24.91±2.16[c]	19.57±0.90[c]
ALT activity(IU/L)	27.70±0.64[a]	24.43±1.86[b]	20.24±1.04[c]	17.77±0.54[c]
AST activity(IU/L)	46.17±4.77[a]	35.45±1.78[b]	30.23±0.48[c]	28.78±0.61[c]

Values are Means + standard deviation of triplicate determination. Superscripts with different alphabets = p≤0.05; Superscripts with same alphabets = p>0.05.

spectrophotometer.

Determination of aspartate transaminase activity

The Reitman and Frankel method was used to determine the serum aspartate transaminase activity (Reitman and Frankel, 1957). The principle of the reaction is that the AST catalyses an exchange reaction of an amino group between aspartate and a-ketoglutarate forming oxaloacetate and glutamate. The oxaloacetate thus formed reacts with 2,4-dinitrophenylhydrazine in sodium hydroxide to form oxaloacetatehydrazone which has a reddish brown colour.

The absorbance of the sample was read against the reagent blank at 546 nm using a DRE 3000 HACH spectrophotometer.

Determination of serum alanine transaminase activity

The Reitman and Frankel method was used to determine serum alanine transaminase activity (Reitman and Frankel, 1957). The principle of the reaction is that ALT catalyses the transfer of the amino group between L-alanine and a-ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with 2,4-dinitrophenylhydrazine in sodium hydroxide to give a complex with a reddish-brown colour. The absorbance of the sample was read against the reagent blank at 546 nm using a DRE 3000 HACH spectrophotometer.

Determination of alkaline phosphatase activity

The method of Wilkinson and Vodden was used in determining serum alkaline phosphatase activity (Wilkinson and Vodden, 1966). The principle of the reaction is that ALP acts upon phenolpthalein monophosphate in 2-amino-2methylpropan-1-ol buffer at pH of 10.15. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen. The absorbance of the sample was read against the reagent blank at 590 nm using a DRE 3000 HACH spectrophotometer.

RESULTS

As represented in Table 1, the total protein and bilirubin concentrations and serum liver enzyme activity in albino rats administered graded concentrations of ethanol extract of *F. capensis* leaf was studied. The results showed a significant p≤0.05 decrease in total protein and globulin concentrations in albino rats administered 250 and 150 mg/kg of plant extract (52.81±2.61 umol/L; 62.20±1.93 umol/L; and 21.24±2.50 umol/L; 29.68±2.83 umol/L) when compared with 100 mg/kg and Control. There was a significant p≤0.05 increase in bilirubin concentration in albino rats administered 250 and 150 mg/kg of plant extract (36.23+3.46 and 32.31+3.46 umol/L respectively) and a significant p≤0.05 increase in the albumin/globulin concentration in albino rats administered 250 mg/kg of plant extract (1.50±0.20 umol/L) when compared with other concentrations of administration and Control. There was also a slight decrease in the albumin concentration in albino rats administered 250 and 150 mg/kg of plant extract (31.57±1.14 and 32.52±1.11 umol/L respectively) when compared with 100mg/kg and control.

As represented in Table 2, the administration of the extract caused significant p≤0.05 increase in the enzyme activity of Alkaline Phosphatase in albino rats administered 250 and 150 mg/kg of plant extract (49.32±2.19 IU/L and 38.53±4.26 IU/L) when compared with the 100 mg/kg and Control. There was also a significant p≤0.05 increase in the activity of Alanine

Transaminase (ALT) in albino rats administered 250 and 150 mg/kg of plant extract (27.70+0.64 and 24.431±1.86 IU/L) when compared with 100 mg/kg and Control. For Aspartate Transaminase (AST), there was a significant $p \leq 0.05$ increase in albino rats administered 250 mg/kg of plant extract (46.17+4.77 IU/L) when compared with the other concentrations of administration.

DISCUSSION

The liver maintains homeostasis in living system. It is involved in biochemical pathways necessary for growth and fighting against diseases (Ward and Daly, 1999). The *F. capensis* plant is used for a number of therapeutic purposes, and this raises the concern of safety and possible toxicity. This study pointed towards possible hepatotoxicity which is shown in the decreased serum total protein concentrations and may be attributed to toxicants in the phytochemical constituents of the leaves or may have been due to increased release of tissue specific enzymes and other intra cellular proteins secondary to parasite-induced cell membrane disruption. Total protein (albumin and globulin) are produced by the liver and in the case of a liver damage, production of these proteins are reduced or completely ceased. The concentrations of the total protein, bilirubin and albumin may indicate the state of the liver and the type of damage (Yakubu et al., 2005). The study also suggests that toxic metabolites in *F. capensis* extract at high concentrations may be responsible for the significantly high value of bilirubin of rats administered extracts at 250 mg/kg. This assertion is supported by the work of Ovuru et al. (2004) who reported an increase in the total serum bilirubin concentration and attributed this to a metabolic disturbance in the liver arising from a defective conjugation and/or excretion of bilirubin. The albumin/ globulin ratio concentration significantly increased indicating conditions causing underproduction of globulins which include liver cirrhosis. There was also significant increase in the serum liver enzyme activities at higher levels of administration. Alkaline phosphatase is a hydrolytic enzyme which is responsible for removal of phosphate group from many types of molecules including nucleotides, proteins etc. ALP is particularly concentrated in the liver, bile duct, kidney bone and placenta. An increase in ALP is an indication that there is an obstruction of bile duct consequently affecting the liver. An increase in ALP may also be as a result of celiac diseases (Tamas et al., 2002). Alanine transaminase usually increases where liver has been diseased or damaged and is also a test used for screening liver problems. Aspartate transaminase is a commonly measured clinical marker for liver health (Ghouri et al., 2010). Hence the marked increases in the serum liver enzyme activity, at high levels of administration of plant extract indicate possible hepatotoxicity at high doses.

CONCLUSION AND RECOMMENDATION

From the study, it is shown that high doses of administration of the ethanol extract of *F. capensis* leaf caused hepatotoxicity in albino rats and if the therapeutic power of this plant must be utilized, lower doses would be recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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