

## Research Article

# Liver toxicity of Crude extract of *Ficus natalensis* traditionally used in South Western Uganda

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**Background:** Traditional healers have used medicinal plants to treat infectious diseases since time immemorial. These natural products have not only played a vital role in healing, but have also contributed to the discovery of many pharmaceutically active agents.

**Objectives:** This research was aimed at assessing the effects of crude extract of *Ficus natalensis* on the liver.

**Methodology:** Test and control Wistar albino rats were fed on either Water or Ethanolic extract of *Ficus natalensis* and water-only (control) respectively and their serum harvested. Biochemical analysis of liver function tests was performed and Human Diagnostic Test Kits were used to assay for the enzymes ALT (alanine aminotransferase), (AST (serum aspartate aminotransferase) and  $\gamma$ -GT (gamma glutamyl transpeptidase). The organ body weight ratio was also recorded.

**Results:** The cold water decoction once administered to the rats showed adverse effects leading to death of the experimental animals by day 3. The ethanolic extract results showed that there was a dose-dependent alteration in the indices of liver function as well as enlargement of the liver following feeding on the ethanolic extract of *Ficus natalensis*. All the serum enzyme activity of ALT, AST and GGT were increased in a dose-dependent manner and the groups of animals being fed on the ethanolic extract, showed a reduction in weight.

**Discussion:** The cold water extraction might have extracted all the active ingredients including some that were toxic to the laboratory animals leading to their death. The ethanolic extracts exhibited alterations in the indices of liver function as well as enlargement of the liver in a dose-specific manner. All the serum enzyme activity of ALT, AST and GGT was increased in a dose-dependent manner. This could possibly be due to hepatotoxicity resulting from the metabolites of the *Ficus natalensis*.

**Key words:** *Ficus natalensis*, Ethanolic extract, Liver toxicity

**Received:** October, 2014

**Published:** December, 2014

## 1. Introduction

Africa is a continent endowed with an enormous wealth of plant resources. Over 5000 distinct species are known to occur in the forests regions alone and most of them have been used for several centuries in traditional medicine for the prevention and treatment of human diseases (Iwu 1993). It has been estimated that up to

90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002).

*Ficus natalensis* is an evergreen tree of 6-21 meters high, with a rounded spread and dense crown. The bark on young branches is hairy but smooth and grey on older branches and often with aerial roots hanging

down from branches. The whole plant exudes copious milky latex. Leaves are dark green, thin and papery or slightly leathery. Figs are hanging in leaf axils, sometimes below the leaves enclosing many small flowers (Adriens, 2005). The native range of the plant is Kenya; S. Africa (Natal); Uganda; Zaire (Oriental and Shaba) and Sudan through tropical Africa to and including southern Africa (hear.org, 2008).

*Ficus natalensis* is one of the 800 species under the genus *Ficus* (Woodland 1997). In Uganda, *Ficus natalensis* is commonly used as a natural fabric for the formation of bark cloth. It is widely propagated in many areas of South Western Uganda where it is an important agro-forestry tree and highly appreciated for other purposes besides bark cloth (Reitzenstein 2003). It is referred to as; "Omutuba" among the Baganda, Omutoma" among the Toro and "Omuthoma among the Konjo communities.

Its bark aerial, roots and leaves are used medicinally. It is used in the treatment of colds, cough, sore throat, dysentery, wounds, acne and pimples, cataracts, worms, cholera and stomach ulcers (Monik 2005). Phytochemical studies of a number of *Ficus* species undertaken thus far have led to identification of over 100 compounds. A substantial number of these compounds are phenthroindolizidine alkaloids, several coumarins, flavonoids and triterpenoids, (Ephraim et al, 2008).

Toxicologically, as one of the oldest known human foods, figs as a fruit have a very high safety profile. However, the toxicological evaluation of other fig products is still at its infancy. It has been reported that skin contact with latex may provoke allergic reactions like dermatitis, asthma and anaphylaxis (Chelminska 2004); while orally administered latex may cause hallucinosis (Luna, 1984a, b). Also, it must be noted that not all medicinal plants are safe for consumption in the crude form. Some levels of toxicity may arise when crude drugs are used as a result of potentially toxic compounds they contain and pesticide application during cultivation (Amdur et al, 1991; Evans et al, 1999).

The liver serves as a barrier between potentially harmful substances absorbed from the gastrointestinal tract and the systemic circulation. Its detoxification functions include the process of hydrolysis, hydroxylation, oxidation, reduction, carboxylation and de-methylation. These mechanisms convert substances into forms that are either less toxic or more water soluble and therefore easily excreted. The detoxification of drugs is a function of the drug metabolizing system of Cytochrome P450. The Cytochrome P450 system is found in the microsomes of the hepatocytes and facilitates the transformation of drugs to more extractable end products by conjugation with such moieties as glycine, glutathione, sulfuric acid, acetate or glucuronic acid (Ingram, 2000).

*Ficus natalensis* is used for the treatment of many ailments. The leaves or bark are usually crushed and mixed with half a liter of water to make a cold decoction. At times the bark and leaves are mixed with half a liter of water, boiled and then drunk. (Monik 2005). While the decoction is believed to heal many

ailments, little research has been done to characterize the natural products present and to evaluate possible risks such as undesirable effects, overdose and poisoning.

## 2. Materials and Methods

### 2.1 Collection of plant materials

The study involved the collection of the leaves of *Ficus natalensis* from a rural setting of Bushenyi District of Uganda; Mitooma Sub County whose natural habitat has not been interfered with much in terms of agriculture and other man-made activities. The plant was identified by a botanist, Mr. Rwaburindore Protase, of the Department of Botany; University of Makerere and the voucher specimen deposited in the Pharmacy laboratory of Kampala International University.

### 2.2 Experimental animals

Twenty-five male Wistar albino rats were purchased, housed and fed on NUVITA animal feeds manufactured by Nuvita Industries Limited Kampala, and provided with tap water. The animals were maintained at room temperature throughout the study period, 12 hours alternative day and night and ambient humidity. The animals were handled according to the NIH guideline for care and use of laboratory animals in teaching and research.

### 2.3 Crude Extraction of *Ficus natalensis* leaves

The leaves were air dried at room temperature for two weeks and further drying was done in a dry oven at 30°C for 24 hours. The dry leaves were then ground to powder form and sieved. Each 200 mg of ground material was cold extracted using Distilled water first then the residue was followed by a cold extraction using 70% ethanol at a ratio of 1:10 respectively. The extracts were shaken and left to stand for 24 hours at room temperature. The extracts were filtered using a Buckner Funnel and Whatman No 1 filter paper. Each ethanol filtrate was concentrated by evaporating the ethanol at 40 °C over a water bath while the water filtrates were freeze-dried. Further drying was done in a hot air oven at 40°C. The extract was collected and stored in small bottles at 4 °C.

### 2.4 Toxicity studies

Using a modified method of Cruz et al, 1979, the male rats were randomly divided into three groups of 5 animals each. The animals were weighed on day 1 and day 14 of the study. Group A served as the control and received 10 mls/kg of distilled water. Group B received 500 mg/kg and Group C received 1000 mg/kg of the crude extract daily by means of an oral intra-gastric cannular for a period of 14 days.

### 2.5 Collection of blood and serum samples

The animals were anaesthetized using chloroform and blood collected from the heart using a needle and syringe. The blood was collected in non-heparinized bottles and allowed to clot. The serum was separated from the clot and centrifuged onto clean bottles for biochemical analysis.

The organ body weight ratio was also recorded.

## 2.6 Determination of Serum biochemical markers

ALT parameters were determined using: GPT (ALAT) IFCC mod. LiquiUV Test package size 12012 complete test kits; AST was determined using GOT (ASAT) IFCC mod. LiquiUV Test package 12011 and  $\gamma$ -GT was determined by  $\gamma$ -GT colorimetric test package size 12013 all manufactured by Human Diagnostics Company based in Germany. The Human Diagnostic Test Kits were used to assay for the enzymes according to the manufacturer's instructions. The optical densities were obtained using a colorimeter. All other reagents and chemicals used were of analytical grade.

## 2.7 Statistical Analysis

Results were expressed as mean and standard error of mean. Where applicable, the data were subjected to one way analysis of variance (ANOVA) followed by

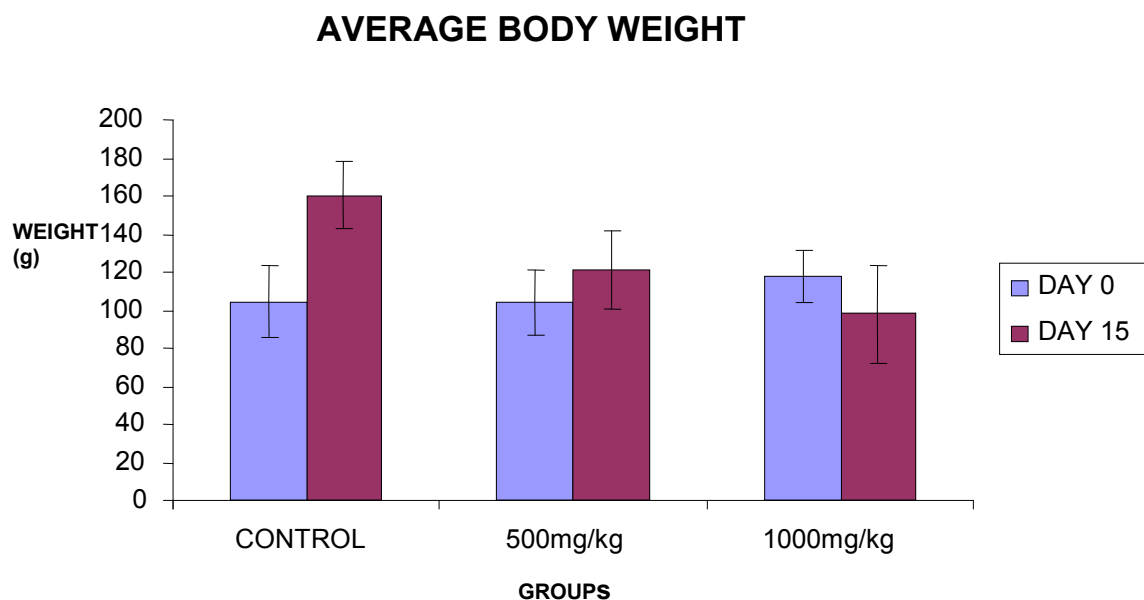
Dunnett's post hoc test using Statistical Package for Social Sciences (SPSS) version 11.5. P values <0.05 were regarded as significant.

## 3. Results

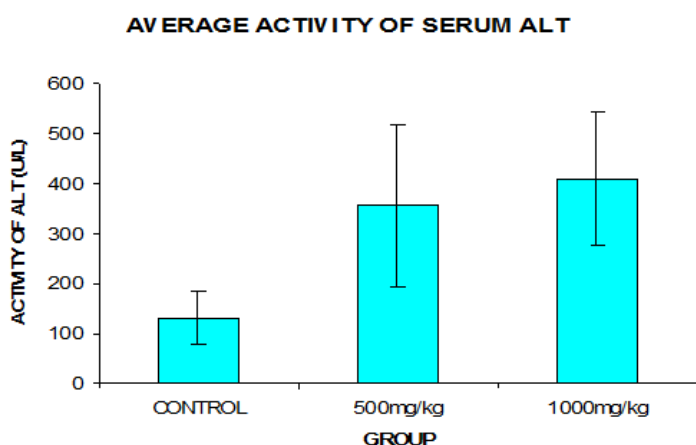
Extract yield from 200 grams of the plant powder used was calculated and found to be 9.6%.

### Weight of the animals

The mean change in weight in the control group had a remarkable increase in weight during the period of the study. The group receiving 500 mg/kg b.w p.o had an increase in weight between day 0 and day 15 but the increase was less than that observed in the control group. The group receiving 1000 mg/kg b.w p.o on the other hand lost weight between day 0 and day 15 and the weight of the rats in this group was significantly lower compared to that of the rats in the control group (**Figure 1**)



**Figure 1:** Average body weights of the rats on day 0 and day 15



**Figure 2:** Mean level of serum ALT

### Alanine aminotransferase (ALT)

There was an increase in the level of serum ALT in groups receiving 500mg/kg and 1000mg/kg as shown in **Figure 2**. This increase is statistically significant from the control at  $P < 0.05$

### Serum aspartate aminotransferase (AST)

There was an increase in serum activity of AST in both groups receiving 500mg/kg and 1000mg/kg but only the increase in the 1000mg/kg group was statistically significant ( $P < 0.05$ ) (**Figure 3**).

### Gamma glutamyl transpeptidase

There was an increase in serum activity of GGT in both groups receiving 500mg/kg and 1000mg/kg, both of which were statistically significant ( $P < 0.05$ ) (Figure 4).

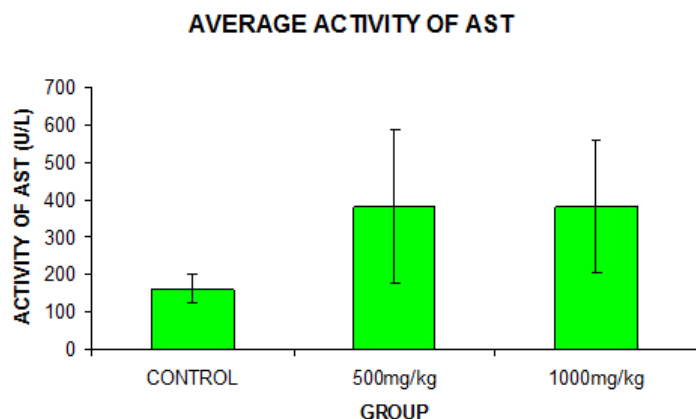


Figure 3: Average activity of serum AST

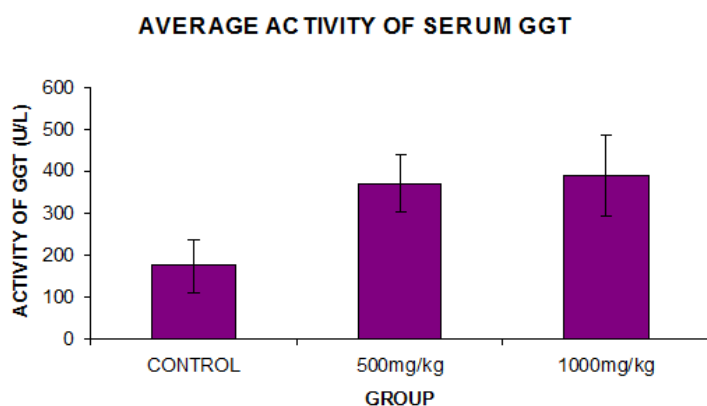


Figure 4: Average activity of serum GGT

### Organ (liver)/ body weight ratio

The liver/ body weight ratio was higher in the treated groups as compared to the control. These differences were statistically significant at  $P < 0.05$  (Figure 5).

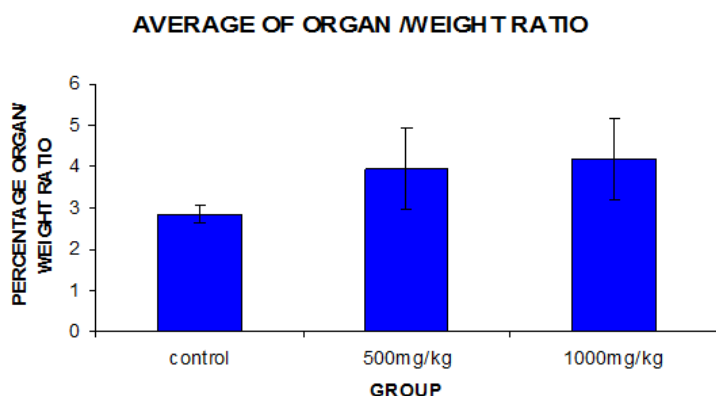


Figure 5: Mean organ/body weight ratio

### 4. Discussion

Herbal medicine is gaining popularity in developing countries (Obici S, et al, 2008). About 80% of the world's population still depends on traditional medicine and traditional treatments involve mainly the use of plant extracts (Akelere et al, 1993, Saggiu et al, 2007). The increase in popularity and the scarcity of adequate scientific studies on their safety and efficacies have raised concerns on the toxicity and adverse effects of herbal medicines (Saad et al, 2006). These remedies contain bioactive principles with potential to cause adverse effects (Bent and Ko 2004).

The present study shows that *Ficus natalensis* at dosages tested is capable of affecting the liver of male Wistar albino rats. The biochemical markers monitored in serum are useful markers for assessing the functional capacity of the liver. Biochemical indices of organ function if impaired will alter the normal functioning of the organ. The change in weight may mean that the extract has an effect on feed conversion rate or the animals were off-fed. It has been reported that polyphenols, particularly proanthocyanidins are known to interfere with intake and digestibility of feeds when fed to animals (Dube et al, 2001). Other members of the *Ficus* family have been found to have proanthocyanidins in their leaves (Ahmed et al, 1998; Teixeira et al, 2006) and this may also be the case with *Ficus natalensis*.

Organ-body weight ratio can be used to indicate organ swelling, atrophy or hypertrophy. Therefore the increase in the liver-body weight ratios in groups receiving the extract may be attributed to hypertrophy.

The aminotransferases (ALT and AST) considered in this study are useful "marker" enzymes in liver cytolysis (Shahjahan et al, 2004). The enzymes occupy a central position in the metabolism of amino acids as they help to retain amino groups (to form a new one) during the degradation of amino acids. The elevated liver aminotransferases activities at all dosages tested could be explained by an increase in the functional activity of the organ resulting in enzyme synthesis. In addition, the soluble enzymes ALT/AST are released when injury involves organelles such as the mitochondria (Senthil et al, 2003). As a result, the establishment of liver injury in this study caused both plasma membrane and organelle membrane damage. Rajesh and Latha 2004 also reported that the elevated activities of these enzymes were indicative of cellular leakage and loss of the functional integrity of the cell membranes. However similar increase in aminotransferases activity may be attributed to the release of the enzymes from the cells of the other damaged organs, aside the liver in this case which might have resulted in changes in membrane permeability of these cells (Latha 1998).

GGT is another membrane-localized enzyme that plays a major role in glutathione metabolism and reabsorption of amino acids from the glomerular filtrate and intestinal lumen. The increase in serum GGT activity at all doses tested may be explained by enzyme induction arising from *de novo* synthesis of the enzyme molecules. The activity of  $\gamma$ -GT in plasma is raised whenever there is cholestasis and it is a very sensitive index of liver pathology. Alcohol and drugs such as phenytoin induce

enzyme activity. In acute hepatic damage, changes in  $\gamma$ -GT activity parallel those of aminotransferases (Ingram, 2003).

From the results it can be concluded that the effects of ethanolic extract of *Ficus natalensis* on liver function; that brings alterations in the indices of liver function as well as enlargement of the liver following treatment with the ethanolic extract of *Ficus natalensis* are dose specific. All the serum enzymes ALT, AST and GGT were affected probably because the liver is a vulnerable target to a number of toxicants since it metabolizes foreign substances to compounds that maybe hepatotoxic as well. The ethanolic extract of *Ficus natalensis* has an effect on the feeding habits of the animals shown by the reduction in weight of the groups receiving the extract. Thus, the plant is not completely safe in male rats when consumed repeatedly over a period of 14 days.

The cold water extraction of *Ficus natalensis* had fatal effect on the rats after 3 days of administration. This may have been caused by an overdose attributed to the fact that active compounds in the *Ficus natalensis* are more soluble in water (Denloye et al, 2000, Denloye 2010). Traditionally, the decoction is prepared by boiling the plant extract; this may result in reduction of toxic compounds by evaporation. We therefore recommend that there is need to carry out acute toxicity testing using the boiled decoction of *Ficus natalensis* as traditionally used to better gauge its toxicity.

There is also need to carry out acute toxicity testing to determine the lethal dose of the plant extract and the effect of the extract on other organs such as the kidney. Moreover the phytochemical screening of this plant should be done to characterize natural products that would directly contribute to its toxic effect.

### Conflict of Interest declaration

The authors declare no conflict of interest.

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