International Journal of Tropical Medicine 4 (4): 136-139, 2009
ISSN: 1816-3319
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# Effects of Ethanolic Extract of *Tetrapleura tetraptera* on Liver Function Profile and Histopathology in Male Dutch White Rabbits

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**Abstract:** *Tetrapleura tetraptera* fruit has wide use in South East and Western Nigeria as a spice as well as a plant to treat various medical ailments. It has been reported that feeding of extracts to animals produced some toxic effects and pathological lesions in some organs. This study was designed to investigate the hepatotoxic effects of 10 days oral administration of the ethanolic extract of Tetrapleura Tetraptera (TTE) in mature male rabbits. Twenty healthy acclimatized male rabbits (1.4-1.6 kg) body weight were randomly assigned to 4 groups. Animals in group 1 served as the control and received only water and no extract. Animals in groups 2-4 were administered 50, 100 and 150 mg $kg^{-1}$ bodyweight of TTE. All animals were fasted for 18 h after withdrawal of treatment and sacrificed after anaesthesia. Venous blood sample of groups were analyzed for liver function parameters. Histopathological examination of internal organs was also carried out. TTE caused elevation in serum AST and alteration of various metabolic parameters and did not induce any marked pathological lesions in the liver.

**Key words:** *Tetrapleura tetraptera*, hepatotoxicity, dutch-white, histopathology, liver, phytochemical

## INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of the world's population (Tripathi and Tripathi, 2003). Plants have been an important source of medicine for thousand of years. Even today, the world health organization estimates that up to 80% of people still rely on traditional remedies such as herbs for their medicines. Plants are also, the source of many modern medicines (Roberts, 1988). Medicinal plants have one or more part with medicinal properties (Sofowora, 1993). Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered. Large numbers of plants are constantly been screened to determine their toxicity level. Traditional use of any plant for medicinal purposes warrant the safety of such plant, particularly with regards to mutagenicity, nephrotoxicity, carcinogenicity and hepatotoxicity (Ashafa *et al*., 2009).

*Tetrapleura tetraptera* belongs to the mimosaceae family. It is referred locally to as Aridan-Yoruba and oshosho in Ibo. It is generally found in the lowland forest of tropical Africa. The fruit consist of a fleshy pulp with small, brownish-black seeds. The dry fruit has a pleasant aroma (Aladesanmi, 2007). It is therefore, used as a popular seasoning spice in Southern and Eastern Nigeria (Okwu, 2003; Essien *et al*., 1994). The fruit is used to prepare soup for mothers from the first day of birth to prevent post partum contraction (Nwawu and Akali, 1986). Its fruits are used for the management of convulsions, leprosy, inflammation, rheumatism (Ojewole and Adesina, 1983), flatulence, jaundice and fevers (Bouquet, 1971). The anticonvulsant activity of the volatile oil from fresh fruits of *T. tetraptera* in mice has been reported (Nwawu and Akali, 1986). Its leaves are essential for the treatment of epilepsy (Aka and Nwabie, 1993) and present strong molluscicidal activity (Adewunmi, 1991). The aqueous fruit extract has also been shown to possess hypoglycaemic properties (Ojewole and Adewunmi, 2004). The root extract has also been proven to be used for the treatment of gastrointestinal related clinical problems (Noamesi *et al*., 1994). The varied use of this plant makes it important to determine the safe dose at which the plant extract is not toxic to the liver. The objective of this study is to determine the effect of varied dose of this extract on the biochemical and histopathological indices of liver function.

## MATERIALS AND METHODS

**Plant materials:** The 1 kg of Tetrapleura tetraptera fruits were purchased from the herbal market in Mushin, Lagos

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State, Nigeria and identified and authenticated in the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria.

**Preparation of plant extract:** The fruit was shade dried and pounded in a mortar before being subjected to soxhlet extraction using 80% ethanol as the solvent. Thereafter, the solvent was distilled off and the extract was successively rinsed with distilled water to eliminate any ethanol still present. The extract was further dried using a lyophilizer. The dried extract was stored in air tight amber bottles. The dried extract was weighed and percent yield was calculated using the expression:

$$\text{Yield}(\%) = \frac{\text{Weight of dried extract}}{\text{Weight of sample used}} \times 100\%$$

**Phytochemical screning:** Phytochemical screening for alkaloids, saponins, flavonoids, tannins, anthraquinones and cardiac glycosides were carried out according to the methods of Sofowora (1993), Harbonne (1984) and Evans (1989).

**Experimental animals:** Twenty male Dutch-White rabbits weighing between 1.4 and 1.6 kg were obtained from and acclimatized in the animal house of the college of medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The study was conducted in January, 2008.

**Treatment of animals:** The rabbits were allowed to acclimatize for 2 weeks prior to administration of extracts. They were randomly divided into four groups of 5 rabbits each such that differences in average body weights were minimal. Each group was kept in a metal cage at uniform temperature with 12 h dark light$^{-1}$ periodicity and fed with standard rabbit pellets (Neimeth Livestock feeds Ltd, Ikeja, Lagos) and water ad-libitum. Group 1 received orally distilled water only, while groups 2-4 were orally administered graded doses (50, 100 and 150 mg kg$^{-1}$ bodyweight) of ethanolic extract of *Tetrapleura tetraptera* fruits daily for 10 days. Treatment was stopped on the 10th day and animals were fasted overnight.

**Collection of blood and organs:** On the 11th day, all animals were sacrificed after anaesthesia with chloroform in a desiccator. The rabbits were quickly dissected and venous blood was collected via left ventricular cardiac puncture into labelled sterilized drug bottles. The blood collected was allowed to clot by standing at room temperature for 1 h and centrifuged at 2500 g for 10 min. The serum (supernatant) was isolated and stored at -30°C, until it was analyzed. All animals were subjected to autopsy. The livers were examined in situ for signs of any gross malformation. The liver was then excised, blotted dry and weighed.

**Biochemical analysis:** Albumin (ALB), total protein, bilirubin (total and direct),as well as the activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were determined in the serum using standard assay kits from Randox Chemicals, UK using Bayer Instrument.

**Histological preparation:** Histological tissue studies of the vital organs from each animal were fixed in 10% formaldehyde and processed for haematoxylin-eosin staining. Photomicrographs of the prepared slides haematoxylin-Eosin stained tissue sections were taken with a camera attached to the compound light microscope in the Department of Morbid Anatomy, College of Medicine, University of Lagos.

**Statistical analysis:** The SPSS 11.0 software was employed for data entry and validation. Statistical analysis was carried out between the groups and control using the student's t-test. A $p<0.05$ was considered statistically significant.

## RESULTS

Phytochemical screening of the plant extract revealed the presence of alkaloids, saponins, tannins, sugar, flavonoids and cardiac glycosides. Phlobatannins were however not detected (Table 1). There was a significant increase in the activity of AST in experimental animals receiving low and medium doses of the extract (Table 2). Significant increase in activity of ALP in experimental animals was observed only at a concentration of 150 mg kg$^{-1}$ bodyweight of the extract. Activities of ALT however, increased significantly in animals receiving low dose of the extract. However, the activities of ALT in animals receiving medium and high doses of the extract decreased significantly (Table 2). There was however, no significant change in the level of albumin. A significant

Table 1: Phytochemical profile of the ethanolic extract of tetrapleura tetraptera fruits

| Phytochemical component | Tetrapleura tetraptera extract |
|---|---|
| Alkaloids | ++ |
| Saponins | ++ |
| Phlobatannins | ND |
| Tannins | + |
| Cardiac glycosides | + |
| Sugar | + |
| Flavonoids | + |

++ = Highly present, + = Present, ND = Not Detected

Table 2: Effect of *T. tetraptera* ethanolic extract on liver function profile in male dutch-white rabbits

| Groups | ALT (IU $L^{-1}$) | AST (IU $L^{-1}$) | ALP (IU $L^{-1}$) | TBIL (mmol $L^{-1}$) | DBIL (mmol $L^{-1}$) | TPROT (g $L^{-1}$) | ALB (g $L^{-1}$) |
|---|---|---|---|---|---|---|---|
| Control | 23.00±0.00 | 11.00±0.00 | 12.00±1.73 | 71.33±0.78 | 20.00±1.04 | 33.00±1.31 | 27.00±1.53 |
| Low dose | 29.00±1.73* | 16.00±1.73* | 12.00±1.16 | 30.87±0.44* | 10.70±0.12* | 36.00±0.00 | 29.00±1.00 |
| Middle dose | 18.00±1.16* | 21.00±1.16* | 12.00±0.00 | 64.43±8.08 | 11.27±0.71* | 49.00±0.58* | 30.00±0.58 |
| High dose | 11.00±1.16* | 9.00±1.16 | 26.00±4.04* | 50.47±3.09* | 12.27±0.72* | 36.00±2.31 | 27.00±1.16 |

n = 5. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, T. BIL: Total Bilirubin, D. BIL: Direct Bilirubin, T. PROT: Total Protein, ALB: Albumin. Results are given as mean of 5 determinations ± standard error mean

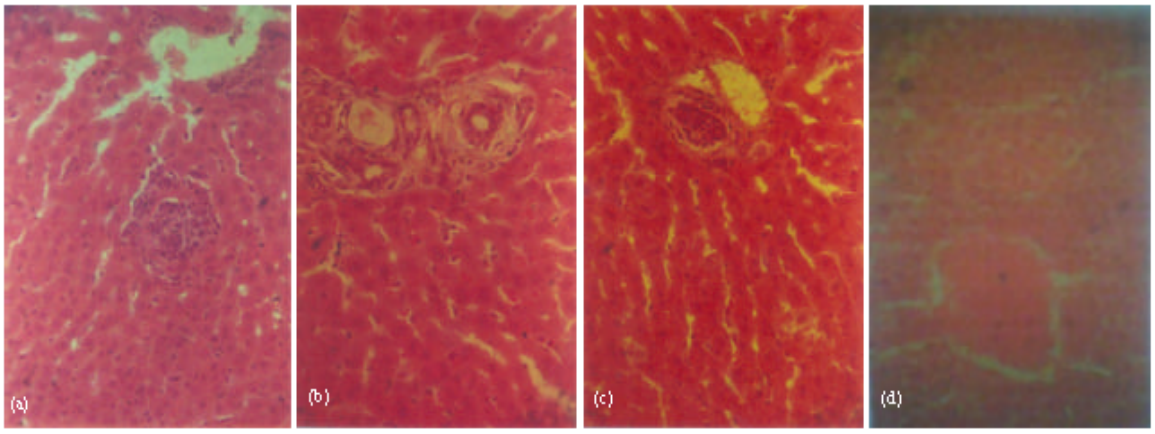



Fig. 1: Histological sections of the liver of rabbits administered varied doses of the extract a) 50 mg $kg^{-1}$, b) 100 mg $kg^{-1}$, c) 150 mg $kg^{-1}$ and Control (No extract)

increase in total protein was only observed in animals receiving medium doses of the extract. Histopathological studies of the tissue sections of the organs of male rabbits administered ethanolic extract of TTE showed no gross tissue damage when compared to those of control rabbits. The liver sections showed normal plates of hepatocytes bounded by portal tracts and having a central vein. Physical histology on post mortem also did not reveal any distortions to the liver (Fig. 1).

## DISCUSSION

The liver is the largest solid organ in the body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver. Essential functions of the liver tend to be lost in the development of hepatic disease or disorder (Bolarin and Bolarin, 2005). Drugs and toxins could cause hepatic cell damage. The damage to hepatocytes will lead to release of intracellular constituents into circulation. Serum enzyme measurement therefore, provides a valuable tool for clinical diagnosis of liver damage as well as toxicity studies (Ashafa *et al.*, 2009). Results indicate that at low dose, there was an increase in ALT activity. However, increased doses caused a decrease in ALT. AST activities increased at the low and middle doses but remained unchanged at high dose. ALT is more specific for liver disease. When, there is persistent elevation of both enzymes, then it indicates continuing destruction of liver cells, which may progress to chronic liver disease. The results as shown in Table 2 is indicative of acute hepatitis where, the serum levels of AST is higher than ALT. Alkaline phosphatase is also an indicator of liver cholestasis. The activity of ALP only increased in animals receiving high dose of the extract. The increase in serum alkaline phosphatase at high dose and AST at low and medium dose implied cellular damage (Ashafa *et al.*, 2009). Significant reduction where recorded in the serum levels of Total and Direct bilirubin. This could be due to a reduced hepatocellular function and reduced synthesis of bilirubin in the reticuloendothelial cells of the liver (Bolarin and Bolarin, 2005). There was no significant change observed in the albumin levels. The fact that the level of albumin remained unchanged on administration of the extract indicates that the secretory function of the liver was not impaired. There was a significant increase in the total protein in animals receiving medium dose of the extract. This increase may be indicative of reduced catabolism in the liver.

The presence of bioactive agents identified during phytochemical screening could also, play a role in the selective toxicity observed (Okwu, 2005). The presence of saponins in large amount in the extract are known to lyse red blood cells by destroying the erythrocyte membrane

(Sodipo and Akiniyi, 2000; Abii and Elegalam, 2007). Saponins therefore could serve as agent contributing to hepatotoxicity. Flavonoids also, found to be present have antioxidant properties (Okwu, 2004).

Results of histopathological studies do not reveal any gross damage to tissues of the liver in experimental animals receiving varied dose of the extract. However, liver function indices indicate that the extract may not be completely safe for usage.

This result is at variance with a work done by Nwogu *et al.* (2008) where, the presence of these phytochemicals in the plant extract did not adversely affect the liver function of the animals.

## CONCLUSION

The ethanolic extact of *T. tetrapleura* fruit exhibit selective toxicity in male Dutch-White rabbits. It is suggested that oral intake of the extract should be at doses $\leq 50$ mg $kg^{-1}$ bodyweight.

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