Protective Effect of Ethanolic Extract of *Palisota hirsuta* on CCl₄ Induced Hepatotoxicity

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**Abstract**

The effects of ethanolic extracts of *Palisota hirsuta* on carbon tetrachloride induced hepatotoxicity were investigated. Biochemical and histological analyses were carried out, biochemical parameters examined include; total protein, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities. Consequently, 40 rats (Wistar strain) were divided into 5 groups of 8 rats each. The animals were fed on rats’ chow and water *ad libitum*. With the exception of group 1 (positive control), all the groups were induced with CCl₄ alongside vegetable oil (1:1). Animals in groups 2, 3, 4 and 5 were administered 0, 200, 400, and 800 mg/kg body weight of drug extract respectively. Measurements of body and liver weights were recorded. Results showed increase in body weights of rats administered the extracts compared with the negative control, increase in liver weights were observed in all the rats induced with CCl₄. Reductions in total bilirubin, ALT, AST and ALP concentrations of rats administered the drug extracts compared with the negative control (group 2) indicate the ameliorative effects of the extract especially at the 400 & 800 mg/kg dose levels. Histological examination of the liver of the rats in the negative control group showed periportal inflammatory cell infiltration and hepatocyte fatty degeneration, these effects were slightly abated on administration of the drug extract. This work therefore demonstrates the hepatoprotective effects of the ethanolic leaf extract of *P. hirsuta*.

**Keywords**: Ethanolic Extract, Palisota hirsuta, Carbon Tetrachloride, Induced Hepatotoxicity, Wister Rats

1. Introduction

The liver plays a central role in transforming and clearing chemicals and is susceptible to toxicity from these agents. Certain medicinal agents e.g. tetracycline, sulphonamides etc., when taken in overdose and sometimes when introduced within therapeutic ranges may injure the liver. Chemical like CCl₄ and some herbal remedies may cause liver injury. Liver disorder is one of the world’s major problems. Despite its frequent occurrence; high morbidity and high mortality associated with it, therapeutic management is currently inadequate. So far no known therapy has successfully prevented the progression of hepatic disease. Most conventional drugs used for managing these disorders often have serious side effects (Aliyu et al, 1995 and Friedman et al, 2003).

Liver injury due to chemicals or infections agents may lead to progressive liver fibrosis and ultimately, cirrhosis and liver failure. The use of plants and plant products for treatment of diseases has brought about a new breakthrough in science today. In recent years, the use of medicinal plants for the treatment of liver damage or diseases has been on the increase all over the world. These plants are easily available, although these plants are believed to be harmless and free from serious adverse reactions more research is needed to ascertain the safe use of this plants. Millions of people in various traditional systems including Nigeria, have resorted to the use of medicinal plants to treat ailments. This could be as a result of the high cost of orthodox health care, or lack of faith in
it or may be as a result of the global shift towards the use of natural rather than synthetic products (Omonkhua & Onoagbe, 2008).

*Palisota hirsuta* K.Schum, (family, Commelinaceae) is a tropical West African plant. It is a robust herb found in forest regrowths, it is about 2-4 m high. Phytochemical analysis of the leaves reveals the presence of alkaloids, flavonoids tannins, and terpenoids. Tannins and flavonoids are the most dominant constituent (Woode et al, 2009). Some works have been done to determine the medicinal potential of this plant e.g. Anani et al, (2000) and Hudson et al (2000) reported the antiviral activity of this plant against *Herpes simplex* and poliovirus; Benson et al (2008) observed that the methanolic extract and the flavonoids isolated from the leaves has sexual stimulant effects in rats. Ethanolic extract of the roots of *P. hirsuta* have been observed to have anti-inflammatory and antipyretic effects (Boakye-Gyasi et al, 2008).

*P. hirsuta* is a common plant used widely in West African folklore medicine for treating liver diseases and other ailments. This invariably proffers the need for a scientific research on the curative and protective properties of this plant. This work was done to validate the claims of some traditional health practitioners on the medicinal properties of this plant.

2. Materials and Methods

2.1. Animals

Twenty-five male wistar strains of albino rats (180-200 g) used for this study were purchased from Anatomy Department, University of Benin. They were divided into five groups of 5 animals each and kept in separate cages. All animals were fed with commercially formulated rat feed and water *ad libitum* and were allowed to acclimatize for two weeks. Their cages were cleaned daily; food and water were also changed daily.

2.2. Chemicals

All chemical used in the study were of analytical grade.

2.3. Preparation of Ethanolic Extract

The leaves of *P. hirsuta* were collected at Ugbowo, Benin City, Edo State, Nigeria, and were identified at the department of Plant Biology and Biotechnology, University of Benin, Edo State. Nigeria. The leaves were sun-dried pulverized and sieved. 25 g of the powdered leaf was dissolved in 250 ml ethanolic extract for 48 hours. The filtrate was evaporated to dryness at room temperature in a rotary evaporator.

2.4. Experimental Design

Animals were randomly assigned to groups, each with similar body weights. The CCl₄ model described by Obi et al (1998) was employed for inducing liver damage [0.5 ml/kg i.e. CCl₄ diluted in vegetable oil (1:1)].

- **Group 1 (Positive control):** Animals in this group received 0.5 ml/kg body weight groundnut oil only.
- **Group 2 (Negative control):** Animals in this group were induced with CCl₄: groundnut oil (1:1), 0.5 ml/kg
- **Group 3 (P/200 mg/kg):** Animals in this group received CCl₄: groundnut oil (1:1) alongside 200 mg/kg ethanolic extract of *P. hirsuta* for 7 days.
- **Group 4 (P/400 mg/kg):** Animals in this group received CCl₄: groundnut oil (1:1), alongside 800 mg/kg ethanolic extract of *P. hirsuta* for 7 days.
- **GROUP 5 (P/800 mg/kg):** Animals in this group received CCl₄:groundnut oil (1:1) alongside 800 mg/kg ethanolic extract of *P. hirsuta* for 7 days.

All the animals were allowed three accesses to food and water. Weekly measurements of weights were recorded.

2.4.1. Blood and Tissue Sample Collection

At the end of the treatment, blood samples were collected by direct cardiac puncture into sterile containers without anticoagulant. The liver from both control and test animals were excised, and a part, sliced and fixed in 10% buffered formaldehyde solution and used for histological examination.

2.4.2. Biochemical Analysis

Biochemical analysis were carried out to determine the serum concentrations of protein, albumin and total bilirubin and the activities of liver enzymes such as AST, ALT and ALP using diagnostic kits (Randox laboratories Crumlin, Co. Antrim, UK). Total protein was determined by the Biuret method (Tietz, 1995), albumin by the bromocresol green method (Doumas et al, 1971). Bilirubin was estimated by the method described by Jendrassik and Grof (1938). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated by the method of Reitman & Frankel (1957), alkaline Phosphatase (ALP) by the phenolphthalein monophosphate method (Babson, 1965).

Histological examinations were done using the method of Humason (1962). The liver tissues were fixed in 10%
neutral formalin dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

2.5. Statistical Analysis

All data were expressed as mean ± SEM. One way analysis of variance was used to test for difference among all the groups. Duncan’s multiple range tests was used to test for significant differences among the means. A P-value of <0.05 was considered statistically significant.

3. Results

All results are shown in Figures 1-7.

Results showed weight loss of animals in group 2 which were administered CCl₄ but not treated with P. hirsuta extracts. Increase in weight was observed in the group administered the extract.

Liver weight of test rats were significantly (P < 0.05) lower than the liver weight of rats in the negative control group, and significantly higher than those in the positive control group.

Induction with CCl₄ (as seen in the NC group) produced a reduction in total protein (Figure 3) and ALP levels (Figure 7) and an increase in ALT and AST activities (Figure 5 and 6) compared with the positive control. Albumin and total bilirubin (Figure 3 and 4) concentrations were not significantly altered in both test and control animals. AST and ALT activities were normalized on administration of the drug extract. AST and ALT activities at the 400 and 800 mg/kg dose levels were not significantly altered when compared with the positive control but were significantly reduced when compared with the negative control rats.
4. Discussion

Carbon tetrachloride is used as hepatotoxic agent in animal research work to study the hepato-curative agent in plants and other compounds (Aliyu et al., 1995). Treatment with CCl₄ successfully induced liver damage. Increases in liver weight, total bilirubin, ALT, AST and ALP concentrations and reductions in total protein and albumin concentration indicate liver malfunction. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an induction of the toxic effect of that chemical (Orisakwe et al., 2003). The increase in liver weight on induction with CCl₄ was ameliorated on administering the ethanolic extract of *P. hirsuta* (Figure 2). In addition, there were significant losses in weights of rats in group 2 (induced with CCl₄, drug extract not administered). But there were no losses in weights of rats induced with CCl₄ and administered different dose levels of the extract (Figure 1).

Any change in the concentration of serum protein and albumin indicates a change in the normal liver function (Ahmad et al., 1992). The reductions observed in total protein concentration (Figure 3) were ameliorated only at the 400 and 800 mg/kg dose levels. Similarly increases in total bilirubin concentrations (Figure 4) and in the activities of liver enzymes such as ALT, AST, and ALP (Figures 5, 6 and 7) on induction with CCl₄ were also ameliorated mainly at 400 and 800 mg/kg dose levels. This demonstrates the dose dependant hepatoprotective effect of the ethanolic extract of *P. hirsuta* leaves. This hepatoprotective effect can be attributed to high content of flavonoids and terpenoids (Woode et al., 2009). Flavonoids have been reported to exhibit antioxidant activity (Ramanathan et al., 1989). CCl₄ treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis (Obi et al., 1998), natural antioxidants like flavonoids and terpenoids may counter the effects of these free radicals thereby producing an antihepatotoxic effect.

5. Conclusion

The results of this study shows the protective effects of ethanolic extracts of *P. hirsuta* on CCl₄ induced liver damage. It was observed that the hepatoprotective effects were dose-dependent with greater effects at higher doses of administration. This hepatoprotective effects can be attributed to the presence of natural antioxidants e.g. flavonoids, present in the leaves of this plant. More research work is needed however, to ascertain its mechanism of action.

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