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ACUTE TOXICITY STUDY OF METHANOLIC EXTRACT OF *ASPARAGUS PUBESCENS* ROOT IN RATS

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The acute toxicity study of methanolic extract of Asparagus pubescens root was studied on rats. The indices of the study were the liver enzymes (transaminases), cholesterol, creatinine and urea serum levels as well as the ionic analysis. Both alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) showed a significant ($p<0.01$-0.001) non dose-dependent increases in serum levels. There was a dose-dependent increase in cholesterol serum level (p+) and Hydrogen carbonate ($HC0_3^-$) ions showed non-discriminatory rise relative to control while converse was correct for Sodium (Na^+) and Chloride (Cl^-) ions. The physical signs of toxicity ranged from decreased motor activity, loss of appetite to increased respiratory rate which was followed by restlessness, gasping and death. The effects may in part be due to its chemical (steroid) constituent.

Keywords: *Asparagus pubescens, Acute toxicity, Methanolic extract, Rat*

INTRODUCTION

Several numbers of plants have been screened for contraceptive activities in an attempt to replace hormonal contraceptives. Some have shown promising activity (Farnsworth *et al*. 1980; Shukia *et al*., 1988; Okwuasaba *et al*,1991). One of these plants is Asparagus pubescens Bak (Liliaceace). It has been reported that most contraceptive agents are in the liver through biochemical process of ring reduction, inactivation and conjugation L before elimination either through the bile or the kidney (Harper et al., 1979). In addition, these agents are known to interfere with the integrity of the liver and kidney to varying degrees (Elias, 1984).

Nwafor *et al.*, (1998) have reported that the methanolic extract of Asparagus pubescens possesses contraceptive and non -estrogenic effects, however, he did not elucidate the acute toxicological effects in these organs of metabolism. The present study was designed to investigate the acute toxicity of the extract on these organs of metabolism.

MATERIALS AND METHODS

Preparation of extract: The plant material used in this study was collected from rocky hills and valley of

Jos Metropolis, Plateau State between the months of April and May 2000. The plant was identified and authenticated by Dr. S.S. Sanusi, Department of Botany, University of Maiduguri. Specimen vouchers (CMS 020) were made and deposited at the Department of Pharmacology Laboratory, University of Maiduguri.

The dried root was pulverized by grinding using pestle and mortar. Then, 59g of the ground root was subjected to exhaustive soxhlet extraction in methanol (250 ml) for 72h at 60℃. This gave a mean yiel d 1 5 0.43 g w/w of extract. The extract was stored at -4℃ from where it was used when required.

Animal stock: Adult albino rats (Wistar strain) weighing 160-200g were used in this study. All the animals were housed in a cross-ventilated room (22 ± 2.5℃), 12h light 12h dark cycle) and were fed with standard growers marsh feeds (Sanders Nig. Ltd. Maiduguri) and water ad-libitum.

Acute toxicity study: The animals were divided into six groups of six rats per cage . Group 1 was administered with normal saline (5m1/kg, p.0). Groups 2-4 received 250-1000mg/kg (p.0) respectively. Group 5 was given carbon tetrachioride (3m1/kg,Sc), Group 6 was pretreated with extract (SOOmg/kg,p.o), lh later, carbon tetrachioride was administered. The animals were observed for physical signs of toxicity for 24h. The blood samples were collected and centrifuged (5000rpm) and clear sera was separated and collected for the following investigations. Serum transaminases (ALT & AST), cholesterol, creatinine, urea and electrolytes (sodium, potassium, bicarbonate and chloride). These biochemical parameters were measured in the diagnostic Laboratory of Department of Chemical Pathology, University of Maiduguri Teaching Hospital Maiduguri, Nigeria.

Statistical analysis: Results were expressed as the mean value ±S.E.M and significance was determined by Student's t-test. A probability level of less than 5% was considered significant.

RESULTS

Acute toxicity: The physical signs of toxicity which ranged from decreased motor activity, loss of appetite to increased respiratory rate which was followed by restlessness, gasping and death. The median lethal dose (LD 2700 173mg/kg) showed no variation from our earlier report (Nwafor *et al,* 1998).

Table1: Effect of ethanolic extract of *Asparagus pubescens* root on transaminases (AST& ALT) levels in rats.

Dose (mg/kg)	AST(Mmol/L)	ALT(Mmol/L)
Control	85.83 ± 0.44	25.00 ± 0.10
250	130.66± 0.23**	44.00± 0.31**
500	82.33 ± 0.3 5*	26.33± 0.11**
1000	102.50 ± 0.01*	38.66 ± 0.05*
CCL_4 (3ml/kg)	210.50± 1.30**	131.33 ± 1.10
CCL_4+ 500AP	179.16± 1.02**	126.90±1.01**

*Significance relative to control: *p<0.01; **p<0.001; AP=Asparagus pubescens n=6*

Biochemical analysis: The results obtained were not dose-dependent. At the lowest dose tested (250mg/kg), there were rises in both ALT and AST levels. At 500mg/kg, the AST level was significantly lowered while the increase in ALT level was marginal. At 1 000mg/kg, there were increased AST and ALT levels relative to control. Carbon tetrachloride (toxicant) showed elevated serum level (p<0.001) relative to control. However, in the presence of 500mg/kg, the serum levels of both enzymes were attenuated (Table 1).

Table 2 showed mean serum levels of cholesterol, and kidney profiles. There was a dose-dependent increase in cholesterol level. Carbon tetrachloride (CCL decreased the cholesterol level; however, in the presence of extract (500mg/kg), it was slightly elevated. On creatinine level, there were no regular pattern of changes in the serum levels, while the lowest dose increased the serum level , the highest dose slightly lowered it with no significant change at the medium dose. However, CCL increased the creatinine level, this was higher in the presence of extract. Urea showed a non dose- dependent increase in serum level.

Table 2: Effect of methanolic extract of *Asparagus pubescens* root on cholesterol, creatinine and urea

levels in rats.

Dose (mg/kg)	Cholesterol (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control	1.28 ± 0.14	89.50 ± 0.09	2.37 ± 0.16
250	1.63 ±0.10	96.50 ± 012	4.18 ± 0.01^{d}
500	1.87 ±0.11^{c}	89.00 ± 0.13	3.17 ± 0.05a
1000	2.05 ± 0.16^{b}	87.00 ± 0.05	4.25 ± 0.24^{a}
CCL_4 (3ml/kg)	0.78 ± 0.10^{c}	96.00 ± 0.41^{d}	3.73 ± 0.20a
CCL_4+500AP	0.90 ± 0.13^{a}	111.00 ± 0.23^{d}	3.67 ±0.11^{a}

Significance relative to control: $^{a}p<0.01$; $^{b}p < 0.02$; $^{c}p<0.05$; $^{d}p<0.001$; n=6

Table 3: Effect of *Asparagus pubescens* root on ionic levels in rats

Dose (mg/kg)	Na^+	K^+	HCO_3^-	Cl^-
Control	130.83 ±0.15	6.10 ± 0.01	19.16 ± 0.12	97.50 ± 0.02
250	140.67 ± 0.01	7.02 ± 0.21a	22.33 ± 0.14^{c}	108.67 ± 0.11^{c}
500	143.67± 0.23^{c}	7.07 ± 0.05^{C}	21.33 ± 0.06^{o}	113.0 ± 0.23^{c}
1000	143.67 ± 0.04^{C}	6.92 ± 0.30^{b}	22.17 ± 0.14^{c}	110.33 ± 0.14^{c}
CCL_4	136.33 ± 0.13^{C}	6.78 ±0.04^{C}	19.00 ± 0.21	105.66 ± 0.23^{c}
CCL_4 + 500AP	138.67 ± 0.210	6.60 ± 0.22	16.83 ± 0.07	112.37 ± 0.05^{c}

Significance relative to control: ap< bp<005; ℘p<00 01 n=6

The effects of the extract on electrolyte levels were as shown on Table 3. There were increased sodium, bicarbonate and chloride serum levels; however, potassium did not show much change. In the presence of CCL sodium (Na^+) and chloride (CU) levels were increased while bicarbonate (HCO_3^-) showed some decrease, potassium (K remained fairly unchanged.

DISCUSSION

Carbon tetrachloride (CCl_4 is a typical hepatotoxin causing centrilobular necrosis. The mechanism responsible for CCL associated liver damage include the haemolytic cleavage to C-CL bond yielding CCL and CU free radicals at the site where membrane proteins having —SR groups and membrane component of liver endoplasmic reticulum occur (Recknagel, 1983., Snvastava *et al*., 1997). ALT is a hepatospecific enzyme that is principally found in the cytoplasm of rats (Benjamin, 1978; Ringler and Dabich, 1979).

AST is an enzyme that is present in high quantities in the cytoplasm and mitochondria of liver, also present in the heart, skeletal muscle, kidney and brain (Benjamin, 1978; Ringler and Dabich,1979) It is known that increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture (Wittwer and Bohmwald, 1986; Benjamin, 1978). The extract caused increase in both ALT and AST serum levels with that of AST higher as reflected in number of organs capable of releasing it. Hepatotoxin (CCL caused an astronomical increase in these enzymes, however, this effect was ameliorated by the presence of extract indicating a possible antihepatotoxic potential. It is likely also that the increase in transaminases might in part be due to its (extract) estrogenic (steroid) properties since steroid is known to interfere with the integrity of liver and kidney (Nwafor *et al.*, 1998; Elias, 1984).

The dose-dependent increase in cholesterol is in part a reflection of steroidogenetic effect of the extract (Nwafor *et al.,* 1998). There is an unsteady increase in creatinine level as compared to urea. However, these non-protein nitrogens which are functions of kidney integrity do not affect the physiological functions as much as hydrogen (H^+) and potassium (K^+) ions (Guyton, 1981).

On the ionic analysis, the concentration of intracellular ion (K and extracellular ions (Na', CU and I{C0 do not raise any physiological function alteration since the serum concentrations of K and HC0 were not much affected (Anderson and More, 1980).

In conclusion therefore, the extract may in part possess some antihepatotoxic and steroidogenic effects. The results of non-protein nitrogen compounds and ionic analysis showed that the integrity of the kidney was not compromised. However, work is continuing on the histopathology as way of elucidating its organ of localization.

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