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Acute Toxicity Potential of Methanolic Extract of *Smilax kraussiana* Leaves in Rats

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Abstract: The acute toxicity study of methanolic extract of *Smilax kraussiana* leaves was investigated in rats. The parameters studied were the liver enzymes (transaminases), cholesterol, protein, 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 decreases in serum levels. There were also non dose dependent decreases in cholesterol, protein and creatinine serum levels ($p<0.001$). The serum level of urea did not indicate a significant change. Sodium and chloride ions showed discriminatory rise in serum levels especially in high doses ($p<0.05$-0.001) while the potassium serum level remained statistically insignificant. The physical signs of toxicity ranged from decreased motor activity with loss of appetite to increased respiratory rate, restlessness, gasping and death. The median lethal dose was calculated to be (240.00±49.44 mg kg^{-1}). The extract may in part possess some antihepatotoxic effects.

Key words: *Smilax kraussiana*, acute toxicity, methanolic extract, antihepatotoxic, leaves, rats

INTRODUCTION

In ethnomedicine, several plants are employed for the treatment of infertility (Nwafor *et al*., 2004). Many of them have been screened for contraceptive activities in an attempt to replace hormonal contraceptives. Some have shown promising activity while others have limited usage based on their toxicities (Farnsworth *et al*., 1980; Nwafor *et al*., 1998).

It has been reported that most fertility regulating agents are metabolized in the liver through a biochemical process of ring reduction, inactivation and conjugation before elimination either through the bile or the kidney (Harper *et al*., 1984). In addition, these chemicals are known to interfere with the integrity of the liver and kidney to varying degrees (Elias, 1984).

One of the plants used in the treatment of infertility is *Smilax kraussiana* (Liliaceae). It is used for the treatment of infertility in the Eastern Tanzania tribes of South Africa (Chhabra *et al*., 1993), while it enjoys good reputation for the treatment of inflammation among the Ibibios of South Eastern Nigeria (Okokon, Personal Communication, 2005). Although Iwu and Anyanwu (1982) reported on its anti-inflammatory properties, no report to our knowledge has been seen on these organs of metabolism. Therefore, the present study was designed to investigate the acute toxicity of the extract on these organs of metabolism. Based on the results that would be obtained, further research on fertility regulating potential of the plant would be investigated.

MATERIALS AND METHODS

Preparation of extract: The plant material used was collected from Uruan in Uruan Local Government of Akwa Ibom State, Nigeria, in March 2005. The plant was identified and authenticated by Dr. (Mrs.) M. Bassey of the Department of Botany, University of Uyo, Nigeria. A specimen voucher (UU/HER. NO 44e) has been made and deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo. The dried leaves were pulverized by grinding using pestle and mortar. Thereafter, 65 g of the ground bark was subjected to exhaustive Soxhlet extraction in 250 mL methanol for 72 h at 60°C. The extract was stored at -4°C until required for use.

Animal stock: Adult albino rats of Wistar strain weighing about 150-200 g were used for the study. All the animals were housed in a cross-ventilated room at 22±2.5°C with a 12 h light/12 h dark cycle and were fed with standard growers mash feeds (Bendel feeds Limited, Benin City Nigeria) and water ad libitum.

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Phytochemical screening: Phytochemical screening of the extract was performed according to the methods of Clarke (1975), Odebiyi and Sofowora (1978) and Trease and Evans (1989). Tests for alkaloids, saponins, tannins, terpenes, simple sugars, flavonoids, anthraquinones and cardiac glycosides were carried out.

Acute toxicity studies

Median lethal dose (LD_{50}): For determination of the median lethal dose (LD_{50}), mice were divided into seven groups each containing 7 animals. The extract was administered intraperitoneally (ip) in a dose range of 0.01-1.0 g kg^{-1} body weight. A maximum of 0.15 mL was given. All the animals were observed for physical signs of toxicity for 24 h. The LD_{50} was calculated by the method of Miller and Tainter (1944).

Biochemical analysis: Biochemical analyses were carried out in adult albino rats. The rats were divided into six groups of 6 rats per cage. Group I animals were administered with 2 mL kg^{-1} normal saline (po). Groups 2 to 4 animals received extract (24, 48 and 72 mg kg^{-1} body weight of the animal, respectively), while group 5 rats were given carbon tetrachloride (CCL_4, 3 mL kg^{-1} Sc. dissolved in corn oil 1:1 v/v). Animals in group 6 were pretreated with 48 mg kg^{-1} body weight of the extract (po) and 1 h later, CCL_4 was administered subcutaneous (Sc) to the animals. The animals were observed for 24 h for physical signs of toxicity. The blood samples were collected by sacrificing the animals following ether anaesthesia and centrifuged at 5000 rpm for 15 min and clear sera was separated and collected for the following investigations: Serum transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST), cholesterol, creatinine, urea, protein and electrolytes (sodium, potassium and chloride). These biochemical parameters were measured in the Diagnostic Laboratory of the Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo, Nigeria, by standard methods.

Statistical analysis: Multiple comparisons of mean±SEM were carried out by one way analysis of variance (ANOVA), followed by Tukey-Krammar multiple comparisons tests. A probability level of less than 5% was considered significant.

RESULTS

Acute toxicity test: The physical signs of toxicity which ranged from decreased motor activity, with concomitant increase in the movement of whiskers, loss of a appetite to increased respiratory rate were followed by restlessness, gasping and death. The median lethal dose (LD_{50}) was calculated to be 240±49.44 mg kg^{-1}.

Biochemical analysis: Generally, the results obtained were not dose-dependent. AST showed a non dose dependent decrease in serum concentration. Similarly, the decrease observed in ALT values were quite inconsistent with the dose. Carbon tetrachloride (toxicant) showed exaggerated increase in both the ALT and AST values. This effect was not significantly attenuated in the presence of extract (48 mg kg^{-1} body weight of animal) especially with AST (Table 1).

The extract also showed no significant increase in urea level. However, creatinine, protein and cholesterol showed significant decrease in their respective levels relative to control (Table 2). In the presence of the toxicant, the extract failed to reverse the values to the normal or control levels.

With the exception of potassium (K^+) ions which remained fairly statistically insignificant, Sodium (Na^+) and chloride (Cl^-) ions showed a non dose-dependent increases in the ionic levels. (Table 3) increases were statistically significant ($p<0.001$). These effects were accentuated in the presence of carbon tetrachloride (CCL_4).

Phytochemical screening: Phytochemical screening of the extract showed that it contained saponins, tannins, simple sugars, cardiac glycosides and flavonoids. Alkaloids were however absent.

Table 1: Effect of methanolic extract of *Smilax kraussiana* leaves on transaminases (AST and ALT) levels in rats

Dose (mg kg^{-1})	AST (mMol L^{-1})	ALT (mMol L^{-1})
Control	58.67±0.86	12.00±1.90
24.00	37.00±0.07[b]	6.83±0.22[b]
48.00	37.00±0.70[b]	8.66±0.32[a]
72.00	40.17±1.05[b]	12.50±1.80
CCL_4	86.00±0.37[b]	71.66±0.77[b]
48.00+CCL_4	82.67±1.15[b]	76.33±1.86[b]

Values represent Mean±SEM (n = 6), Significance relative to control: [a]$p<0.01$; [b]$p<0.001$, CCL_4 = Carbon tetrachloride (3 mL kg^{-1})

Table 2: Effect of methanolic extract of *Smilax Kraussiana* leaves on urea, creatinine, protein and cholesterol levels in rats

Dose (mg kg^{-1})	Urea (mMol L^{-1})	Creatinine (mMol L^{-1})	Protein (mg dL^{-1})	Cholesterol (mg dL^{-1})
Control	8.80± 0.50	46.03±1.94	61.83±0.70	63.00±0.04
24.00	8.56±0.36	42.01±0.51	49.66±0.20[b]	55.50±0.18[b]
48.00	9.63±1.06	36.66±1.07[b]	57.16±0.50	55.33±0.10[b]
72.00	9.00±1.34	43.36±0.82	62.50±2.52	65.00±1.07
CCL_4	10.15±0.94	39.31±0.60[a]	50.83±0.28[b]	90.66±1.20[b]
48.00+CCL_4	9.70±0.75	47.33±1.70	74.16±0.24[b]	126.16±1.30[b]

Values represent Mean±SEM (n = 6), Significance relative to control: [a]$p<0.01$; [b]$p<0.001$, CCL_4-Carbon tetrachloride (3 mL kg^{-1})

Table 3: Effect of methanolic extract of *Smilax Kraussiana* leaves on ionic levels in rats

Dose (mg kg^{-1})	Na^+ (mMol L^{-1})	K^+(mMol L^{-1})	CL- (mMOl L^{-1})
Control	131.16± 0.04	5.25± 0.32	95.83±0.10
24.00	138.33±1.01[a]	3.91±0.18	85.66±0.12[b]
48.00	214.83±0.85[b]	4.71± 0.40	111.83±0.90[b]
72.00	108.33±0.80[b]	5.46±0.64	106.00±0.04[b]
CCL_4	151.50±1.80[b]	4.70±0.36	102.00±0.09[b]
48.00+CCL_4	155.16±2.30[b]	6.86±0.54	112.50±1.02[b]

Values represent Mean±SEM (n = 6), Significance relative to control: [a]$p<0.05$; [b]$p<0.001$, CCL_4-Carbon tetrachloride (3 mL kg^{-1})

DISCUSSION

Carbon tetrachloride (CCL_4) is a typical hepatotoxin causing centrilobular necrosis. The mechanism responsible for CCL_4 associated liver damage include the haemolytic damage to C-Cl bond yielding CCl_3 and CL^- free radicals at the site where membrane proteins having-SH groups and membrane components of liver endoplasmic reticulum occur (Recknagel, 1983; Srivastava *et al*., 1997; Nwafor *et al*., 2004).

Aspartate aminotransferase (AST) is an enzyme that is present in high quantities in the cytoplasm and mitochondria of liver, heart, skeletal muscle, kidney and brain while alanine aminotransferase (ALT) is a hepatospecific enzyme that is principally found in the cytoplasm of rats (Benjamin, 1978; Ringler and Dabich, 1979). It is known that increase in the enzymatic activity of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in the serum directly reflects a major permeability or cell rupture (Benjamin, 1978; Wittwer and Bohmwald, 1986). The extract produced a non dose-dependent decrease in both AST and ALT. The toxicant (CCL_4) produced significant rise in serum concentrations of both enzymes (ALT and AST) which were not attenuated in the presence of the extract especially alanine aminotransferase (ALT). The relatively higher rise in the serum level of AST observed may in part be due to nonspecific organ nature of the enzyme. The lower serum level of ALT may suggest that the extract may not be toxic to the liver and could explain its high acceptability and relatively low/non toxic effects observed among the consumers. The phytochemical screening of the plant revealed that it does not contain sterol and/or triter penes. It is known that sterol (steroids) and triter penes possess estrogenic properties which interfere with the integrity of liver and kidney (Elias, 1984; Nwafor *et al*., 1998; Jacks *et al*., 2004). Therefore, the absence of these compounds further indicate a possible antihepatotoxic potential of the extract.

The non-protein nitrogen compounds include urea, uric acid, creatinine and a few other less important compounds. These are end products of protein metabolism and must be removed continually to ensure continued protein metabolism in the cells (Guyton, 1981). Urea, creatinine, protein and cholesterol showed slight to significant decreases in serum concentrations which suggest a nontoxic potential of the extract.

On the ionic analysis, the concentration of intracellular potassium (K^+) ion did not indicate a rise in serum concentration. However, the concentrations of extracellular ions of Na^+ and Cl^- showed rise in their serum levels. Regulation of sodium concentration in plasma and urine is related to regulation of total body water. Hypernatremia is usually associated with an excess accumulation of body water. Intrinsic factors causing hypernatremia are increased aldosterone production or decrease in its inactivation. Steroids also cause hypernatremia (Ruppel and Scanlen, 1995). Therefore, the rise in sodium serum level way not be unrelated to intrinsic factors.

In conclusion therefore, the extract may in part possess some antihepatotoxic effects. The results of non-protein nitrogen compounds and ionic analysis showed that the integrity of the kidney was not can promised. The low toxic level may have encouraged its increased utility by the local populace.

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REFERENCES

Benjamin, M.N., 1978. Outline of Veterinary Clinical Pathology. University Press, IOWA, USA., pp: 229-232.

Chhabra, S.C., R.L.A. Mahunnah and E.N. Mshiu, 1993. Plants used in traditional Medicine in Eastern Tanzania vi. Angiosperms (Sapotaceae to Zingiberaceae). J. Ethnopharmacol., 39: 83-103.

Clarke, E.G.C., 1975. Isolation and Identification of Drugs, Vol. 2 Pharmaceutical Press, London, pp: 905.

Elias, E., 1984. Jaundice. In: Oxford Textbook of Medicine ECRS Edn. (Weatherhall, D.J., J.G.G. Ledingham and D.A. Warrel Eds.), Oxford University Press, Oxford, England, pp: 12. 175-00012.182.

Farnsworth, N.R., A.S. Bingel and D.D. Soejarto, 1980. Prospects for higher plants as a source of useful fertility regulating agents for human use. Symposium on Recent Advances in Fertility Regulation, 2-5 September, 1980. Beijing, China, pp: 330-364.

Guyton, A.C., 1981. Textbook of Medical Pathology, 6th Edn. W.B. Saunders Company, Philadelphia, USA., pp: 463-474.

Harper, H.A., V.W. Rodwell and P.A. Mays, 1984. Chemistry and Functions of the Hormones. In: Review of Physiological Chemistry. 17th Edn. Lange medical Publications, California, USA., pp: 548.

Iwu, M.M. and B.N. Anyanwu, 1982. Phytotherapeutic profile of Nigerian herbs I. Anti-inflammatory and anti-arthritic agents. J. Ethnopharmacol., 63: 263-274.

Jacks, T.W., P.A. Nwafor and A.U. Ekanem, 2004. Acute toxicity study of methanolic extract of *Pausinystalia macroceras* stem-bark in rats. Nigerian J. Exp. Applied Biol., 5: 59-62.

Miller, L.C. and M.L. Tainter, 1944. Estimation of LD_{50} and its errors by means of log probit graph paper. Proc. Soc. Exp. Biol. Med., 57: 261-264.

Nwafor, P.A., F.K. Okwuasaba and O.O. Onoruwe, 1998. Contraceptive and non-estrogenic effects of methanolic extract of *Asparagus pubescens* root in experimental animals. J. Ethnopharmacol., 62: 117-122.

Nwafor, P.A., T.W. Jacks and O.O. Longe, 2004. Acute toxicity study of methanolic extract of *Asparagus pubescens* roots in rats. Afr. J. Biomed., Res. 7: 19-21.

Odebiyi, O. O. and E.A. Sofowora, 1978. Phytochemical Screening of Nigerian Medicinal plants. Lloydia 41: 234-235.

Recknagel, R.O., 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci., 33: 401-408.

Ringler, D.H. and L. Dabich, 1979. Haematology and Clinical Biochemistry. In: The Laboratory Rat. Baker, J., J.R. Lindsey and S.H. Weisbroth (Eds.), Academic Press London, 1: 105-118.

Ruppel, G.L. and Scanlan, C.L., 1995. Solutions, Body Fluids and Electrolytes. In: Eglan's Fundamentals of Respiratory Care. Ed. Craig L. Scanlan, Charles Bud Spearman, Richard L. Sheldon and N.Y. Mosby, pp: 288-302.

Srivastava, S., A.K. Srivastava, G.K. Patnaik and B.N. Dhawan, 1997. Effect of Picroliv and Silymarin on Liver regeneration of Carbon tetrachloride treated rats. J. Pharm. Res., 2: 9-13.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. English Language Book Soc. 13th Edn. Bailliere Tindall. London, pp: 683-684.

Wittwer, F.M. and L.H. Bohmwald, 1986. Manual de Patologia Clinica Veterinaria. Publicia, Santiago, Chile pp: 53-93.