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Full Length Research Article

Effects of chromatographic fractions of *Euphorbia hirta* on the rat serum biochemistry.

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ABSTRACT
The effects of the chromatographic fractions of *Euphorbia hirta* **Linn** on the serum biochemical parameters in rats were investigated. The ethanolic extract of this plant was subjected to chromatographic separation using the vacuum liquid chromatographic technique, a modified form of classical column chromatography. With the aid of thin layer chromatography, six fractions of this plant were obtained and were administered to rats in graded doses of 400mg/kg, 800mg/kg and 1600mg/kg orally for fourteen days. Some fractions of this plant caused significant increase in the levels of total protein, albumin, globulin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin, creatinine, and blood urea nitrogen (BUN). Some fractions also caused significant decrease in the level of conjugated bilirubin. The result from this study thus showed that some chromatographic fractions of *Euphorbia hirta* have potentially deleterious effects on the serum chemistry of rats; therefore caution should be exercised in the use of *Euphorbia hirta as* medicinal plant. It also means that the presence of this plant in the pasture could serve as a source of toxicosis to grazing animals.

Keywords: *E.hirta;* chromatographic fractions; serum biochemistry; rats; toxicity

INTRODUCTION

Euphorbia hirta belongs to the family Euphorbiaceae which is a large family of trees, shrubs and herbs, of rainforest, Guinean, Soudanian, and xereophylactic habitats [Burkill, 1994]. Although plants of this family have important economic uses as foodstuffs, medicinal, and industry, particularly as sources of rubber and timber, nonetheless most members of this family are poisonous [Garner, 1957; Evans and Kinghorn, 1975; Burkill, 1994; Adedapo, 2002].

The plant contains relatively abundant white latex. The latex is capable of causing dermatitis. The analysis of the latex has revealed 1-inositol, pyrrogalic and catechuic tannins and the alkaloid xanthoramnine [Oliver, 1960]. Gupta and Gargi [1966] found taxerol, frieldelin, P-sitosterol, myricyl alcohol, ellergic acid and hentriacontane in extracts of the stem whilst Blanc *et al,* [1972] reported ellagic, gallic, chlorogenic and caffeic acids, kaempferol, quercitol, quercitrin (as a genin of a heteroside), and a number of amino acids. The use of latex on warts, whitlows and the like is worldwide [Hartwell, 1969]. The plant has a diuretic and purgative action and is also known to have a remedy for inflammation of the respiratory tract, and for asthma as it has a special reputation for causing bronchial relaxation [Johnson *et al,* 1999]. The plant shows antibiotic activity [Sofowora, 1993]. A number of substances have been detected in the plant; tannins, gallic acid,

quercetin, phenols, phyto-sterols, alcohols, alkaloids etc. [Kerharo and Adam, 1974; Burkill, 1994]. The alcoholic extract of the whole plant had an anticancer action against Friemd leukaemia virus in mice [Burkill, 1994]. It further showed hypoglycaemia action in albino rats and an antiprotozoal effect [Dhar *et al,* 1968]. The plant has also been shown to have anthelmintic activity [Ayensu, 1979; Sofowora, 1993; Adedapo *et al,* 2005].

This plant grows wild among the pastures in Ibadan and its environs hence livestock animals are often exposed to this plant. The study was carried out to provide insight into the activities of chromatographic fractions of *Euphorbia hirta* on the serum biochemistry of rats because biochemical changes are the earliest indicators of organic damage.

MATERIALS AND METHODS

Animals, Groupings and Experimental Design

Seventy six eight-week old albino rats (of both sexes), bred and maintained at the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan were used in this study. They were divided into seven groups. Six groups (n = 12) were dosed with six chromatographic fractions of *Euphorbia hirta while* the seventh group (n = 4) served as controls. Each fraction was dosed orally at 400mg/kg, 800mg/kg and 1600mg/kg for 14 days to 4 rats per group. The animals in the controlled experiment received only distilled water for the same number of days.

Preparation of the Chromatographic fractions of Euphorbia hirta

Fresh leaves of the plant were collected within the campus of the University of Ibadan and were identified at the Department of Botany and Microbiology, University of Ibadan. A voucher specimen was thereafter deposited at the Department of Botany and Microbiology herbarium. Some of the air dried leaves were ground into fine powder and the extraction procedure is as described by Harbone [1984]. The powdered leaves were continuously extracted using absolute ethanol in a soxhlet extractor until all the pigment was in the extract. The extract was concentrated in vacuo using a rotatory evaporator. The ethanol remaining in the extract was finally removed by placing the extract in porcelain dishes in a vacuum oven at 60^{o}C. The semi-solid extract was used for chromatography.

The crude extract was dissolved in just sufficient quantity of ethanol. Silical gel powder (TLC grade) was added to the solution with stirring until all the sample was adsorbed on silical gel and there is no free flowing liquid. The impregnated silical gel was spread on a watch glass and the solvent was allowed to evaporate in the fume cupboard on standing. The almost dried extract was afterwards dried in the oven at 60^{o}C.

Buckner filter assembly was used for the vacuum liquid chromatography. TLC grade of silical gel was used as stationary phase. The stationary phase (20g) was packed on top of a thick filter paper in the Buckner funnel. Another filter paper was placed on the column of silical gel, before the impregnated silical gel (4g) was carefully spread on it with the vacuum line on. The mobile phase comprises of a gradient ranging from hexane, ethyl acetate and methanol, with successive 10% increment in the next polar solvent. A total of 50ml was prepared for each gradient mixture. Each gradient mixture was added to the column and eluted with the vacuum line until the bed is sucked dry. The eluates were collected successfully as separate fractions.

The fractions were monitored by thin layer chromatography using silical gel GF_{254} as stationary phase and ethanol as mobile phase. The plates were visualized under UV lamp at 254nm. With this procedure, fractions with similar resolutions were pooled together bringing the number of fractions to 6. Samples were then obtained from these fractions after evaporation. These were designated fractions A-F based on their profile in terms of non-polarity to increasing polarity. In other words, while fraction A is non-polar, fraction F is polar.

Blood Samples Collection.

The animals were anaesthetized using diethyl ether and the blood samples were collected by cardiac puncture into non-heparinised clean bottles and allowed to clot. The sera were then separated from the clot and centrifuged according to groups into clean bottles for biochemical analysis.

Biochemical studies involved analysis of parameters such as total protein, serum albumin, globulin, blood urea nitrogen (BUN), bilirubin (total and direct), creatinine, and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Statistical Analysis

All data were expressed as means $\pm$ SE. The data were subjected to the pooled variance "t" test for

comparison and Duncan Multiple Range Test (DMRT) as described by Steel and Torrie [1986].

RESULTS

The results are shown in Tables 1. All fractions except E and F (800, 1600mk/kg) caused significant increase ($P<0.05$) in the level of total protein. For albumin, fractions A (400, 1600mg/kg), B (400, 1600mg/kg), D (400, 800mg/kg) and E (1600mg/kg) caused significant increase when compared with the control. In the case of globulin, only fraction A (800mg/kg) caused significant increases in the level of this parameter.

All the fractions except D (400mg/kg) and E (400mg/kg) caused significant increases in the level of ALT. All the fractions caused significant increase in the levels of ALP, AST and total bilirubin when compared with the control. Fractions A (1600mg/kg) B (1600mg/kg)), D, E (400, 1600mg/kg) and F all caused significant decrease in the level of conjugated bilirubin. For creatinine, it was only fraction D (400mg/kg) that caused a significant increase in the level of this parameter. In the case of BUN, fractions A (800, 1600mg/kg), B, C (400, 1600mg/kg), D, E (800mg/kg) and F (800, 1600mg/kg) all caused significantly increased level of this parameter.

DISCUSSION

Four of the chromatographic fractions showed an increase in the level of protein compared with the control. Five of the fractions caused an elevation of serum albumin and globulins. The increase noted in these parameters may be due to haemoconcentration. Haemoconcentration due to dehydration is reported to lead to elevation of total plasma protein through concentration in reduced blood volume [Caroll *et al,* 1965; Duncan *et al*, 1994]. One may assume that if the animals refuse to take water due to the fact that the fractions are bitter, dehydration may occur and then result in the elevation of these parameters. It was actually observed that the animals have reduced appetites, thus the possibility of the animals refusing to take water because of the bitter taste of the fractions is very high.

All the chromatographic fractions of this plant caused a significant increase in the levels of AST, ALT and ALP. Variation in the concentration of certain enzymes as measured by their biochemical activity occurs primarily as a result of elevation due to the escape of the enzyme from the disrupted parenchyma cells with necrosis or altered membrane permeability [Bush, 1991; Duncan *et al,* 1994]. Elevation in the activity of AST can be associated with cell necrosis of many tissues. For example, pathology involving the skeletal or cardiac muscle and or the hepatic parenchyma allows for the leakage of large, amounts of this enzyme into the blood [Kaneko, 1980; Duncan *et al,* 1994]. The elevation in AST produced by this plant is an indication of tissue necrosis. ALT on the other hand is present in cells. It is particularly useful in measuring hepatic necrosis, especially in small animals [Cornelius, 1989; Bush, 1991]. Since it is one of the specific assayable liver enzymes, its elevated level in this study may indicate hepatic damage by this plant. ALP is reported to be present in large number of cells but only in a few cells is the activity sufficient to be of clinical importance. It is found in liver cells and is associated with osteoblastic activity in the bone [Hoffman and Dorner, 1977; Saini and Saini, 1978; Bush, 1991]. It means that these fractions may contain the toxic component and hence cause injury to several tissues of the body.

All the chromatographic fractions of *E. hirta* caused an elevation in the level of the total bilirubin, but a decrease in the level of conjugated bilirubin. This implies that the increase in total bilirubin is associated with increase in unconjugated bilirubin. This unconjugated bilirubin is generally associated with hepatic disease and extrahepatic biliary obstruction. Extra hepatic biliary obstruction (i.e. choletasis) should be strongly considered when conjugated bilirubin is 25% of the total [Knoll, 1998]. Since unconjugated bilirubin is less than 25% of total extra hepatic biliary obstruction is therefore ruled out. The increase is caused by an increase in indirect reacting bilirubin.

Hepatocellular injury is usually accompanied by cellular swelling that can compress bile canaliculi and invariably cause some cholestasis. Cholestasis implies that components of bile occur in abnormal concentrations in blood. It may develop from extra-or intra hepatic physical obstruction of biliary flow or by abnormal secretion by hepatocytes [Duncan *et al,* 1994]. Metabolic causes of cholestasis involve alterations in uptake, conjugation, and secretion. Since unconjugated bilirubin predominates over conjugated bilirubin, this may indicates interference in conjugation process. Hepatotoxic chemicals, sepsis, anorexia are known to cause acquired metabolic cholestasis [Duncan *et al,* 1994].

Table 1:
Effects of Fractions of *Euphorbia hirta* on the serum biochemistry of rats

Parameters	Treatments																			
	Control	Fraction A			Fraction B			Fraction C			Fraction D			Fraction E			Fraction F		
		400 mg/kg	800 mg/kg	1600 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg	400 mg/kg	800 mg/kg	1600 mg/kg
Total Protein (g/dl)	63.0 ± 0.8	67.0 ± 2.0[a]	70.0 ± 8.0[a]	71.0 ± 2.0[a]	70 ± 8.0	71 ± 8.0	65 ± 0[a]	70± 8.0	71± 1.4[a]	70± 8.0	75± 4.0[a]	70± 7.0	65± 3.0	60 ± 8.0	61 ± 9.0	62 ± 5.0	66 ± 1.0[a]	62 ± 8.0	60± 8.0
Albumin (g/dl)	32.0 ± 1.6	35.0 ± 2.0	30.0 ± 4.0	40.0 ± 2.0	35 ± 0.8	32 ± 1.6	35 ± 0[a]	31± 0.8	33± 1.4	32± 0.8	35± 3.0	40 ±4.0[a]	32± 2.0	33 ± 2.0	31 ± 0.8	35 ± 0.8[a]	30 ± 2.0	30 ± 2.0	28± 0.8[a]
Globulin (g/dl)	31.0 ± 2.5	32.0 ± 0	40.0 ± 7.0[a]	33.0 ± 6.0	35 ± 7.0	39 ± 7.0	30 ± 0	39± 7.0	38 ± 0[a]	38± 8.0	40± 4.0[a]	26± 4.0	33± 2.0	24 ± 7.0	28 ± 3.0	28 ± 3.0	36 ± 2.0	35 ± 5.0	32± 8.0
Aspartate aminotransferase (AST) (IU/L)	41.5 ± 1.3	146 ± 1.6[a]	366 ± 2.8[a]	183 ± 1.6[a]	146 ± 1.6[a]	156 ± 1.6[a]	167 ± 2.0[a]	355± 4.1[a]	366± 2.8[a]	230± 3.3[a]	209± 0.8[a]	324± 2.8[a]	261± 1.6[a]	183 ± 1.4[a]	219 ± 0.8[a]	261 ± 1.4[a]	193 ± 0.8[a]	271 ± 0.8[a]	279± 2.7[a]
Alanine aminotransferase (ALT) (IU/L)	43.0 ± 0.8	62.0 ± 1.6[a]	48.0 ± 1.6[a]	94.0 ± 1.6[a]	62 ± 1.6[a]	62 ± 0.8[a]	52 ± 2.0[a]	52± 1.6[a]	67± 0.8[a]	48± 1.6[a]	39± 0.9	67± 1.4[a]	94± 0.8[a]	39 ± 2.2	52 ± 1.6[a]	62 ± 0.8[a]	62 ± 1.6[a]	52 ± 2.2[a]	60± 1.6[a]
Alkaline Phosphatase (ALP) (IU/L)	42.8 ± 0.5	180 ± 8.2[a]	175 ± 0.8[a]	200 ± 41[a]	250 ± 1.6[a]	190 ± 8.2[a]	250 ± 2.0[a]	210± 8.2[a]	245± 2.8[a]	180± 2.5[a]	190± 8.2[a]	180± 4.1[a]	190± 3.3[a]	200 ± 1.6[a]	210 ± 1.6[a]	215 ± 1.4[a]	180 ± 2.5[a]	190± 2.8[a]	200± 1.6[a]
Total Bilirubin (umol/l)	34.0 ± 1.8	68.3 ± 2.9[a]	68.3 ± 7.0[a]	103 ±13.9[a]	120 ± 13.9[a]	137 ± 14[a]	85.6 ± 17.5	103± 14[a]	119.8 ± 5.2[a]	120± 14[a]	120± 14[a]	137± 14[a]	120± 14.0[a]	120 ± 14.0[a]	154 ± 0[a]	120 ± 14.0[a]	120 ± 14.0[a]	103± 14.0[a]	99.3± 2.9[a]
Conjugated Bilirubin (umol/l)	3.4 ± 0.3	3.4 ± 0.3	3.4 ± 0.3	1.7 ± 0[a]	3.4 ± 0.4	3.4 ± 0.3	1.7 ±0[a]	3.4± 1.2	2.6± 0.5[a]	3.4± 0.3	1.7± 0.2[a]	1.7± 0.3[a]	2.6± 0.7	1.7 ± 0[a]	3.4 ± 0.7	1.7 ± 0[a]	1.7 ± 0[a]	1.7 ± 0.3[a]	1.7± 0[a]
Creatinine (umol/l)	53.0 ± 7.4	71 ± 7.4	62 ± 14.7	62 ± 7.4	71 ± 7.4[a]	53 ± 7.4	62 ± 9.0	62± 12.7	53± 7.4	62± 7.4	80± 0[a]	62± 14.7	53± 7.4	53 ± 7.4	62 ± 7.4	62 ± 7.4	62 ± 7.4	71 ± 12.7	71± 7.4
Blood Urea Nitrogen (mmol/l)	6.2 ± 0.1	6.0 ± 0.3	8.8 ± 0.3[a]	8.5 ± 0.2[a]	6.8 ± 0.3[a]	7.7 ± 0.7[a]	7.7 ± 0.4[a]	8.3± 0.2[a]	5.4± 0.1[a]	6.8± 0.4[a]	8.5± 0.3[a]	7.7± 0.2[a]	6.8± 0.3[a]	6.0 ± 0.2	6.6 ± 0.1[a]	4.6 ± 0.2[a]	5.3 ± 0.3[a]	6.5 ± 0.1[a]	7.1± 0.3[a]

Superscripted items indicate significant values from control at P<0.05.

All the chromatographic fractions of *E. hirta* also caused an elevated level of serum creatinine compared to the control. It thus shows that with prolonged exposure of animals to these fractions, renal disease could be precipitated. Five fractions of this plant caused increased level of blood urea nitrogen (BUN) compare with the control. It is therefore an indication that the urea nitrogen formed in rats poisoned by these fractions was not being adequately excreted. It should be noted that blood urea nitrogen (BUN) and creatinine are the substances in the blood most often used to assess renal function test [Iyayi and Tewe, 1998]. It is also known that any agent that precipitates nephrotoxicity can cause hepatoxicity hence extreme caution should be exercised in the use of this plant as a medicinal agent.

It is therefore obvious from this study that the plant *E. hirta* contains substances that have potential deleterious effects on serum biochemistry. Since the plant is found among pasture, livestock animals are potentially at risk and this might have serious effects on livestock production in Nigeria.

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REFERENCES

Adedapo A. A. (2002): Toxicological effects of some plants in the family Euphorbiaceae on rats. Ph.D. Thesis, University of Ibadan.

Adedapo, A. A., Shabi, O. O. and Adedokun, O. A. (2005): Assessment of the anthelmintic efficacy of the aqueous crude extract of *Euphorbia hirta* Linn in local dogs. *Veterinarski Arhiv,* 75(1): 39-47.

Ayensu, E. S. (1979): Medicinal Plants in West Africa. Reference Publication Inc. Algonac, Michigan, USA. pp. 4-7.

Blanc, P., Bertrand, P., de Saqui Sanner, G., Ane, M. (1972): Identification par chromatographie et etude spectral de quelque acides phenols, acides ellaqique, gallique, chologenique, cafeique dans une Euphorbiaceous exotique; *Euphorbia hirta*, L. Annales des Pharmacie francaises, 30: 720-721

Burkill, H. M. (1994): The useful plants of West Tropical Africa. Royal Botanical Gardens, Kew.

Bush, B. M. (1991): Interpretation of laboratory results for small animal clinicians. Blackwell Scientific Publications, Oxford.

Caroll, E. J., Schalm, O. W. and Wheat, J. D. (1965): Endotoxaemia in a horse. J. Am. Vet. Med. 46: 1300.

Cornelius, C. E. (1989): Serum enzyme activities and other markers for detecting hepatic necrosis, cholestasis or carcinoma. In: Clinical Biochemistry of Domestic Animals. Academy Press, Toronto, pp. 381-386.

Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotara, B. N., Ray, C. (1968): Screening Indian plants for biological activity. Part I. Indian J. Exptal. Biol. 6, 232-247.

Duncan, J. R., Prasse, K. W., Mahaffey, E. A. (1994): Veterinary Laboratory Medicine (Clinical Pathology). Iowa State University Press.

Evans, F., Kinghorn, A. (1975): The succulent Euphorbias of Nigeria. Part1. LLoydia 38, 363-365.

Garner, R. J. (1957): Veterinary Toxicology. Balliere Tindall, London.

Gupta, D. R., Gargi, S. K. (1966): A chemical examination of *Euphorbia hirta* L. Bull. Chem. Soc. Jap. 39, 2532-2534.

Harbone, J. B. (1984): Phytochemical methods. Chapman and Hall, London.

Hartwell, J. L. (1969): *Plants used against cancer; a survey,* Lloydia 32: 153-205.

Hoffman, W. E., Dorner, J. L. (1977): Disappearance rates of intravenously injected canine phosphatase-iso enzymes. *Am. J. Vet. Res.* 38(10), 1637-1638.

Iyayi, E., Tewe, O. (1998): Serum total protein, urea and creatinine levels as indices of quality of cassava diets for pigs. *Trop. Vet.* 16, 59-67.

Johnson, P. B., Abduraham, E. M., Tiam, E. A., Abduaguye, I., Hussaini, I. N. (1999): *E. hirta* extracts increase urine output and electrolytes in rats. J. Ethnopharmacol, 65, 63-69.

Kaneko, J. J. (1980): In: Kaneko, J. J. (Ed), Clinical Biochemistry of Domestic Animals. American Press, Orlando, Fla, pp. 21-24.

Kerharo, J., Adam, J. G. (1974): *la Pharmacopie Senegalese traditionelle. Plants medicinales et toxique*, Vigot Freres, Paris.

Knoll, J. S. (1998): Diagnostic procedures for the private practice laboratory, In: Aiello, S.E. (Ed.), Merck Veterinary Manual. Merck & Co., Inc., New Jersey, pp. 1193-1196.

Oliver, B. (1960): Medicinal plants in Nigeria. Nigerian College of Arts, Science and Technology.

Saini, P. K., Saini, B. K. (1978): Origin of serum alkaline phosphatase in the dog. *Am. J. Vet. Res.* 39, 1510-1513.

Sofowora, A. (1993): Medicinal Plants and Traditional Medicine in Africa. Spectrums Books Limited, Ibadan, Nigeria. pp 4-10.

Steel, R. G. D., Torrie, J.I.I. (1986): *Principles and procedure of Statistics. A Biometric approach.* 2nd ed. McGraw Hill, New York.

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