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Haematological and biochemical evaluation of the *n*-hexane extract of *Ricinus communis* var. minor (RICOM-1013-J) in adult Sprague-Dawley albino rats

Iornmube J. Usar[1*], Ekwere O. Ekwere[2] and Francis K. Okwuasaba[1]

[1]*Department of Pharmacology, University of Jos, PMB 2048, Jos. Nigeria.*
[2]*Department of Anatomy, University of Jos, PMB 2048, Jos. Nigeria.*

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Abstract
The study undertook to evaluate the sub-chronic toxicological profile of the seed of *Ricinus communis* var. minor (RICOM-1013-J), widely used as anticonceptive agent among Bassa people of Plateau State, Nigeria, on haematological and biochemical parameters in adult rats. Thirty-six (36) adult female rats were divided into four groups of 9 rats each, and allowed to acclimatize for 1 week. Group 1 rats served as control and were administered 0.12 ml/Kg vegetable oil subcutaneously. The other rats housed as groups 2, 3 and 4 were treated with 5, 20 and 30 mg/Kg of the extract of RICOM-1013-J as single dose, respectively. Another set of thirty-six (36) adult female rats, also divided into four groups of 9, were similarly treated. 3 rats from each group and from both sets were anaesthetized with 60 mg/Kg phenobarbitone intraperitoneally, and blood samples obtained from the femoral vein for the determination of haematological and biochemical parameters on days 7, 42 and 56 post-treatment. There was no statistically significant difference in haematological parameters between the experimental and control groups in the study. Similarly, although RICOM-1013-J caused general increase in mean levels of the biochemical parameter in treated rats relative to controls, there was also no statistically significant adverse effect on both liver and renal function tests. The study concludes that RICOM-1013-J may have no adverse effect on both haematological and biochemical parameters in rats, and presents a potentially significant breakthrough in family planning technology.

Keywords: *Ricinus communis*; RICOM-1013-J; antifertility; haematological parameters; biochemical indices; safety

INTRODUCTION

The seeds of *Ricinus communis* var. minor (RICOM-1013-J) have been used in traditional medicine as anticonceptive agent for centuries by the Bassa speaking people of Plateau State, North Central Nigeria [1,2]. It was reported to prevent conception in women over a duration of one year [3]. The seeds of *Ricinus communis* (commonly called castor plant) are large, smoothly oval, shiny bean-like and vary widely in size and colour. Generally, they may be brown, black, grey or a variable brownish mottling, and based on seed size, *Ricinus communis* has been classified as major, intermediate and minor varieties. Usually, the seeds are harvested in the dry season, and are made up of 80% kernel and 20% shell [4]. The chemical composition include; 20% proteins: albumin, enzymes (lipase, chymase), nucleoalbumin and amino acids. Other constituents are globulins, glycol proteins, alkaloids and

* Corresponding author. *E-mail*: jiusat@yahoo.com *Tel*: +234 (0) 8033935661
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steroids [5,6]. Castor bean also contains magnesium, calcium and manganese in very high amounts which is said to contribute to its toxicity [4]. The stem, leaves and seed of *Ricinus communis* have been reported to contain the highly toxic glycoprotein ricin, which is however absent in the oil [7,8]. Okwusaba *et al.,* [1,2] have demonstrated significant antifertility activity in the diethyl fraction of methanolic extract of the seed in treated adult cyclic rats. In addition, McNeil *et al.,* [9] showed that RICOM-1013-J possess antiovulatory properties in rats.

There is consensus regarding therapeutic value of herbal medicine and corresponding agreement in respect of their toxicities, which often times limit their therapeutic usefulness. The latter has thus generated keen interests in the study of toxic effects of herbal preparations as an important public health agenda given that most herbal drugs are used in their crude, un-standardized form [10-12]. Therefore the present study aims to evaluate sub-chronic toxicological risk of RICOM-1013-J.

EXPERIMENTAL

Plant material. The seeds of *Ricinus communis* var. minor were collected by the traditional herbal practitioner and consultant herbalist in the Department of Pharmacology, University of Jos, Mrs. O. Azija. The seed were authenticated by Prof. S. W. H. Huseini of the Department of Botany, University of Jos, and voucher specimen deposited in the Herbarium of the Department of Pharmacognosy, University of Jos.

Preparation of extract. Sun dried seeds of *Ricinus communis* var. minor were decoated and finely grounded with mortar and pestle. The resulting powder weighing 130.4g was subjected to exhaustive Soxhlet extraction using 250mls of n-Hexane at 67-69^{o} C for 72hrs, with a yield of 49.5%. The oily residue was stored at 4^{0}C until required for use.

Animals. Thirty-six (36) adult female rats were divided into four groups of 9 rats each, and allowed to acclimatize for 1 week. Group 1 rats served as control and received 0.12 ml/kg vegetable subcutaneously. The other rats housed as groups 2, 3 and 4 were administered with 5, 20 and 30 mg/kg of the extract of RICOM-1013-J as single dose respectively. Three rats from each group were sacrificed following administration of phenobarbitone anaesthesia 60 mg/Kg intraperitoneally, and blood samples obtained from the femoral vein were used for the determination of haematological parameters and biochemical indices on days 7, 42 and 56 as described below.

Bleeding time determination. A rat was placed in a restrainer with its tail outside. The tail of the rat was sterilized with 70% alcohol and allowed to dry. A sterile lancet was used to prick the portion sterilized and the stop clock was started. A blotting paper was used to blot off blood gently from the pricked spot every 10 seconds until the bleeding ceased. The time bleeding stopped was noted and recorded as the bleeding time.

Clotting time. The tip of the tail of a rat was sterilized with 70% alcohol and allowed to dry. Clotting time was determined by cutting off the tip of the tail using a sharp pair of sterile scissors. The first drop of blood was cleaned off and 3 subsequent drops of blood were placed on a clean, grease-free glass slide and a stop watch started immediately. The tip of an office pin was then passed through the drop of blood once every 10 seconds until the pin lifted a fine strand of coagulation. This was recorded as the clotting time.

Packed cell volume (PCV). Blood samples were collected using plain micro-capillary tubes until each tube was about three quarter filled. The end of each tube not in contact with blood was sealed with plasticine. These were then placed in micro-haematocrit and centrifuged (version K x 2300L) at a rate of

12000 revolutions per minute for 5 minutes. The packed cell volume was read directly using a micro-haematocrit reader, as described by Dacie and Lewis (1995).

Haemoglobin (Hb) estimation. Haemoglobin concentration was estimated by collecting blood samples and diluting in Drabkin solution in a ratio 1:250. The resulting mixture was read colorimetrically against a blank (Drabkin solution).

Determination of Red Blood Cell (RBC) count. 0.5 ml of blood was collected in a red cell pipette and diluted with Haymen's solution to the 101 mark, mixed and placed in a clean, greaseless counting chamber of the improved Nebauer haemocytometer. The chamber was placed on a microscope and red blood cells counted under high power (X40) objective.

White Blood Cell (WBC) count. The procedure employed is similar to that described for red blood cell count, except that the white cell pipette and Turk's diluting fluid were used.

Platelet count. Blood was diluted with brilliant Cresyl blue in a white cell pipette. Platelets were then counted using improved Neubeaur counting chamber (Boar's Method).

Biochemical analysis. 10ml of blood was collected from each group of rats for biochemical analysis. The samples were centrifuged at 12000 rpm for 10 minutes and the resulting clear sera taken to the biochemistry laboratory, University of Jos, for the following liver and renal function tests: total protein (Biuret Method), Bilirubin (Diazotization Method), serum glutamate oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), Alkaline Phosphatase (ALP) and Urea (by method of Berthelot's Reaction).

RESULTS

The results showed that RICOM-1013-J had no significant effect on haematological parameters in the studied rats ($P > 0.05$). For biochemical indices, although RICOM-1013-J caused an increase in levels of analyzed parameters, these were also not statistically different from control groups. Both findings are presented pictorially in Tables 1 and 2 respectively.

Table 1: Effect of RICOM-1013-J on haematological parameters in rats

Days	Treatment (mg/kg)	RBC 10^6 cell/cm^3	WBC $x10^3$ cell/cm^3	Platelet $x10^3$ cell/cm3	Bleeding time(sec)	Clothing time(sec)	PCV (%)	Hb gHb/dl
7	Control	8.14±2.46	16.75±4.55	129.0±9.9	152.0±22.63	126.0±65.05	49.5±0.71	15.7±0.56
	5	6.85±1.36	17.3±6.90	144.5±3.54	167.0±79.20	142.0±21.21	51.0±4.24	16.5±1.34
	20	6.39±0.18	18.1±3.50	136.0±8.49	170.0±21.21	141.5±20.51	47.5±2.12	14.9±1.13
	30	7.06±0.04	17.7±3.10	141.5±2.90	154.0±60.81	124.5±9.19	45.7±0.56	15.18±1.77
42	Control	6.13±0.64	23.05±3.75	180.0±4.24	187.5±10.61	127.5±10.61	47.0±1.41	13.25±1.2
	5	6.89±0.7	23.33±4.02	170.3±2.52	200.0±34.64	141.7±18.92	48.7±5.03	12.57±0.67
	20	6.11±0.97	23.03±3.19	182.7±22.1	197.7±11.24	143.4±7.64	48.0±3.0	15.73±0.45
	30	6.34±0.18	22.67±3.25	176.7±30.44	215±31.22	145.0±22.9	51.0±3.2	14.37±0.46
56	Control	6.36±1.32	20.0±5.21	115.3±5.51	170.0±8.6	111.7±40.72	54.0±4.36	17.0±1.39
	5	6.05±0.64	19.35±2.06	116.5±3.51	187.5±3.51	125.0±21.21	55.5±3.54	16.8±0.56
	20	7.18±0.84	22.8±6.79	119.0±1.41	180.0±12.0	120.0±26.16	56.0±3.9	18.2±1.16
	30	7.62±0.46	25.17±4.06	116.0±3.00	161.7±29.0	119.0±18.63	53.0±4.36	16.83±1.34

No significant difference between treated rats compared with the control ($P > 0.05$).

Table 2: Effect of RICOM-1013-J on biochemical parameters in rats

Days	Treatment (mg/kg)	Alkaline Phosphatase (iu/L)	SGOT (iu/L)	SGPT (iu/L)	Total Protein (g/100ml)	Urea (mg/100ml)	Bilirubin (µmol/L)
7	Control	245.0±33.0	45.5±4.6	28.4±3.9	18.11±1.2	57.14±3.3	-
	5	220.0±28.0	43.2±9.8	30.6±9.8	21.33±2.4	51.14±3.1	-
	20	250.0±41.0	27.0±4.5	27.0±4.5	18.66±2.0	56.24±2.4	-
	30	236.0±39.0	31.5±4.2	31.5±4.2	19.11±1.6	58.11±2.0	-
42	Control	224.0±28.0	45.5±6.6	29.4±4.5	24.89±2.4	57.44±2.0	-
	5	242.0±32.0	43.0±11.0	29.6±9.8	21.44±1.8	56.14±2.2	-
	20	259.0±41.0	42.2±10.2	32.6±4.2	25.11±2.1	57.44±2.0	-
	30	234.0±38.0	46.6±6.0	30.8±9.0	24.66±2.1	57.14±1.8	-
56	Control	241.0±42.0	45.3±5.8	29.2±4.5	23.9±1.8	57.1±3.0	-
	5	254.0±43.0	49.4±6.1	35.6±3.8	24.1±2.3	54.6±3.2	-
	20	245.0±38.0	44.0±5.8	30.1±4.6	25.4±2.0	57.6±2.8	-
	30	258.045.0	49.6±5.0	31.6±4.3	26.4±1.9	57.1±2.8	-

No statistically significant difference in biochemical parameters between rats with RICOM-1013-J compared with control group ($P > 0.05$).

DISCUSSION

The study findings have demonstrated that RICOM-1013-J does not have deleterious effect on blood elements including erythropoietic tissue of the bone marrow, even at the toxic dose of 30mg/kg. The blood cells (RBCs and WBCs), blood coagulating indicators such as platelet count, bleeding and clotting times were unaffected in the experimental groups. In a similar way, estimates of haemoglobin (Hb) and Packed Cell Volume (PCV) between treated and control groups of rats were not affected by the extract of *Ricinus communis* var. minor. These are evidenced by the absence of identifiable pattern of change across all haematological parameters studied, as well as lack of statistically significant difference between treated rats and controls ($P>0.05$). This result supports early findings reported by Das *et al*. [13]. On the contrary, the study did not observe a significant increase in the WBC count as reported by McNeil *et al*., [9]. These findings are not surprising, considering that RICOM-1013-J exerts oestrogenic activity [2] and eostrogens enhance protein synthesis.

Also, this study has shown that although RICOM-1013-J caused a general increase in the mean levels of biochemical parameters in treated rats relative to controls, there was no statistically significant adverse effect on both liver and renal functions. The liver is the site of several enzyme related chemical reactions, which leak out of liver cells into the blood stream during liver injury. In particular increased levels signify major pathological changes in liver cell membrane integrity [14,15]. Both serum glutamate oxaloacetic transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) levels in control and experimental groups were comparable, suggesting no acute or extensive hepatic injury. More specifically, the activity of SGPT outside the liver is low and serves a more specific indicator of hepato-cellular damage [15,16]. Alkaline phosphatase is also routinely used in the diagnosis of liver injury especially by chemicals. In liver injury, there is an increase release of the enzyme into the blood stream due to increase synthesis [17]. In this study, there was no statistical difference in alkaline phosphatase levels in sera of control and experimental groups. The liver also synthesizes many proteins, but albumin is quantitatively the most important. The finding of approximately identical blood concentrations of albumin in all rats indicated that there was no impaired protein production by the liver. During liver injuries, changes

may occur in the synthesis of albumin or in the intracellular transport and release, resulting to hypoalbuminaemia. The liver also plays a central role in the metabolism of bile pigments [18], most importantly bilirubin, which was found to vary insignificantly between control and treated groups.

Urea is formed almost exclusively in the liver, by catabolism of amino acids and is the main excretion product of protein metabolism. The concentration of urea in blood represents closely the balance between urea formation from protein catabolism and urea excretion by the kidney, and measures kidney functioning [18]. The study has demonstrated that the concentration of urea in the sera of control and treated rats were statistically identical. These findings of absence of adverse effects of RICOM-1013-J on liver and kidney function tests supports earlier findings by Das *et al* [13].

This study concludes that RICOM-1013-J possesses a high margin of safety. Coupled with its high contraceptive efficacy, RICOM-1013-J presents a potentially significant breakthrough in family planning technology and provides possible alternative for current hormonal contraceptive agents.

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