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Full Length Research Paper

Haematological and histopathological effects of oil from castor seeds (*Ricinus communis* LINN) on albino-rats

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The effects of soxhlet extraction of oil from castor seeds (*Ricinus communis* Linn) using n-hexane as solvent on hematological and histopathological properties of albino rats was investigated using standard method. The haematological analysis of the animals' blood showed that the extract caused a reduction in the packed cell volume (PCV) from 49.3 to 46.7%. Histopathological analysis of the organs of the animals showed that the extract caused dilation of the sinusoid with less prominent nucleus of the liver, homogeneity of the muscle fibres of the heart with inflammatory cell infiltration, infiltration of kidney cells with increased hyaline casts, while the small intestine showed acute erosion of superficial and middle parts of the intestinal villi. Since the oil has deleterious effects on the organs of the animals used and also reduced the PCV, it is conceivable that when the oil is consumed by humans, it will have the same effect. Therefore, it is advocated that the oil should not be consumed until further work is done on it.

Key words: Castor, oil, albino rats, haematology, histopathology.

INTRODUCTION

Herbal medicine is one of the oldest, if not the oldest forms of healing, starting with the origin of human life. The importance of aromatherapy as a branch of herbal medicine cannot be overemphasized. The use of herbal medicine is relatively cheap, easily accessed and blends with socio-cultural life of people (Celikel and Kavas, 2008). The use of essential oils as parts of medicinal and aromatic plants for the treatment of diseases is on the increase. Castor plant, *Ricinus communis*, is a species of flowering plant in the spurge family, Euphorbiaceae. The family contains a large variety of phytotoxins (toxic substances produced by plants), mainly diterpene esters, alkaloids, glycosides, and ricin-type toxins. *R. communis* belongs to a monotypic genus, *Ricinus*, and sub-tribe, Ricininae. Its seed is the castor bean which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (Phillips and Martyn, 1999). The oil from the castor seed is colourless or faintly yellow, almost odorless, viscid liquid, having a taste at first bland but subsequently avid and nauseating. It is fixed and dries very slowly, having a specific gravity, 0.958. It is slightly dextrorotatory, about + 4° 30^1. It has a refractive index, 1.4790 to 1.4805 and solidifies at -10 to -18°C. Its acidity is expressed as oleic acid which is 1.5%. The oil extracted from the seed have been used in small doses in clinical setting for numerous medical conditions such as liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyper activity in children, and to avert threatened abortion in pregnant women (Mc Garey, 2008). Traditionally, the Ebira people in Kogi State of Nigeria use it for skin diseases, purgative, heal irritated or inflammed nipples and to aid delivery in delayed expectant mothers. Though, this has no literature backup,

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it is still being used till date in this part of Nigeria. This work is therefore aimed at investigating the biosafety of the oil on the organs of laboratory animals.

MATERIALS AND METHODS

Plant and preparation

Castor seeds used for this work were the white variety of the *R. communis* L. This was collected from a farm at Eika village, Okene, Kogi State, Nigeria. Five hundred grams of the seeds were air dried for two week and crushed to fine powder in mortar using pestle. Extraction was done using 98% N-hexane in soxhlet extractor and the N-hexane extract was concentrated using water bath at 45°C.

Hematological assay

The animals were sacrificed and their blood was collected by cervical collection into labeled ethylenediamminetetraacetate acid (EDTA) bottles. The following are the various analyses carried out on the blood of the animals.

Erythrocyte sedimentation rate (ESR)

A Wintrobe tube was filled to the top 0 mark and one end of it blocked with plastacine. It was stand in an upright position undisturbed for 60 min (1 h). The distance of the fall of red cells in it was read and expressed as millimeter fall in an hour as the ESR.

Packed cell volume (PCV)

Blood collected into anticoagulant bottle was mixed and a capillary tube was filled up to 75% (3/4) of its length and was placed in the micro-haematocrit centrifuge with the sealant at the outer end and centrifuged at 12,000 rpm for 5 min. The result was read as a percentage of packed red cells to total volume of whole blood using a haematocrit reader.

Red blood cell count (RBC)

The blood sample was diluted 1:200 and mixed properly. 0.02 ml of the blood was pipetted into 4 ml of diluting fluid in a Bijou bottle and was washed thoroughly by alternately drawing up and expelling the diluting fluid. A fine Pasteur pipette was used to fill the counting chamber and it was counted using a counter under ×40 objective.

White blood cell count (WBC)

The blood was first diluted in ratio 1:20 and 0.05 ml of the blood was pipetted into 0.95 ml of diluting fluid. Little portion was charged into the counting chamber and observed using ×10 objective to count the white cells/cubic millimeter.

Haemoglobin (Hb)

Using mouthpiece, sucker and a 0.02 ml pipette, blood was withdrawn and expelled into 4 ml Drabkin's solution in a tube. The tube was stoppered, mixed and allowed to stand for 5 min for full colour development. A standard blood sample of known haemoglobin concentration was prepared. Using a green (624) filter, the calorimeter was set to zero using plain Drabkin's solution as a blank. The readings of the sample and the standard were taken and the result calculated as follows:

$$\text{Sample haemoglobin concentration} = \frac{\text{Reading of test} \times \text{standard haemoglobin concentration}}{\text{Reading of standard}}$$

White blood cell differential (WBC Differential)

These are divided into granulocytes and agranulocytes. The granulocytes are further divided into three which are neutrophils, eosinophils and basophils. These were counted after staining with Giesma stain and their numbers were recorded. The agranulocyte are equally further divided into two, which are lymphocytes and monocytes.

Histopathological analysis

Histopathologic tests were carried out on the organs (liver, heart, kidneys and small intestine as suggested by Akparie,2004) of the animals as follows: the organs of the animals were collected and fixed in 10% formalin to prevent decay. They were dehydrated in different percentage (50, 70, 80, and 100%) of alcohol 1½ h each. After dehydration, they were cleared with 100% xylene and were left for 2 h to remove any remnant alcohol, and were impregnated in liquid wax for 2 h for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin (Silva et al., 1999). Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40°C and then placed under the microscope with a digital camera connected to a computer system to be examined by an expert and the photographs were taken.

Statistical analysis

The data gathered were processed using descriptive one way analysis of variance, Statistical Package for Social Sciences (SPSS) Version 10 Microsoft Windows 7. The Duncan Multiple Range Test was used as a follow up test.

RESULTS

Haematology results

The results of the haematological analysis showed that the extract had significant effects on all the parameters. The control (Group 1) had the highest mean PCV value of 49%, while the experiment had 46%. Also, the highest Hb value and RBC value was seen in the control, while the experimental recorded higher values in ESR and WBC which is an indication that the extract has a negative effect on the blood of the animals. Table 1 shows the values obtained for the haematological analysis. Table 2 shows the results of the white blood cell differential. Neutrophils were higher in the experimental with a value of 30% when compared with that of the control with a value of 27%. Since high neutrophil is an indication of systemic infection on animals, the oil is the cause. Also, there is a significant difference in values

Table 1. Effect of administration of the extract on the haematological parameters of albino rats.

Group	ESR (mm/h)	PCV (%)	RBC (cm-^{3})	WBC (cm-^{3})	Hb (cm-^{3})
1	1.33 ± 0.33^a	49.33 ± 0.88^b	859.67 ± 12.78^b	166.67 ± 3.84^a	16.67 ± 0.53^b
2	2.41 ± 0.33^b	46.56 ± 0.88^a	776.33 ± 12.44^a	174.00 ± 9.94^b	15.12 ± 0.55^a

Values are means of triplicates ± standard deviation. Values followed by similar alphabets along the same column are not significant at P = 0.05. 1: Control group; 2: Extract only. RBC: Red blood cell, ESR: erythrocyte sedimentation rate, PCV: packed cell volume; WBC: white blood cell, Hb: haemoglobin.

Table 2. Effect of administration of the extract on the white blood cell differential of albino rats.

Group	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	27.67 ± 0.57^{cd}	63.33 ± 0.58^{ab}	6.30 ± 0.33^a	2.00 ± 0.00	0.70 ± 0.33^b
2	30.47 ± 0.33^a	60.53 ± 0.33^d	6.30 ± 0.33^c	2.00 ± 0.00^a	0.23 ± 0.33^c

Values are means of triplicates ± Standard deviation. Values followed by similar alphabets along the same column are not significant at P = 0.05. 1: Control group; 2: Extract only.

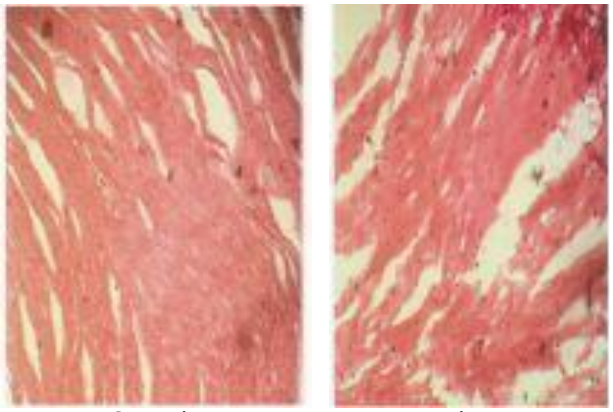

Figure 1. Histopathology of the heart.

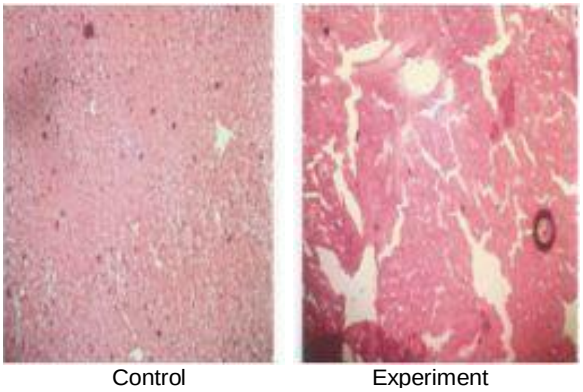

Figure 2. Histopathology of the Liver.

obtained in lymphocyte and basophil count with the control having a higher value. For instance, while the lymphocyte count in the control was 63%, while the basophil had 60%.

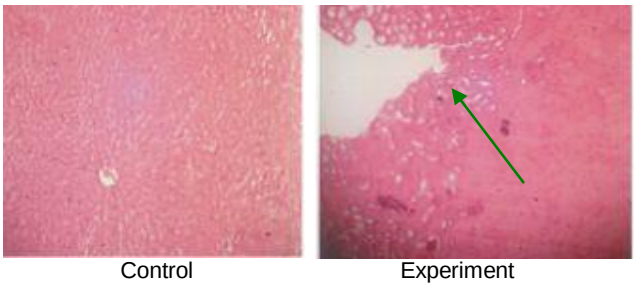

Figure 3. Histopathology of the Kidneys.

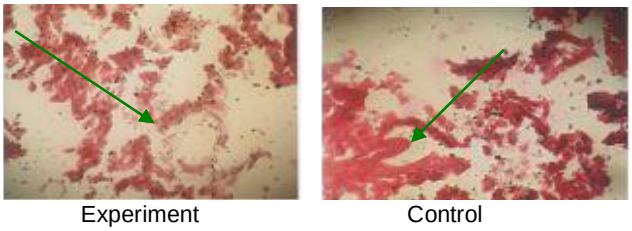

Figure 4. Histopathology of the small intestine.

Histopathology results

The muscle fibre of the heart of the control was normal without any deformation, while that of the experiment shows homogenous muscle fibre with cell infiltrations. The liver showed dilated sinusoids with less prominent nucleus in the experimental, while the control group showed no form of distortion. The kidney for the experimental showed infiltration of cells and increase hyaline casts, this was absent in the control. Only the small intestine of the experimental showed acute erosion of the superficial and middle parts of the intestinal villi (Figures 1, 2, 3, and 4).

DISCUSSION

Castor seeds as well as its oil are known to contain ricin and this is a poisonous substance to mammals (Abrami et al., 2005). Reduction in the PCV, ESR and other blood parameters of the animals may be attributed to this poisonous substance according to Al-faraj (1995). This is because, the extract may possess a portion of the ricinoleic acid, the active component of castor oil, since cineole, terpenes, sabinene and other components of the essential oil are harmless (Celikel and Kavas, 2008). Though, the muscle fibres of the heart of the group fed with the extract only become homogenous with cell infiltration, it did not show any prominent histological deformation. According to www.scribd.com (2009) and Mitchell et al. (2009), once a toxin is in the blood, all organs are exposed to its effects unless a membrane barrier intervenes. This may be correlated with the fact that the essential oil passes rapidly with blood through the heart cavities, which are well protected by the endocardium, in contrast to its relative delay in the infiltrative tissues of the liver and kidney and the absorbent mucosa of the small intestine. Since the oil has deleterious effects on the organs of the animals used and also reduced the PCV, it is conceivable that when the oil is consumed by humans, it will have the same effect on human organs. It could also endanger the health of a growing foetus in the womb when consumed by expectant mothers. Therefore, it is advocated that the oil should not be consumed until further work is done on it.

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