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TOXIC EFFECT OF DICHLOROMETHANE EXTRACT OF CASTOR OIL SEED ON HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

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Abstract

In this study, the rodenticidal potentials of castor oil seeds on Heamatological parameters of an experimental rats (Albino) was evaluated for the benefits of reducing rodents pest population. Thirty experimental rats (Albino) were divided into 3 replicates and each replicate had 5 cages. Each cage had 2 rats of both sexes. The age of the rats used for this experiment was 9 to10 weeks. The rats were fed ad libitum and fresh water was given once daily from a suspended water feeder. 1000g of castor oil seed were crushed into coarse meal using pestle and mortar prior to extraction. The coarse meal of castor seed were then soaked into 2000ml of Dichloromethane for 24 hour before it was later sieved with muslin chloth, the Dichloromethane extract derived from castor oil seed was air-dry completely, 10ml, 20ml, 30ml and 40ml Dichloromethane extract derived from castor oil seed were weighed, each was mixed thoroughly with 50 g of commercial rat feed, fifth one is Commercial rat feed only which was used as the control. Alterations of hematological parameters were analyzed by a quantitative automated hematology analyzer. The result show that ricin intoxication affects various biochemical variables when compared with control group. Dichloromethane extract from castor oil seed can caused a significant change or elevate the enzymatic activities of Alanine transaminase (ALT), Aspartate transaminase (AST), Albumin, Total Protein and Globin levels when compared with the control group. The results indicated that even very low dose of castor oil seed can promote hematological and hepatic alterations. Also, the increase in the WBC and other parameters observed in rats administered extract from castor oil seed suggests that extract of ricin from castor oil seed contains agents that could stimulate the production of leucocytes.

Key words:
Dichloromethane extract, Castor oil seed, Albino rat, Haematological parameters.

Introduction

More than 2000 species of rodents in the world. Out of all of the non-native species have been accidentally or intentionally introduced, the rat has, and continues to be, one of the most destroy invading species. Species are considered "alien" or "invasive" when they are not native to an area, but become established and cause, or are likely to cause, economic or environmental harm or harm to human health National Invasive Species Council (2008).

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Information compiled on the economic and environmental costs of introduced species around the world Pimentel (2011). It was reported in each year, rats destroy approximately 20% of total agricultural products in the world Brennan, (2008). Invasive mammal eradications are powerful conservation tools to protect biodiversity and prevent extinctions on islands (Aguirre-Munoz *et al.,* 2008; Bellingham *et al* 2010; Campbell *et al*., 2011). The opportunity to scale up existing eradication techniques is being realized, with larger and more challenging projects being undertaken (Phillip, 2010; Sutherland *et al.,* 2014). Yet despite these advances it is clear there are limits to what can be achieved and there is an urgent need to overcome these barriers. Rodents need to be controlled for various reasons which may include transmit disease in humans, farmed livestock and serve as companion animals. Among the diseases rats can transmit to humans or livestock are Lassa fever marine typhus, etc. They Cause injury to growing crops, stored animal feed and food materials, they also chewing packaging materials, containers which foods and feed are stored. Structural damage to property and installations by chewing electrical wires, wooden structures include doors, ledges, wall and corners material, rats tear up insulation in walls and make nesting on ceilings. They can damage by gnaw on wide variety of materials and example are soft metals such as copper and lead, with plastic and wood.

There are numerous species of plants that are poisonous and can be deadly to humans and animals, example of this is castor oil seed containing protein toxin, ricin, is effectively a bi-product of the castor oil extraction process and at least 100 million metric tonnes of castor beans are grown and processed every year with the purpose of preparing castor oil. In addition to cultivation for oil production, the *Ricinus* plant also grows like a weed in warmer climates of the world, including Africa, the Middle East, India and Southern France. A highly toxic preparation may fairly easily be produced from castor bean seeds and recipes have been circulated on the internet (Gareth, 2014); and in manuals that would assist the would-be terrorist. The terrorist "cookbooks" have been examined and, in the case of ricin, deemed incapable of achieving a good product for causing a large number of casualties by any exposure route, mainly because of the low content of toxin of the final extracts. It is a large step from preparing a poisonous mixture from castor beans, to delivering this material in a suitable form, on a large enough scale, to cause harm to a number of target subjects. Ricin is harmful and can have potentially lethal consequences when delivered by several routes, including those which are considered relevant to military or terrorist scenarios, namely inhalation, parenteral administration (e.g., transdermal, sub-cutaneous or intramuscular) or by ingestion. However, the toxicity of the toxin may be very different depending upon the route of intoxication. A collection of toxicity assessments have presented for ricin pertaining to different routes of exposure based on studies in mice (Franz.; Jaax, 1997). The toxicity of ricin in mice causing death in 50% of the target population (LD50) by inhalation is given as 3–5 μg kg–1 with a mean time to death of 60 h; 24 μg kg–1 and death in 100 h by subcutaneous injection and 20 mg kg–1 and death in 85 h by ingestion. The main lesion caused by ricin is impairment of the protein synthesis machinery of the cell (Olsnes, 1972) but, in the living organism, a number of symptoms arise leading to an overall picture of ricin intoxication. In the following section, the toxicity, signs and symptoms of ricin poisoning will be examined, as far as is possible, by route of intoxication.

If ricin were released in an attack, it is essential that the toxin is detected on materials and this might entail extremes such as sampling soil to foodstuffs, particularly if contamination of foods had been threatened. As a result of this risk, a considerable amount of work has been undertaken over the past few years to develop detection methods for ricin in a range of substrates. The

detection of ricin will be examined, giving a flavour of methods attempted and limits of detection achieved.

Finally, the ability to diagnose exposure to ricin toxin is an important prelude to subsequent therapy, time being of the essence. Attempts currently underway that inform the diagnosis of ricin poisoning will be examined.

Blood is a good indicator to determine the health of an organism. It is also a good pathological mirror of the entire body. Cellular component of blood is valuable in immunotoxicology to evaluate immunotoxic potential of a compound. To this end, haematological parameters are important in establishing the body's functional status as a result of exposure to toxicants (Joshi *et al*., 2002). Therefore, in this study, the rodenticidal potentials of castor oil seeds on Heamatological parameters of an experimental rats (Albino) was evaluated.

METHODOLOGY

Experimental Site: The experiment was carried out in the year 2015 at the Toxicology Laboratory in the Department of Crop and Environmental Protection, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomosho.

Plant Material

The castor oil seed used for this experiment were obtained from the Botanical Garden Ladoke Akintola University of Technology, Ogbomoso. The seeds were collected when ripe as the capsule, becomes dry, open and discharge the seeds. The 1000g of castor seed were crushed into coarse meal using pestle and mortar prior to extraction. The coarse meal of castor seed were then soak into 2000ml of Dichloromethane for 24 hour before it was later sieved with muslin chloth, the Dichloromethane extract derived from castor oil seed was air-dry completely, 10ml, 20ml, 30ml and 40ml Dichloromethane extract derived from castor oil seed, each was mixed thoroughly with 50 g of commercial rat feed, fifth one is Commercial rat feed only which was used as the control.

All the treatments were replicated three (3) times.

Animals and experimental design. The albino rats were obtained from the Virology Department, University College Hospital (U.C.H) Ibadan, Oyo State. They were kept in rat cages and fed with commercial rat feed (Growers mash) (Bova Jay Livestock Feeds, Nigeria Ltd). Thirty (30) experimental rats were divided into 3 replicates and each replicate had 5 cages. Each cage had 2 rats of both sexes. The age of the rats used for this experiment was 9 to10 weeks. The rats were fed ad libitum and fresh water was given once daily from a suspended water feeder.

Grouping

Rats were weighed and acclimatized for two (2) weeks and randomly grouped into 5 treatment (1, 2, 3, 4 and 5) of 2 rats per treatment with treatment 5 served as the control fed with Commercial rat feed only. The remaining 4 treatments were oral-ly administered 10, 20, 30 and 40 ml extract of castor oil seed respectively for 5 days.

Techniques for obtaining blood and serum samples. Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into heparinised test tubes for haematological studies. Blood samples were also collected into clean non-heparinised test tubes and allowed to clot. This is done just to evaluate the effect of plant extract on the blood parameters of experimental rats.

Determination of haematological parameters and Method of filling capillary tubes with blood: The Coulter Counter Model S-PLUS JR with Coulter histogram differential, hereafter referred to as

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the Model S-PLUS JR, is a quantitative, automated hematology analyzer. It is intended for determining the following hematologic parameters: White blood cell, Granulocyte, Lymphocyte, Red Blood Cell, Hemoglobin (Hb), Mean Corpuscular (erythrocyte) Volume, Mean Corpuscular (erythrocyte) Hemoglobin Concentration, Platelet.

The Coulter method of cell counting and sizing is based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid traversing a small aperture. When cells are suspended in the conductive diluent, they function as discrete insulators. When a dilute suspension of cells is drawn through a small cylindrical aperture, the passage of each individual cell momentarily increases the resistance of the electrical path between two submerged electrodes, one located on each side of the aperture. An electrical pulse, suitable for counting and sizing, results from the passage of each cell through the aperture.

Data analysis: Data collected were analyzed using one way analysis of variance (ANOVA) and means were separated with Duncan Multiple Range Test (DMRT) at 5% level of probability using Statistical Analysis Software (SAS).

RESULT

The least body weight was recorded on the rats fed with the highest concentration of 30ml extract of castor oil seed (Fig 1). However, the rat fed with the control feed had the greater body weight when compare 10ml extract of castor oil seed but the rats fed with the normal feed (control) had significant greater weight than the rats fed with 20ml, 30ml and 40ml extract of castor oil seed. No significant difference was observed among the rat fed with 20ml, 30ml and 40ml extract of castor oil seed irrespective of the concentration and no significant difference was observed with the control group (Rats fed with the normal feed) and the rat fed with 10ml extract of castor oil seed (Fig 1).

No significant ($P < 0.05$) difference was observed on white blood cell of both treated and untreated rats. This shows that extract from castor oil seed slightly increases the level of white blood cell without visible haematological changes in the blood of experimental rat and the increase was not statistically significant. The crucial role of WBC in defending the body against infection and tissue damage is well known. This supports reports that mistletoe and some commonly prescribed medicinal plants contains agents that stimulate the production of leucocytes (Al-mammary, 2002; Imoru *et al*., 2005). No significant ($P < 0.05$) difference was observed on lymphocyte of rats treated with 10ml concentration and the control group, likewise no significant ($P < 0.05$) difference was observed on lymphocyte of rats treated with 20ml, 30ml and 40ml concentration extract of castor oil seed but there is a significant difference between rats treated with 20ml, 30ml and 40ml concentration extract of castor oil seed when compared with rats treated with 10ml concentration and the control group. No significant ($P < 0.05$) difference was observed on haemoglobin of all the treated rats i.e rats treated with 10ml, 20ml, 30ml and 40ml concentration extract of castor oil seed but due to the effect of extract from castor oil seed, there is a great increase in the level of haemoglobin of all the treated rat when compared with untreated rats (control group). This shows that extract from castor oil seed has a great effect on haemoglobins of experimental rat when compared with the control (Table 1).

No significant ($P < 0.05$) difference was observed on red blood cell of both treated and untreated rats. This shows that extract from castor oil seed has no effect on red blood cell of experimental rat. No significant ($P < 0.05$) difference was observed on mean corpuscular volume of both treated

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and untreated rats. However, extract from castor oil seed slightly increases the level of mean corpuscular volume without visible haematological changes in the blood of experimental rat. No significant ($P < 0.05$) difference was observed on Mean cell (Erythrocyte) Haemoglobin concentration of both treated and untreated rats. This shows that extract from castor oil seed has no effect on Mean cell (Erythrocyte) Haemoglobin concentration of experimental rat (Table 2).
No significant ($P < 0.05$) difference was observed on granulocyte of rats treated with 20ml, 30ml and 40ml concentration but there is a significant difference in granulocyte of rats treated with 10ml concentration when compared with rats treated with 20ml, 30ml, 40ml concentration and untreated rats which is the control group, Again, there is a significant difference in granulocyte of untreated rats which is the control group when compared with rats treated 20ml, 30ml and 40ml concentration extract from castor oil seed. No significant ($P < 0.05$) difference was observed on platelet of rats treated with 20ml, 30ml, 40ml concentration and untreated rats which is the control group, but there is a significant difference in platelet of rats treated with 10ml concentration when compared with rats treated with 30ml concentration extract from castor oil seed (Table 2).

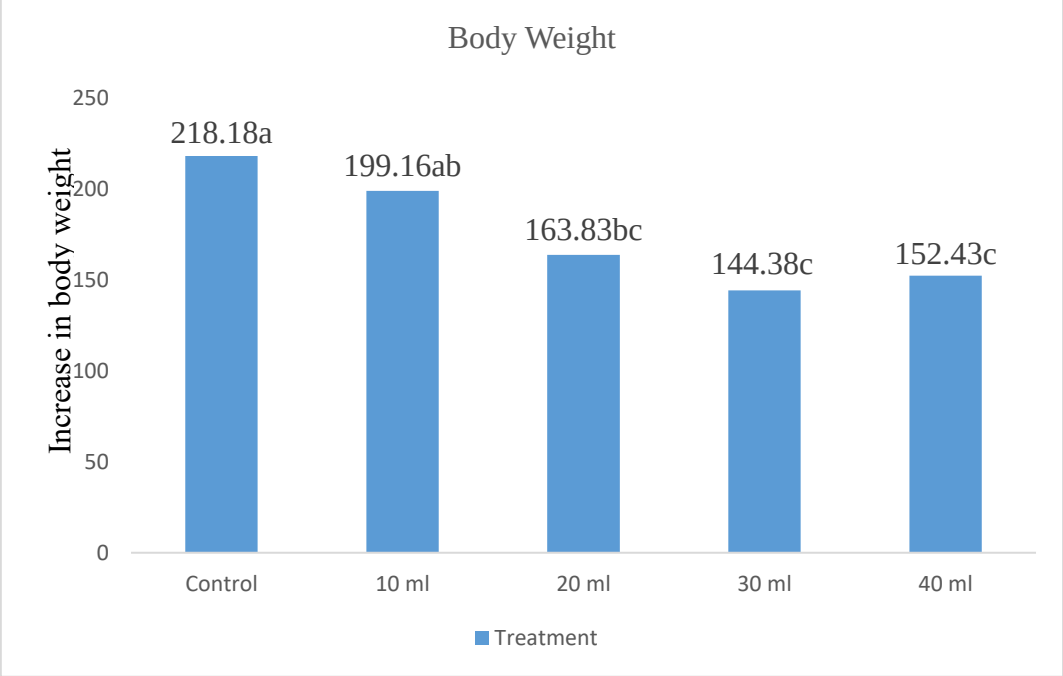

Figure 1. Effect of extract from castor oil seed on the body weight (g).

Table 1 : Effect on heamatological profile following daily oral administration of extract from castor oil seed on the blood parameters of Albino rats (n = 6).

Groups	Treatments	WBC (10^3/ul)	Lymphocyte (10^3/ul)	Hb (g/l)	RBC(10^6/ul)
I	10ml	11.2a	3.5b	12.9a	6.7a
II	20ml	10.8a	5.9a	12.2a	6.0a
III	30ml	10.6a	6.0a	13.0a	5.7a
IV	40ml	10.1a	6.0a	13.9a	6.7a
V	Control	10.0a	4.0b	10.2b	6.5a

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Means followed by the same letters within the same column are not significantly different at p≤0.05, using DMRT.

Table 2 : Effect on heamatological profile following daily oral administration of extract from castor oil seed on the blood parameters of Albino rats (n = 6).

Groups	Treatments	MCV (fl)	MCHC (g/l)	Granulocyte (10^3/ul)	Platelets (10^3/ul)
I	10ml	50.6a	34.4a	5.9a	407.8a
II	20ml	55.0a	36.4a	2.2c	378.8ab
III	30ml	50.8a	36.5a	2.3c	251.2b
IV	40ml	51.0a	35.6a	2.3c	323.0ab
V	Control	49.7a	34.9a	3.9b	344.0ab

Means followed by the same letters within the same column are not significantly different at p≤0.05, using DMRT.

DISCUSSION

This experiment is to determine toxic effect of Dichloromethane extract of castor oil seed on Heamatology (blood Parameters) and the effect on the body weight.

The observation revealed that the extract from castor oil seed caused decrease in the body weight of test rats compared to the control rats which were fed with normal feed ration. Response to decrease in weight depends on the level of concentration applied (Fig 1). This shows that the toxicity of botanical pesticide as rodenticide like conventional pesticides depends on the concentration.

The observation revealed that the extract from castor oil seed has no statistical significant changes in the value of haematological parameters of white blood cell (WBC), Red blood cell (RBC), Mean corpuscular volume (MCV) and Mean cell (Erythrocyte) Haemoglobin concentration (MCHC) of experimental rat when compared with control (Table 1 and 2). It could be concluded from this study that although extract from castor oil seed had no significant effect on most of the hematological parameters in rats but the administration of the extract caused increase in WBC, RBC, MCV and MCHC in rats. This suggests that the use of this extract especially at high doses could increases the haemopoetic system. It was stated that toxic plants do not produce a direct effect on white blood cells, such as neutrophils, lymphocytes, eosinophils, and monocytes (Swenson and Reece, 1993).

The observation on the haemoglobin and lymphocyte of rat treated with 20ml, 30ml and 40ml concentration extract of castor oil seed has greatly increase in the level of haemoglobin and lymphocyte when compared with haemoglobin and lymphocyte of untreated rats and lymphocyte of rat treated with 10ml concentration (Table 1).

Response to increase in the level of haemoglobin and lymphocyte depends on the level of concentration applied (Table 1).This shows that the toxicity of botanical pesticide as rodenticide like conventional pesticides depends on the concentration.

The observation on the granulocyte of rat treated with 20ml, 30ml and 40ml concentration extract of castor oil seed revealed that there is a decrease in the level of granulocyte when compared with granulocyte of untreated rats and granulocyte of rat treated with 10ml concentration (Table 2).

Observation on the platelet of rats revealed that the extract from castor oil seed has no major effect on both treated and untreated group (Table 2).

The result of dichloromethane extract from castor oil seed on some haematology (blood parameters) cause a lot of significant changes in the haematology of experimental rat.

Conclusions

The results indicated that even very low dose of castor oil seed can promote hematological and hepatic alterations. It is therefore advisable that endangered species of plant became endangered mostly because of the low level of importance that utilizers of such plants attached to the plants. Many poisonous plants are so common and seemingly innocuous you do not suspect their toxic qualities. Identification of such plants like castor oil seed can serve as rodenticides and will help plant utilizers to put such plants to conservation. Also, the increase in the WBC and other parameters observed in rats administered extract from castor oil seed suggests that extract of ricin from castor oil seed contains agents that could stimulate the production of leucocytes.

Conflict of interest: The authors declare that there is no conflict of interest.

Author contribution:

Conceptualization of research and preparation of initial draft of the manuscript, research design, result interpretation and improvement of the manuscript.

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