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BIOCHEMICAL BIOMARKERS EFFECT IN EXPERIMENTAL RATS (ALBINO) EXPOSED TO RICIN POISON FROM CASTOR OIL SEED

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Abstract

The Dichloromethane extract gotten from castor oil seed (*Ricinus communis L. Euphorbiaceae*) was used in a Laboratory experiment for its toxicity. The study was undertaken to evaluate the rodenticidal potentials of castor oil seeds on Biochemical Parameters of kidney and liver function of experimental rats. Thirty (30) 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. 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 observation shows that the higher or increase in the toxicity level of extract from castor oil seed the more or increase in the Plasma urea and creatinine levels (i.e dose depend).

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

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Introduction

Ricin is obtained from *Ricinus communis*, the castor oil plant. Castor oil seed is a highly toxic protein that belongs to the type 2 ribosome-inactivating protein (RIP) family (Olsnes, S. and Kozlov, J.V. 2001**;** Yermakova, A. and Mantis, N. J. 2011). The plant belongs to the spurges (i.e. fam. Euphorbiaceae). This beautiful plant is sometimes used to decorate gardens. It is cultivated chiefly to harvest oil from the seeds: ricinus oil or castor oil. In temperate regions it is an annual plant with brownish red stalks and large palmate leaves with long stalks and 5-11 jagged lobes. In tropical areas it grows as a shrub or a tree that can reach 40 feet in height along streams and rivers and on bottomlands with well-drained, nutrient rich soils (Ombrello, Noteworthy 1999). In the tropics it is perennial, and grows even taller. Most varieties are green, but some are red or purple. The flowers may be strikingly green, or a pronounced pink in the pigmented varieties. The seeds are approximately 17 mm long and 8 mm broad. Present-day cultivars have seeds weighing approximately 3g. The seed mantle accounts for approximately ¼ of the weight. Dry seeds contain approximately 50% oil. The seeds also contain a lipase, which can be used commercially.

The active poison in castor seeds is ricin (RYE-sin), a very deadly protein called a lectin. Ricin is found in the meal or cake after the oil has been extracted. Those who occasionally take castor oil may be assured that ricin does not occur in the pure oil. When a gram of ricin is compared with equivalent weights of other toxic substances, it turns out to be one of our deadliest natural poisons. It has been estimated that, gram for gram, ricin is 6,000 times more poisonous than cyanide and 12,000 times more poisonous than rattlesnake venom. Ricin mixed with food and used as bait is highly toxic to certain pest animals, such as rodents and insects. E. A. Weiss (1971) states that a dose of 0.035 milligram (approximately one millionth of an ounce) may kill a man, and even small particles in open sores and in the eyes may prove fatal. According to the Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals (1997), a dose of ricin weighing only 70 micrograms or two millionths of an ounce (roughly equivalent to the weight of a single grain of table salt from a salt shaker) is enough to kill a 160 pound person. As few as four ingested seeds can cause death in an adult human, and lesser amounts may result in symptoms of poisoning, such as vomiting, severe abdominal pain, diarrhea, and convulsions. Of course the degree of poisoning depends upon the amount ingested and the age and general health of the individual. There are numerous documented cases of ricin poisoning and death when horses, livestock, and poultry accidentally ate castor seeds or meal.

The poisoning mechanism of castor beans is very complicated. It causes clumping (agglutination) and breakdown (hemolysis) of red blood cells, hemorrhaging in the

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digestive tract, and irreparable damage to vital organs such as the liver and kidneys. It is most toxic when taken intravenously or inhaled as fine particles. In fact, the possibilities of ricin dust in chemical warfare are horrendous. There are even documented cases of castor bean poisoning in murders by paid assassins.

At the cellular level, ricin is known as a "ribosome-inactivating protein" or Type 2 RIP, one of the most potent cytotoxins in nature. It is synthesized in the endosperm cells of maturing seeds. Ricin is composed of two complex 3-dimensional molecular subunits, a 267 amino acid globular protein called the A chain (RTA) and a 262 amino acid protein called the B chain (RTB). Ricin is one of the most common and potent lethal biological molecules known (Walsh, M.J.*et al,.* **2013)**. Ricin and related proteins display one common feature, i.e. the ability to inhibit ribosomes, and this particular feature laid the foundation for the general name for all these proteins: "ribosome-inactivating proteins" (Tumer, N.E.; Li, X.P. 2012). In general, all RIPs have been classified as single chain (type 1) and double chain (type 2) proteins (Stirpe F.; Barbieri, L. 1986)

In summary, ricin is a potent cytotoxic protein that is lethal to eukaryotic cells by inactivating the organelle sites of protein synthesis called ribosomes. Just one single ricin molecule that enters the cytosol of a cell (the semifluid medium between the nucleus and plasma membrane) can inactivate over 1,500 ribosomes per minute and kill the cell.

MATERIALS AND METHODS

The experiment was carried out at the Toxicology Laboratory in the Department of Crop and

Environmental Protection, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomosho.

Animals and experimental design. Healthy 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.

Preparation of the solvent crude extracts of the plants. 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

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muslin cloth, 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.

The treatments were as follows:

Treatment I: 10 ml Dichloromethane extract gotten from castor oil seed plus 50g of Commercial rat feed

Treatment II: 20 ml Dichloromethane extract gotten from castor oil seed plus 50g of Commercial rat feed

Treatment III: 30 ml Dichloromethane extract gotten from castor oil seed plus 50g of Commercial rat feed

Treatment IV: 40 ml Dichloromethane extract gotten from castor oil seed plus 50g of Commercial rat feed

Treatment V: Commercial rats feed only served as control

All the treatments were replicated three (3) times. The experiment lasted for 5 days

The rats were fed ad libitum (feeding without restriction) once daily with Dichloromethane Castor Seed Extract mixed thoroughly with commercial feed to control rat, They were given fresh water once daily from a suspended water feeder.

Techniques for obtaining blood and serum samples. Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into clean non-heparinised test tubes and allowed to clot. The serum was separated from the clot and centrifuged according to groups into clean test tubes for biochemical analysis. This is done just to evaluate the impact of plant extract on the levels of renal and liver related enzymes in the serum.

Determination of serum biochemical parameters. biochemical parameters were estimated using an auto analyzer (BIOTRAN BTR-830) and diagnostic reagent kits (Johnson & Johnson) supplied by Lab Trade Ltd, Ogbomoso, Nigeria. The sera samples were used for liver function assessment by estimating the concentrations of the enzymes Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Globulin, Albumin and Total protein (TP). Renal function was evaluated from sera samples by estimating the urea and creatinine concentration.

Data analysis:

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

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RESULT

The result show that ricin intoxication affects various biochemical variables when compared with control group. Oral administration of the Dichloromethane extract derived from castor oil seed to rats resulted in significant increase in serum levels of ALT,AST, ALP (figure 1, 2 & 3), the level of Urea and creatinine suggestive of a hepatotoxic effect induced by the extract derived from castor oil seed. Following ricin toxicity, decreased levels of blood glucose, total protein, and an increased level of blood urea were reported (Muldoon, D.F.; Hassoun, E.A. & Stohs, S.J. 1992)
. In the present study, decreased level of total protein, Albumin and Globin was observed (figure 4, 5 & 6). The decreased protein concentration may ***be*** associated with disturbed liver function due to inhibition of protein synthesis. The levels of Urea and creatinine were increased. Initial increase in glucose level may be due to shock and body fluid loss (figure 7 & 8).

Effect of extract from castor oil seed on enzymes (Biochemical parameters) in the kidney and liver of experimental rat.

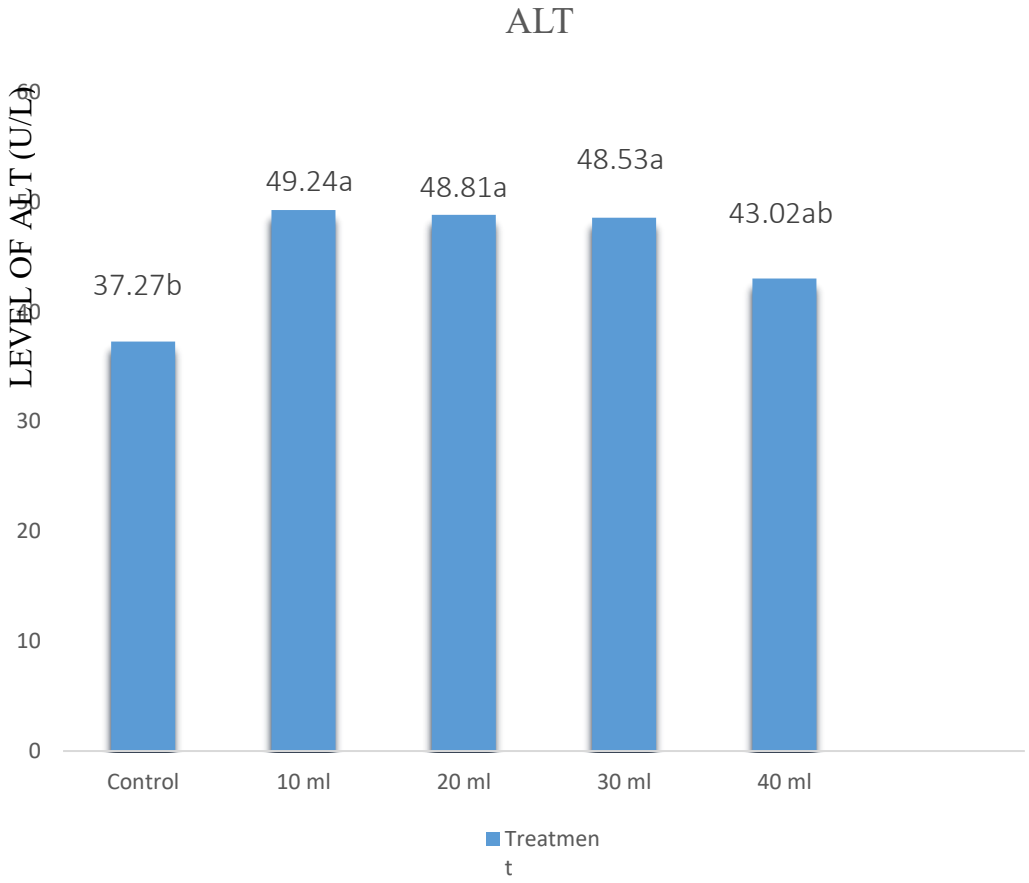

Figure 1. Effect of extract from castor oil seed on Alanine transaminase (ALT**);**

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Alanine transaminase (ALT): No significant ($P < 0.05$) difference was observed for rats treated with 10ml, 20ml, 30ml and 40ml concentration extract from castor oil seed but there was a significant ($P < 0.05$) decrease in the ALT activity in the plasma of control or untreated rats when compared with rat treated with 10ml, 20ml and 30mlconcentration extract from castor oil seed.

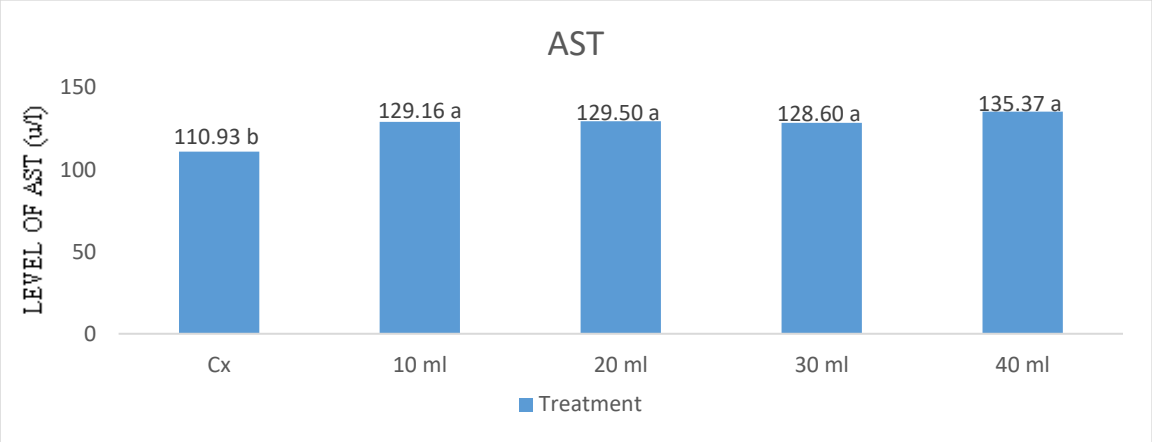

Figure 2. Effect of extract from castor oil seed on Aspartate aminotransferase (AST**);**

Aspartate aminotransferase (AST): There was no significant ($P < 0.05$) difference among the treated rat fed with 10ml, 20ml, 30ml and 40ml concentration extract of castor oil seed but there was a great significant ($P < 0.05$) increase in the AST activity in the plasma of treated rat fed with extract of castor oil seed when compare with untreated rat. The increase in markers enzyme ALT & AST of liver of treated rat fed with extract could be due to botanical rodenticide induced oxidative stress that could have affected various metabolic processes while the decrease in control group or untreated rat could be attributed to the proper metabolism of dietary intake.

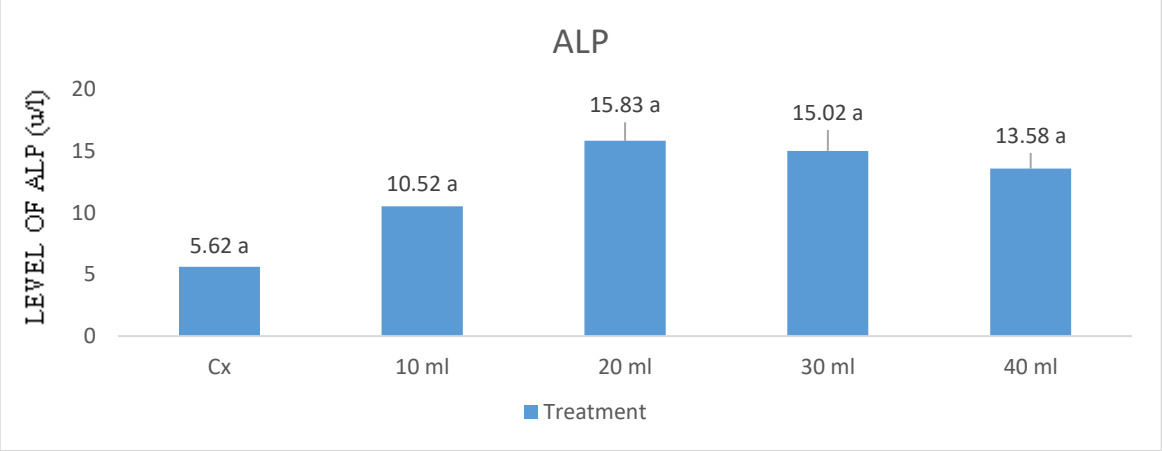

Figure 3. Effect of extract from castor oil seed on Alkaline Phosphatase (ALP)

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Alkaline phosphatase (ALP): No significant ($P < 0.05$) difference was observed both treated and untreated rats. This show that ingesting extract from castor oil seed has no effect on Alkaline Phosphatase (ALP) biochemical markers in the liver.

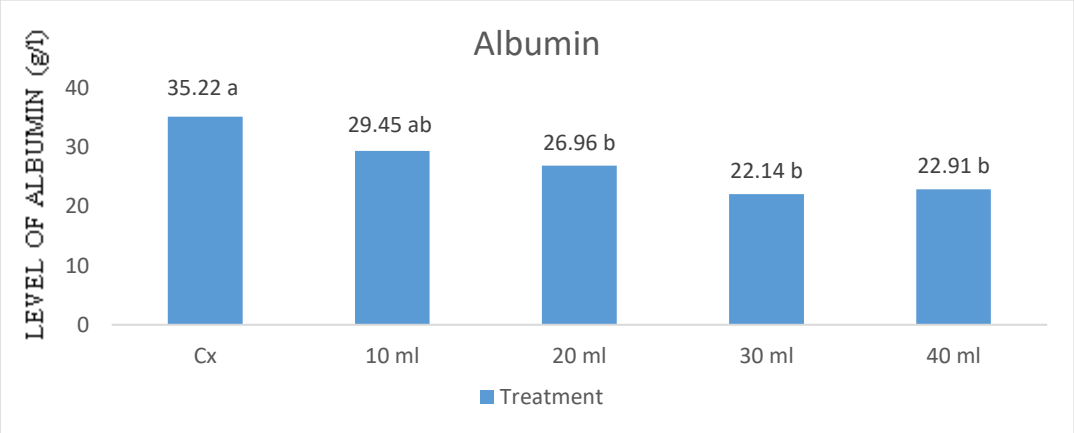

Figure 4. Effect of extract from castor oil seed on Albumin

Albumin: With reference to the marker enzymes of Albumin, the rats fed with the normal feeds (Control) had significant increase in the level of albumin than the rats fed with 20ml, 30ml and 40ml concentration extract of castor oil seed but no significant difference was observed among the treated rat or among the rat fed with treated feed.

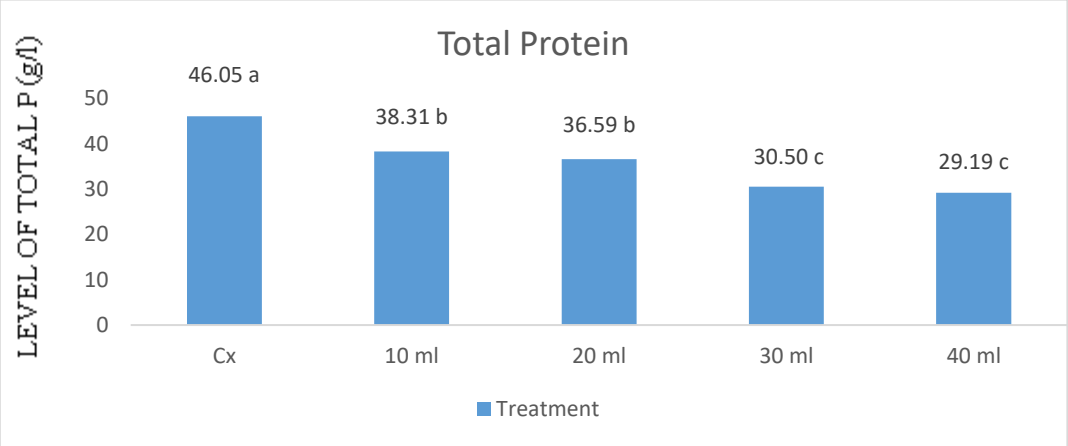

Figure 5. Effect of extract from castor oil seed on Total Protein

Total protein: Under normal condition, enzyme like Total Protein present in the liver must increase except when there is a problem in the liver. But this shows that there is a significant difference between the treated and untreated rats. Again, the result

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shows that as the concentration of the toxic substance increases from 10ml to 40ml concentration, there is a slope decrease in total protein level from 10ml to 40ml concentration extract from castor oil seed. Rats fed with 40ml extract from castor oil seed had the least protein level.

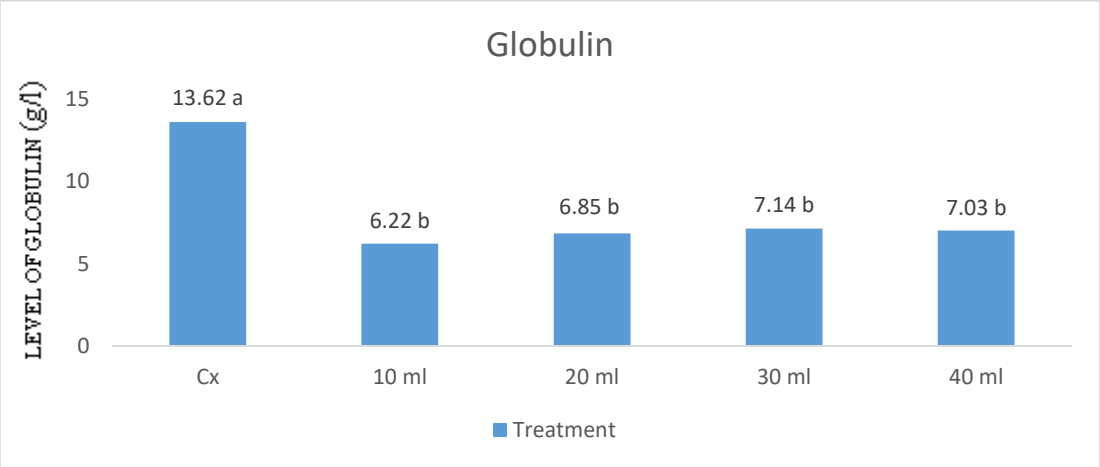

Figure 6. Effect of extract from castor oil seed on globulin

Globulin: Also, there must be increase in haemoglobin, just like Total protein which must increase or rise in the liver. There was a significant ($P < 0.05$) increase in haemoglobin activity in the plasma of untreated rats (Control group) when compared with rat treated with extract of castor oil seed. No significant ($P < 0.05$) difference was observed on rats treated with extract of castor oil seed.

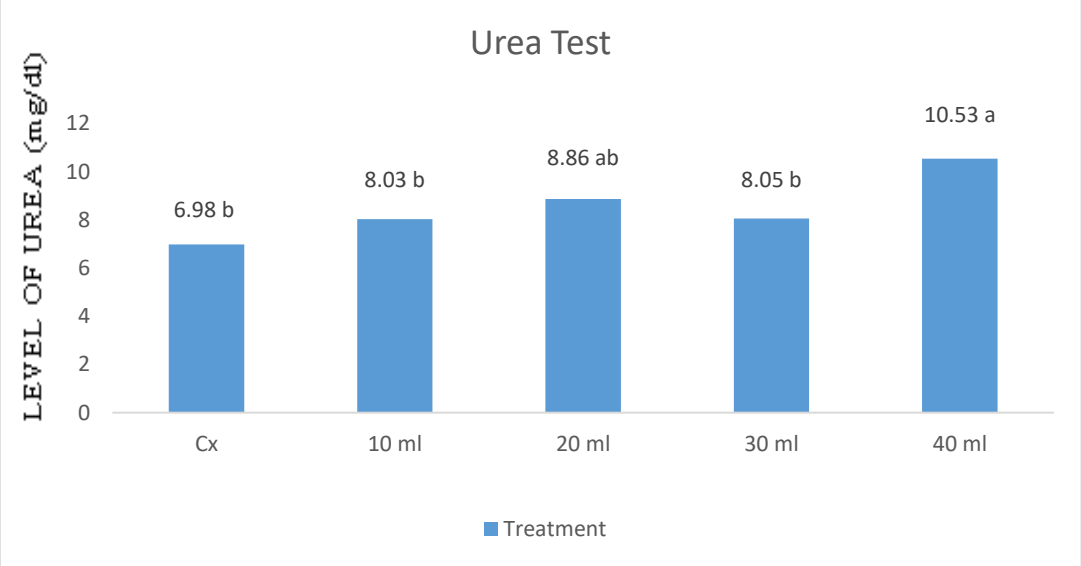

Figure 7. Effect of extract from castor oil seed on Urea test

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Urea clearance test: Urea are kidney function parameters and many toxic substance can cause adverse effects on kidney tissue and alter or increase plasma urea level. The result shows that there is a significant ($P < 0.05$) increase in the level of urea on the rat treated with the highest concentration when compared with those rat treated with the 10ml, 30ml concentration and control group. No significant ($P < 0.05$) difference was observed on the rat treated with 10ml, 20ml, 30ml extract from castor oil seed and the control group.

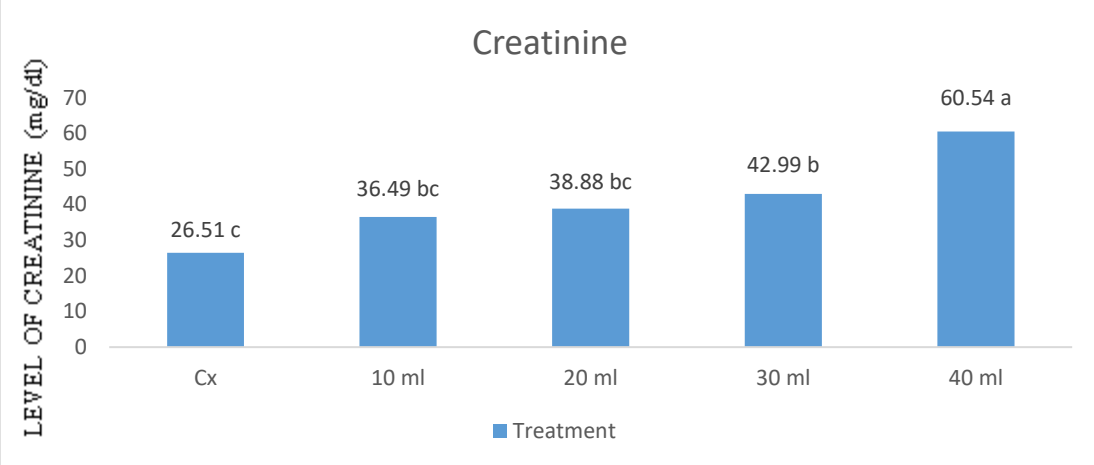

Figure 8. Effect of extract from castor oil seed on Creatinine clearance test

Creatinine clearance test: Creatinine are kidney function parameters just like urea level. These enzymes are present in lower level in the kidney but when there is increase or rise in the level of creatinine in the kidney, it means there is a problem in the kidney.

DISCUSSION

Several studies have shown the effect of oxidative stress on liver and kidney function (Xiao-ying G, et al. 2003). Liver and kidney are important organs of the body which play a vital role in metabolic processes. Liver detoxifies substances that are harmful to the body. Liver function tests are groups of blood assays that provide information about the state of the liver. Hepatotoxicity involves only mild symptoms initially, therefore early detection is vital (Olaniyi J. A., et *al.* 2003). ALT and AST are known markers for liver damage (Loganathan G, et al. 2005). Elevated activity of the enzymes ALT and AST in plasma could indicate their leakage from the intracellular store as a result of possible liver damage.

The result shows that extract from castor oil seed can caused a significant change in all this marker of enzymes: ALT, AST, when compared with the control group. Akanji

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et al., (1993), elevation or increase in the enzymatic activities of ALT and AST may be due to its activation of synthesis caused by extract mixed with commercial feed as a consequence of increase activity of the organs. It has been shown in this study that extract from castor oil seed can elevate the enzymatic activities of ALT, AST. But decrease serum ALP activity in the present study may be attributed to inhibition as a result of type 2 ribosome-inactivating protein (RIP) found in the extracts, prominent which is ricin; this is known to be chelate metal ions and precipitate proteins (Salau A. K. *et al*., 2013; Akanji *et al*., 1993; Akanji *et al*., 2013). The reduction in the activity of ALP in the serum of the animals may also be due to increase plasma clearance of the enzymes (Mayne P. D. 2005), which is consistent with the findings of (Akanji *et al*., 2013). It was found that extract from castor oil seed treated animals had significantly higher ALT, AST, than the control group animals. Serum total protein, Albumin and Globulin are useful indices of the synthetic function of the liver (Yakubu M. T. and Musa I. F. 2012). The increase in the level of total protein without a corresponding alteration in the Albumin, may indicate an imbalance in the rate of protein synthesis and degradation. Increase in the level of total protein in this study is in line with Salau A. K. (2012) previously report after the administration of aqueous extract of *Ficus exasperate* to rats.

The kidney helps in maintaining homeostasis of the body by reabsorbing vital substances and excretion of waste products (Chawla, 1999). Plasma urea and creatinine levels were determined in the present study. Many pesticides can cause some toxic and adverse effect on kidney tissues (Elhalwagy M. E. A., et al., 2008). Urea and creatinine levels are kidney function parameters (Singh S. P., et al. 2002; Yeh Y. H., et al. 2008). Pesticide can alter plasma urea and creatinine levels (Yousef M. I., et al. 2006). Urea is the end product of protein catabolism. Increased blood urea is correlated with an increased protein catabolism in the mammalian body and / or referred kidney dysfunction. The levels of urea in the plasma of rats are tested as indicators for kidney functions (Husain K, Whitworth C and Rybak L. P. 2004). Also, high levels of blood urea result from the increased breakdown of tissues or impaired excretion. In this study, the increased urea level may be due to effect of extract from castor oil seed administered (fig 15). It was observed that the highest concentration which is 40ml extract from castor oil seed had higher significant ($p<0.05$) increase when compared with control group.

Creatinine excretion is almost dependent on the process of glomerulus filtration. A significant rise in the serum creatinine level may be due to the impairment of the glomerular function and tubular damage in the kidneys (Singh S. P., et al. 2002). The creatinine level is a good risk marker for chronic renal insufficiency. Increase creatinine level indicate damage of the glomerular function and tubular damage in the

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kidneys (Mohssen M. 2001; Mora L. de O. et al. 2003). Response to increase in the level of creatinine level (fig 8). This shows that the higher or increase in the toxicity level of extract from castor oil seed the more or increase in the level of creatinine level (i.e dose dependent).

CONCLUSION

The present investigation has given a clear perspective of biochemical alterations associated with ricin poison from castor oil seeds. This examination revealed the presence of an inherent potential toxicity of castor oil seeds at different dose levels. Based on this observations, we therefore conclude that ricin poison exposure was detrimental to the parameters examine in albino rats tested and hence it can be used as an alternative to synthetic rodenticides.

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