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Prophylactic Effect of an Alkaloid-Rich Fraction of *Abrus precatorius* Seed Methanol Extracton Paracetamol-Induced Liver Damage in Rats

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Abstract: This study aims to investigate the prophylactic effect of an alkaloid-rich fraction of the chloroform-methanol extract of *Abrus precatorius* seeds on paracetamol-induced hepatotoxicity in rats. The extract was fractionated in a 17.5 x 2.5cmSephadex G15 swollen, packed and eluted with water. Five millitre fractions were spotted on F_{254} pre-coated Tin Layer Chromatographic plates and sprayed with Drangendoff's reagent. The fractions that turned purple indicating the presence of alkaloids were pulled together and used in the study. Hepatotoxicity was induced using per oral 2500 mg/kg b.w. of paracetamol. The fraction was administered at the doses of 100 and 200mg/kg b.w. while silymarin was used as the standard drug (100mg/kg b.w.). Prophylactic treatment with Fraction I caused dose-dependent significant decreases (p□0.05) in the activity of serum liver marker enzymes (ALP, AST, ALT), bilirubin levels, serum urea, creatinine and MDA concentrations when compared with the positive control, while there was a significant increase (p□0.05) in the SOD activity of the rats pre-treated with Fraction I when compared with the positive control pre-treated with silymarin. The haematological parameters of the rats pre-treated with Fraction Ishowed significant increases (p□0.05) in the PCV levels, Hb concentration and RBC count compared to the positive control. A dose-dependent significant decrease (p□0.05) was observed in the WBC count of all pre-treated groups compared to the positive control.From this findings, the alkaloid-rich Fraction was able prevent liver damage in the paracetamol-intoxicated rats but the standard drug used was more potent.

Abbreviations:
ALP, alkaline phosphate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TB, total bilirubin; CR, creatinine; NA^+, sodium ion; K^+, potassium ion; CL^-, chloride ion; SOD, superoxide dismutase; MDA, malondialdehyde; HB, haemoglobin; PCV, packed cell volume; RBC, red blood cell count; WBC, white blood cell count; NAPQI, n-acetyl-p-bezoquinone imine; GSH, glutathione; UV, ultra violet; TLC, tin layer chromatography; ANOVA, analysis of variance; SD, standard deviation; SEM, standard error of mean; NM, nanometer; HPLC, high performance liquid chromatography; ROS, reactive oxygen species; SPSS, statistical product for service solutions.

Key words: *Abrusprecatorius* · Sephadex · Drangendoff's Reagent · Paracetamol

INTRODUCTION

The liver plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism [1]. The role played by the liver in the removal of toxic substances from the portal circulation makes it susceptible to first and persistence attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction [2,3].

Paracetamol, a commonly used analgesic, is considered safe at therapeutic doses. However, an overdose of paracetamol causes severe hepatotoxicity and necrosis in both humans and experimental animals [4]. After an overdose of paracetamol, elevated levels of the toxic NAPQI metabolite are generated, which extensively deplete hepatocellular GSH and covalently modify cellular proteins resulting in hepatocyte death [5].

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Liver disorders remain a serious health problem and the management of these liver disorders is still a challenge to the modern medicine. Despite tremendous strides in modern medicine there is still need for a drug that stimulates liver function or offers protection to the liver from damage or helps regenerate hepatic cells. Herbs play a major role in the management of various liver disorders along with other system associated diseases [6]. Medicinal plants such as *Aloe vera, Eclipta alba, Phyllanthus niruri, Solanum Indicum, Maytenus emerginata, Panax ginseng* and *Aegle mameloes* are well known for their hepato-protective effects [3,7]. *Abrus precatorius Linn* is a leguminous plant of the Fabaceae family. Its seeds, roots and leaves are widely used for medicinal purposes in Africa and Asia [8].

In Nigeria, the Igbos use the aqueous decoction of the seeds to treat a wide range of conditions including ulcer, infections, hypertension, diarrhoea, infarct and ogbanje [9]. Reports have shown the uterotonic activity of Abrus seeds in rats [10] and the antibiotic potential of three pyridinium constituents of the seed cotyledons [11]. The works of Dipanjan and Tapas [12] and Battu and Kumar [13] have shown that phytochemicals such as isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates and triterpinoids are present in the seeds of *Abrus precatorius* and these phytochemicals have been suggested to be responsible for the medicinal properties observed in most medicinal plants [14]. However alkaloids such as boldine, Protopine, Berberine, columabmine, oxycathine, yatroricine, atropine, reserpine, Pilocarpine have also been shown to be potent against liver disorders [15]. Thus this study aimed at investigating the prophylactic effect of an alkaloid-rich fraction of *Abrus precatorius* seed chloroform-methanol extract on paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Materials

Collection and Identification of Plant Materials: The seeds of *Abrus precatorius* Linn Fabaceae was collected from Igala Area of Kogi State and authenticated by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria.

Preparation of Plant Extract and Alkaloid rich-Fraction: The seeds of *Abrus precatorius* were pulverized using a high speed grinder. Six hundred grammes (600g) quantity of the crushed seeds was macerated in a mixture of 400 ml of methanol and 800 ml of chloroform for 24 hr. The macerate was filtered through Whatman no. 4 filter paper and the filtrate shaken with 0.2 volume water to obtain two layers. The upper methanol layer was collected and the extract concentrated using magnetic stirrer to get a dry weight of 12.51g. Fractionation of the dry residue was done by gel filtration, using Sephadex G15which was allowed to swell for 3hrs and packed in a column of height 27cm and diameter 2.5cm. The extract was diluted with distilled water and introduced into the column and eluted with water. Fractions (3 ml) were then collected in test tubes labelled 1-50 (of about 3ml each). The absorbance reading of various fractions was read in a UV-Visible spectrophotometer at 265nm. A plot of absorbance against the fractions was drawn to produce elution profile with different peaks of fraction range. The Fractions were spotted on a TLC plate (F_{254} pre-coated with silica gel) and was left to dry for about one hour. Afterward, the spotted plates were developed in a chromatographic tank (made up of butanol, acetic acid and water in ratio of 65:13:22 respectively) which was allowed to equilibrate for one hour. After development of the plate, it was sprayed with Drangendoff's reagent. The fractions that turned purple were pulled into a beaker as fraction I while the other fractions in which there was no colour change were pulled together as fraction II. The fractions were concentrated using magnetic stirrer, however fraction I (alkaloid-rich fraction) was used in the study.

Experimental Animals: Twenty-five (25) Wistar albino rats weighing 70-100g were used for this study. They were obtained from the rat house of Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. These animals were fed with standard pellet feed (Vital feed® Jos, Nigeria) and water *ad libitum* and were acclimatized to laboratory conditions for 2 weeks before the experiment. Clearance and approval for the humane use and handling of laboratory animals were given by the ethical committee of Biochemistry Department University of Nigeria Nsukka.

Experimental Protocol: All animals were randomly divided into five groups of five rats per group based on the similarity of their weight.

- Group 1 served as the control group receiving 5 ml/kg of normal saline (p.o).
- Group II served as paracetamol control group and received paracetamol 2500 mg/kg b.w. only [16].
- Group III was treated with silymarin as a reference drug 100 mg/kg b.w. (p.o) for 7days before paracetamol induction.

- Group IV was treated with 100 mg/k.g. b.w. of the alkaloid-rich fraction for 7days before paracetamol induction.
- Group V was treated with 200 mg/k.g. b.w. of the alkaloid-rich fraction for 7days before paracetamol induction.

Biochemical Investigation: All animals were sacrificed 24hrs after the last treatment and blood was collected and allowed to clot for serum separation. The changes in serum liver marker enzymes (ALP, AST, ALT) bilirubin levels, serum urea, creatinine, malondialdehyde (MDA), superoxide dismutase (SOD), serum electrolytes and hematological parameters were measured for biochemical investigations.

Estimation of Biochemical Parameters: Alkaline phosphate activity was estimated using the method of Klein *et al*. [17], Babson [18] and Babson *et al*. [19]. Assay of Aspartate aminotransferase activity and Alanine aminotransferase Activity was done using the method of Reitman and Frankel [20]. Determination of Total Bilirubin Concentration was done using the method described by Jendrassik and Grof [21] while urea concentration was determined using the method described by Fawcett and Scott [22]. Serum creatinine concentration was determined using the method described by Bartels and Rohmen [23]. Serum sodium ion concentration and serum potassium ion concentration were determined by the method of Tietz [24] while Serum chloride ion was determined by the method described by Skeggs and Hochstrasser [25]. The activity of SOD was evaluated by the method of Xin *et al*. [26], more so malondialdehyde (MDA) a product of Lipid peroxidation was determined as described by Wallin *et al*. [27]. Haemoglobin concentration was determined by the method described by Dacie and Lewis [28] while packed cell volume was done using standard technique as described by Ochei and Kolhartar [29]. Red blood cell count was done using the method as described Cheesbrough [30], however white blood cell count was determined using the standards technique as described by Cheesbrough [31].

Statistical Analysis: The results were expressed as means ± SD and tests of statistical significance were carried out using one way analysis of variance (ANOVA). The Statistical Product for Service Solutions (SPSS), version 20 was used. P values<0.05 will be considered significant.

RESULTS AND DISCUSSION

Alkaloids are naturally occurring chemical compounds containing organic nitrogenous bases. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants and animals and are part of the group of natural products (also called secondary metabolites).

Alkaloids are known to have diverse pharmacological effects on humans and animals. Alkaloids like cocaine the first local anesthetic, tubocurarine the first neuromuscular blocking agent, quinine the first antimalarial, ephedrine, morphine, strychnine and nicotine have been shown to be of medicinal importance and also other alkaloids like hyoscyamine and vinblastine have provided lead compounds for the development of synthetic drugs [35].

However *Abrus precatorius* seeds have been shown to contain three alkaloids which are trigonelline, precatorine and a sugar ester of trigonelline [11, 36]. These alkaloids especially trigonelline have been shown to possess numerous pharmacological effects which includes antibiotic, antioxidant, nutritional and anti-toxic effects [11, 36, 37]. Previous works by Battu and Kumar [13] has showed the hepato-protective ability of the hydroalcoholic seed extract of *Abrus precatorius* seeds however this work tends to look at the prophylactic effect of an alkaloid rich fraction of *Abrus precatorius* seeds on paracetamol-induced hepatotoxicity in wistar albino rats. Paracetamol (acetaminophen) is one of the most commonly and widely used analgesic and is considered safe at therapeutic doses while at overdose it produces acute liver damage. It has been established that at therapeutic dose a fraction of acetaminophen is converted via the cytochrome P_{450}pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) [38] which is conjugated with glutathione (detoxification) to form mecapturic acid which is less toxic and excreted in urine. However at an overdose of acetaminophen, NAPQI formation exceeds into the glutathione stores, leading to accumulation of NAPQI which oxidizes tissue macro molecules such as membrane lipids and-SH groups of protein leading to acute hepatic necrosis.

In the assessment of liver damage certain biomarkers of hepatotoxicity are measured and one of such biomarkers is the enzyme level of transaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) [39] because liver damage arising from necrosis or membrane damage normally releases these enzymes into circulation. Thus, measurement of these enzyme activities in serum gives an indication of the health status of the

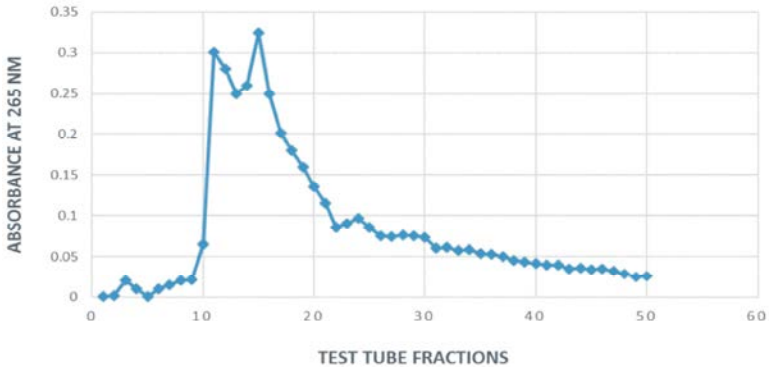

Fig. 1: Absorbance reading of test tube fractions from 1-50 at 265 nm

Table 1: Effect of Alkaloid-rich fraction of *Abrusprecatorius* seed methanol extracton liver marker enzymes and total bilirubin

	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (Mg/dl)
Normal	38.67 ± 1.7*	21.67 ± 0.9*	78.21 ± 1.5*	0.47 ± 0.03*
Paracetamol (2500 mg/kg)	68.0 ± 0.6	42.33 ± 7.2	126.7 ± 15.9	0.73 ± 0.03
Silimarin (100 mg/kg)	41.67 ± 3.5*	23.33 ± 2.9*	80.1 ± 3.4*	0.5 ± 0.00*
Alkaloid-rich Fr (100 mg/kg)	52.67 ± 2.7*	33.0 ± 2.9	91.1 ± 3.7	0.6 ± 0.57
Alkaloid-rich Fr (200 mg/kg)	48.67 ± 2.7*	26.33 ± 3.0*	84.35 ± 3.4*	0.57 ± 0.67*

Values are Mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

liver. Elevated levels of AST and ALT are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [40], however ALT is more specific because it is localized in the liver. In the present study, from Table 1 a significant increase (P<0.05)was observed in the serum ALT and AST levels of the paracetamol control group after paracetamol administration which is a sign of liver damage, but the elevated levels of these enzymes were not observed in the groups pre-treated with the alkaloid-rich fraction at 100 mg/kg and 200 mg/kg indicating that the fraction offered protection by preserving the structural integrity of the hepatocellular membrane against paracetamol which might be through its membrane stabilization activity. This is in accordance with the work of Battu and Kumar [13] which suggest that extracts that exhibit hepato-protective effect could contain substances that act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Also its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1, 1-di phenyl-2-picrylhydrazyl radical (DPPH) which has been shown to be one the major qualities exhibited by medicinal plants with hepato-protective activity [41].

Serum alkaline phosphatase (ALP) activity and bilirubin levels are related to the functionality of the liver. ALP lines the cells in the biliary ducts of the liver and when the bile ducts are blocked maybe by a tumor or inflammation, ALP and bilirubin levels will be increased much more than AST and ALT in the serum. High levels of ALP and bilirubin in serum are due to increased synthesis by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure [42]. Increase in bilirubin concentration might also be as a result of the breakdown of hemoglobin by the free radicals generated by NAPQI. From Table 1 pre-treatment with the alkaloid-rich fraction was able to prevent the observed increases in levels of ALP and bilirubin to a significant level (P<0.05) especially at the dose of 200 mg/kg when compared to the paracetamol untreated groups. Effective control of bilirubin levels and alkaline phosphatase activity points towards an improved secretory mechanism of the hepatic cells by the extract. This work is in tandem with the works of Ebenyi *et al*. [6] which demonstrated effective control of bilirubin and ALP levels by *alliums sativum* extract in paracetamol induced hepatotoxicity in rats.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to an overdose of acetaminophen and generation of highly toxic and reactive compound NAPQI [43]. In the present study, an elevation in the levels of MDA a product of lipid peroxidation was observed in the serum of animals treated with acetaminophen only. From Table 2, an increase in MDA levels of the paracetamol control group suggests enhanced lipid peroxidation leading to tissue damage

Table 2: Effect of Alkaloid-rich fraction of *Abrusprecatorius* seed methanol extracton urea, creatinine, MDA concentrations and SOD activity

MDA (Mg/ml)	SOD(U/L)	UREA(Mg/dl)	CR(Mg/dl)	
Normal	4.38 ± 0.6*	39.72 ± 0.9*	45.0 ± 3.0*	1.3 ± 0.2*
Paracetamol (2500 mg/kg)	6.51 ± 0.3	25.88 ± 0.7	68.0 ± 3.5	2.0 ± 0.1
Silimarin (100 mg/kg)	4.47 ± 0.5*	35.02 ± 0.9*	49.33 ± 1.5*	1.47 ± 0.1*
Alkaloid-rich Fr (100 mg/kg)	5.69 ± 0.3	28.3 ± 0.9	57.67 ± 4.7	1.7 ± 0.00
Alkaloid-rich Fr (200 mg/kg)	5.07 ± 0.4	31.59 ± 0.6*	54.67 ± 4.8*	1.63 ± 0.1*

Values are Mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. However pre-treatment with the alkaloid-rich fraction was able to prevent increase in the levels of MDA but the decrease was not statistically significant (P□0.05). This is in line with the work done by Nashwa and Aita [44] where the hepatoprotective Effect of Spirulina Platensis Against Aluminum Chloride Induced Liver Damage in Rats.

Activity of serum superoxide dismutase (SOD) is the most sensitive enzymatic index in liver injury caused by ROS and oxidative stress [45]. It has been reported as one of the most important enzymes in the enzymatic antioxidant defence system [46]. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Decrease in the activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live damage [45]. In Table 2, it was observed that pre-treatment with the alkaloid-rich fraction at the dose of 200 mg/kg caused a significant increase (P<0.05) in hepatic SOD activity; thus, reducing the reactive free radical induced oxidative damage to the liver. This shows that the fraction can reduce reactive free radicals that could cause oxidative stress and damage to the tissues and improve the activities of the hepatic antioxidant enzyme. This corresponds with work of Seham *et al*. [47] which showed that extracts with hapato-protective activity has the ability to maintain and even improve *in vivo* antioxidant activity.

Several metabolic disorders including urea and creatinine derangements are possible in the presence of acetaminophen overdose [48]. Serum urea and creatinine are kidney markers used to test for the functionality of the kidney in a diseased state. Urea is a waste product of protein catabolism that should be excreted through the urine while creatinine is also a waste product the breakdown of creatine phosphate in the muscle and is usually produced in the body in proportion to body mass. However increase in serum urea and creatinine levels may occur when the rate of breakdown of proteins and intracellular macromolecules of the muscle tissues exceeds the rate of their clearance (glomerular filtration rate). As observed in Table 2, administration of acetaminophen resulted to a significant increase (P<0.05)in the serum urea and creatinine levels which could be as a result of kidney damage or increase in breakdown of intracellular membrane protein, muscle mass or creatinine phosphate by free radicals generated by NAPQI. This might also account for the loss of weight experienced by the rats in the group that received acetaminophen only. However, in the groups pre-treated with the alkaloid-rich fraction at the dose of 200 mg/kg, there was a significant reduction (P<0.05) in serum urea and creatinine levels, which shows that the fraction could inhibit the damage of kidney and improve functionality probably by increasing the cell membrane stability. This result is in line with the works of Ezeonwu and Dahiru [49] where the protective effect of Bi-Herbal formulation of *Ocimum gratissimum* and *Gongronemalatifolium* aqueous leaf extracts on acetaminophen-induced hepato-Nephrotoxicity in Rats was examined which suggest that at an overdose of acetaminophen serum urea and creatinine levels are stable when the kidney functionality is maintained by the extract.

Electrolytes are electrically charged minerals that help move nutrients into and wastes out of the body's cells, maintain a healthy water balance and help stabilize the body's acid/base (pH) level. The levels of electrolytes, urea and creatinine give a strong indication of kidney function, principally in excretion and homoeostasis. From Table 3, low levels of serum electrolytes (Na^+ and Cl^-) was observed in the groups that received only an overdose of acetaminophen and this loss in electrolytes could be as a result of impairment in the kidney function or insensitivity to the antidiuretic, aldosterone and parathyroid hormone in maintaining the electrolyte balance of the system. However, potassium an intracellular ion showed a marked increase in concentration in the groups that received only the toxicant. This could be as a result of renal failure [50] or metabolic acidosis which may be caused by the

Table 3: Effect of Alkaloid-rich fraction of *Abrusprecatorius* seed methanol extracton serum electrolytes

	NA^+(mEqI/L)	k^+ (mEqI/L)	Cl-(mEqI/L)
Normal	120.25 ± 4.46*	5.37 ± 0.73	85.44 ± 2.8
Paracetamol (2500 mg/kg)	90.87 ± 3.57	6.47 ± 0.69	79.60 ± 2.8
Silimarin (100 mg/kg)	116.67 ± 12.73*	5.67 ± 0.37	85.00 ± 3.1
Alkaloid-rich Fr (100 mg/kg)	110.42 ± 4.64	6.10 ± 0.58	81.23 ± 1.9
Alkaloid-rich Fr (200 mg/kg)	114.78 ± 1.16*	5.79 ± 0.34	84.67 ± 4.8

Values are Mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

Table 4: Effect of Alkaloid-rich fraction of *Abrusprecatorius* seed methanol extracton hematological parameters

	Hb (g/dl)	PCV (%)	RBC (x109/l)	WBC (mm^{-3})
Normal	15.67 ± 0.9*	49.00 ± 2.08*	326.00 ± 30.27*	4300.00 ± 264.58*
Paracetamol (2500 mg/kg)	11.00 ± 1.53	34.00 ± 3.06	192.00 ± 38.57	6800.00 ± 346.41
Silimarin (100 mg/kg)	14.67 ± 0.67*	45.00 ± 2.52*	316.00 ± 10.58*	5000.00 ± 230.94*
Alkaloid-rich Fr (100 mg/kg)	14.00 ± 1.15	41.00 ± 2.52	280.00 ± 14.42*	5600.00 ± 115.47*
Alkaloid-rich Fr (200 mg/kg)	14.33 ± 0.33*	43.67 ± 2.03*	291.33 ± 15.07*	5400.00 ± 115.47*

Values are Mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

formation of mercapuric acid a compound formed by the reaction of the toxic metabolite of acetaminophen N-acetyl-p-bezoquinone imine (NAPQI) with glutathione. However, pre-treatment with alkaloid-rich fraction of *Abrus Precatorius* was able to maintain the electrolyte balance of the rats in a dose-dependent manner which was only significant with sodium ion at the dose of 200 mg/kg. This shows that the fraction could contain some active biochemical compounds that have the capacity to maintain the functionality of the cells of the kidney and could be effective in the case of kidney damage. This preservative effect could be as a result of some bioactive compounds such as precatorine and a sugar ester of trigonelline Lakshimi *et al*. [37] inherent in the alkaloid rich fraction of the seeds of *AbrusPrecatorius*.

Hematology is the branch of medicine concerned with the study, diagnosis, treatment and prevention of diseases related to the blood. In the present study from Table 4 hematological parameters such as HB, PCV, RBC showed significant decreases (p □ 0.05) in groups that received only the toxicant (paracetamol) when compared to the test groups. PCV measures the percentage by volume of packed RBC in a whole blood sample after centrifugation, while Hb test measures the amount of Hb in grams in 1 dl of whole blood and provides an estimate of oxygen carrying capacity of the RBCs [51] This significant decrease (p □ 0.05) could be as a result of anemia, haemolysis or inability of the kidney to produce erythropoietin (a hormone that stimulates the production of red blood cells) as a result of kidney failure. However, pre-treatment with the alkaloid-rich fraction at the dose of 200 mg/kg showed a dose-dependent significant increase (p □ 0.05) in these hematological parameters when compared to the paracetamol control group. However, the fraction was able to maintain the haematological parameters within the normal range when compared to the paracetamol control group. This shows that the extract could have the ability to regenerate red cells, prevent haemolysis and maintain the blood level in the body. This is in agreement with the work done by Shereen and Zaghlou [52] where the beneficial effects of Green Tea extract on Liver and Kidney Functions, Ultrastructure, Lipid Profile and Hematological Parameters in Aged Male Rats.

WBC is usually important in fighting against infections, from table 4 a significant increase (p □ 0.05) in the white blood cell count (WBC) was observed in the group that received only the toxicant. This could be as a result of infestation, anaemia, infections, tissue damage inflammation or the body in its mechanism fighting the foreign compounds [51]. However treatment with the alkaloid-rich fraction at the doses of 100 mg/kg and 200 mg/kg caused a significant decrease (p □ 0.05) in the WBC count when compared to the paracetamol control group. This is in line with the work that was done by Anbu*et al*.[53]where the anticancer activity of petroleum ether extract of *Abrus precatorius* on Ehrlich Ascitis carcinoma in mice was evaluated. By this result, it could be deduced that there was no anaemic condition, infection or tissue damage among the pre-treated groups and the alkaloid-rich fraction could have potential of boosting the levels of RBC, HB and PCV and also maintain the WBC count in both normal and pathological conditions. However in all the biochemical parameters measured in

this study, the standard control group that received slymarin had a better effect when compared to other test groups that received the alkaloid rich fraction.

CONCLUSION

From the data obtained from this findings, it is evident that the Alkaloid-rich fraction of *Abrus precatorius* methanol extract prevented liver damage in the paracetamol-intoxicated rats, by preventing leakage of liver enzymes into system, enhancing the synthesis of antioxidants or reducing lipid peroxidation in a dose-dependent manner, hence its use as a hepato-protective agents may have scientific bases. However there is need to test for the bioactive ingredient(s) of this alkaloid rich fraction of *Abrus precatorius* seeds.

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REFERENCES

1. Kumar, K.V., R. Satish, T. Rama, A. Kumar, D. Babul and J. Samhitha, 2010. Hepatoprotective effect of *Flemingia strobilifera* on paracetamol-induced hepatotoxicity in rats. International Journal of PharmTech Research, 2: 1924-1931.
2. Vidhya, M.H.L. and B.S.M. Mettilda, 2009. Hepato-protective activity of *Phyllanthus Emblica* against paracetamol-induced hepatic damage in Wistar albino rats. African Journal of Basic and Applied Sciences, 1(1-2): 21-25.
3. Rasha, H. and G. Hasan, 2015. Antioxidant Effects of Panax ginseng and Zinc against CCl4 Induced Hepatotoxic on Rats. Global Veterinaria, 14(1): 103-111.
4. Garba, S.H., N. Sambo and U. Bala, 2009. The effect of the aqueous extract of *Kohautiagrandiflora* on paracetamol-induced liver damage in albino rats. Nigerian Journal of Physiological Sciences, 24(1): 17-23.
5. Galal, R.M., H.F. Zaki, M.S.E. Mona and M.A. Azza, 2012. Potential protective effect of honey against paracetamol-induced Hepatotoxicity. Archives of Iranian Medicine, 15: 674-680.
6. Ebenyi, L.N., U.A. Ibiam and P.M. Aja, 2012. Effects of Alliums sativum extract on paracetamol-induced hepatotoxicity in albino rats. International Research Journal of Biochemistry and Bioinformatics, 2(5): 93-97.
7. Parmar, S.M., P.H. Vashrambhai and K. Kalia, 2010. Hepatoprotective activity of some plants extract against paracetamol-induced hepatotoxicity in rats. Journal of Herbal Medicine and Toxicology, 4(2): 101-106.
8. Yadava, R.N. and V.M. Reddy, 2002. A new biologically active flavonol glycoside from the seeds of*AbrusprecatoriusLinn*. Journal of Asian National Product Resources, 4(2): 103-107.
9. Nwodo, O.F.C. and E.O. Alumanah, 1991. Studies on *Abrusprecatorius* seeds II: Antidiarrhoeal activity. Journal of Ethnopharmacology, 31: 395-398.
10. Nwodo, O.F.C. and J.H. Botting, 1983. Uterotonic activity of *Abrusprecatorius* seeds. Planta Medica, 47: 230-233.
11. Amuta, O.P., P.O. Nnamani, A.D. Musa and O.F.C. Nwodo, 2011. Three pyridiniumalkaloids may account for the antibiotic effect of the seed of *Abrusprecatorius*. Der Chemical Sinica, 2(2): 44-45.
12. Dipanjan, G. and K.M. Tapas, 2007. Immunomodulatory and anti-tumor activities of native and heat denaturedAbrus agglutinin. Immunobiology, 212: 589-599.
13. Battu, G.B. and B.M. Kumar, 2009. Hepato-protective activity of *Abrusprecatorius* Linn against paracetamol-induced hepatotoxicity in rats. Pharmacologyomline, 3: 366-375.
14. Ukegbu, C., A. Odiba. A. Edeke, O. Anunobi and I. Chukwunonyelum, 2016. Anti-diabetic Effect of the Methanolic Leaf Extract of *Axonopuscompressus* (*P. Beauv*) in Alloxan Induced Diabetic Rats. International Journal of Biochemistry Research and Review, 12(1): 1-5.
15. Valan, M.F., D.B.A. John and R. Venkataraman, 2010. Phytoconstituents with Hepatoprotective Activity. International Journal of Chemical Scientist, 8(3): 1421-1432.
16. Mitchell, J.R., D.J. Jollow, W.Z. Potter, J.R. Gillettee and B.N. Brodie, 1973. Acetaminophen induced hepatic necrosis: Role of drug metabolism. Journal of Pharmacology Explanatory Therapy, 187: 185-194.

17. Klein, B., P.A. Read and L.A. Babson, 1960. Rapid colorimetric method for the quantitative determination of serum alkaline phosphatase. Clinical Chemistry, 6: 269-275.
18. Babson, L.A., 1965. Alkaline phosphatase. Clinical Chemistry, 2: 789-795.
19. Babson, L.A., S.J. Greeley, C.M. Coleman and G.D. Philips, 1966. Alkaline phosphatase determination. Clinical Chemistry, 12: 482-490.
20. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28: 56-63.
21. Jendrassik, L. and P. Grof, 1938. Simplified photometric methods for the determination of bilirubin. Biochem Zschr, 297(8): 1-9.
22. Fawcett, J.K. and J.E. Scott, 1960. Colormetric method of determining serum urea concentration. Journal of Clinical Pathology, 13: 156-159.
23. Bartels, H. and M. Rohmen, 1972. Colormetric method of determining serum creatinine concentration. Clinical Chemistry Acta, 37: 193-199.
24. Tietz, N.W., 1976. Fundamentals of Clinical Chemistry. W.B. Saunders Company, Philadelphia. pp: 874.
25. Skeggs, L.T. and H.C. Hochstrasser, 1964. Colorimetric determination of chloride. Clinical Chemistry, 10: 918-924.
26. Xin, Z., D.E. Waterman, R.M. Henken and R.J. Harmon, 1991. Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. Journal of Diary Science, 74: 3078-3080.
27. Wallin, B., B. Rosengren, H.G. Shertzer and G. Camejo, 1993. Lipoprotein oxidation and measurement of TBARS formation in a single microliter plate: Its use for evaluation of antioxidants. Analytical Biochemistry, 208: 10-15.
28. Dacie, J.V. and S.M. Lewis, 1991. Practical Haematology. 7th Edn. Churchill Livingstone, Edingburgh, pp: 535-544.
29. Ochei, J. and A. Kolhatkar, 2008. Medical Laboratory Sciences: Theory and Practice. Tata McGraw Hill, New York, pp: 663-665.
30. Chessbrough, M., 2005. District Laboratory Practice in Tropical Countries (Part 1). 2ndEdn. Cambridge University Press, pp: 340-349.
31. Cheesbrough, M., 2008. Counting white cells and platelets in district laboratory practice in tropical countries part 2. The Edinburgh: Cambridge University Press, United Kingdom, pp: 314-329.
32. Andre, N., W. Xiaoming, G. Pan, Z. Fan, F. Hong and O. Olajide, 2013. A review on indole alkaloids isolated from Uncariarhynchophylla and their pharmacological studies. Fitoterapia, (86): 35-47.
33. Valli, S., S. Gokulshankar, B.K. Mohanty, M.S. Ranjith, S.R. Ashutosh and V. Remya, 2014. Anticryptococcal Activity of Alkaloid Rich Fraction of Leaves of *ProsopisJuliflora*-A Future Promising Supplementary Therapy for Cryptococcosis and Cryptococcal Meningitis? International Journal of Pharmacy and Pharmaceutical Sciences, 6(2): 491-495.
34. Felix, G.C. and J.A. Gregory, 1996. Screening of Medicinal plants used by the Garifuna of Eastern Nicaragua for bioactive compounds. Journal of Ethnopharmacology, 53: 29-50.
35. Jack, G., 2001. Plant Alkaloids. De Montfort University, Leicester, UK. Encyclopedia of Life Sciences / and 2001 Nature Publishing Group / Www.Els.Net.
36. Rajaram, N. and K. Janardhanam, 1992. The chemical composition and nutritional potential tribal pulse, Abrusprecatorius. Plant food for Human Nutrition, 42(4): 285-290.
37. Lakshmi, P., H. Tajdar, T. Jehangir and S. Sultana, 2006. The effect of gallic acid on renal biochemical alterations in male rats. Human Toxicology, 25: 523-529.
38. Dahlin, C.D., G.T. Miwa, A.Y.H. Lu and S.D. Nelson, 1984. N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen (reactive metabolite/reduction/conjugation). Proceedings from the National Academy of Science, 81: 1327-1331.
39. Emad, A.H. and S.A. Elgam, 2016. Protective Effect of Melatonin against Chromium-Induced Hepatotoxic and Genotoxic Effect in Albino Rats. Global Veterinaria, 16(4): 323-329.
40. Drotman, R.B. and G.T. Lawhorn, 1978. Serum enzymes are indicators of chemical induced liver damage. Drug and Chemical Toxicology, 1: 163-71.
41. Anosike, C.A., N.E. Ogbodo, A.L. Ezugwu, R.I. Uroko, C.C. Ani and O. Abonyi, 2015. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Activity of Some Ethnomedicinal Plants in Nigeria. American-Eurasian Journal of Toxicological Sciences, 7(2): 104-109.
42. Gaw, A., R.A. Cowan, D.S.J. O'Reilly, M.J. Stewart and J. Shepherd, 1999. Clinical biochemistry-an illustrated color text. 1st edn. New York: Churchill Livingstone, pp: 51-3.

43. Muriel, P., 1997. Peroxidation of lipids and liver damage In: *A*ntioxidants, Oxidants and Free Radicals, S.I. Baskin and H. Salem, edtitors, pp: 237.
44. Nashwa, A. and A. Aita, 2014. Hepatoprotective Effect of Spirulina Platensis Against Aluminum Chloride Induced Liver Damage in Rats. Global Veterinaria, 13(4): 552-559.
45. Curtis, J.J. and M. Mortiz, 1972. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. Gastroenterology, 62: 84-92.
46. Hijora, E., F. Nistar and A. Sipulova, 2005. Changes in ascorbic acid and malonaldehyde in rats after exposure to mercury. BratislLekListy, 106(8-9): 248-251.
47. Seham, S.K., M.M. Abdel-Kader, E.M. Al-Sayed, S. El-Din, M.H.A.Z. El-Hawary and M.M. Haggag, 2014. Modulatory Effects of Aerial Parts of Coriandrum sativum L. on Carbon-Tetrachloride Induced Hepatorenal Toxicity. Global Veterinaria, 12(4): 523-531.
48. Kale, R.H., U.K. Halde and K.R. Biyani, 2012. Protective Effect of Aqueous Extract of *Urariapicta*on Acetaminophen Induced Nephrotoxicity in Rats. International Journal of Research in Pharmacy and Biomedical Sciences, 3(1): 110-113.
49. Ezeonwu, V.U. and D. Dahiru, 2013. Protective Effect of Bi-Herbal Formulation of *Ocimumgratissimum*and *Gongronemalatifolium*Aqueous Leaf Extracts on Acetaminophen-induced Hepato-Nephrotoxicity in Rats. American Journal of Biochemistry, 3(1): 18-23.
50. Henry, R.J., D.C. Cannon and J.W. Winkelman, 1974. Clinical Chemistry, Principles and Techniques, 2nd edn, Harper and Row, pp: 525.
51. Nwodo, O.F.C., P.E. Joshua, N.F. Ozoemena and N.J. Onwuzu, 2010. Haematological evaluation of the effect of coconut (*Cocosnucifera*) water on paracetamol-intoxication in laboratory rats. Journal of Pharmacy Research, 3(8): 1831-1834.
52. Shereen, B.G. and D.M. Zaghloul, 2013. Beneficial Effects of Green Tea Extract on Liver and Kidney Functions, Ultrastructure, Lipid Profile and Hematological Parameters in Aged Male Rats. Global Veterinaria, 11(2): 191-205.
53. Anbu, J., V. Ravichandiran, M. Sumithra, B.C. Sudheer, K. Swaroop, S.L.V.V.S.N. Kumar, R. Kannadhasan and R.K. Satheesh, 2011. Anti-cancer Activity of Petroleum Ether Extract of *Abrus Precatorius*on Ehrlich Ascitis Carcinoma in Mice. International Journal of Pharmacy and BioSciences, 2(3): 24-31.