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EFFECTS OF METHANOL EXTRACT OF *PAULLINIA PINNATA* ON LIVER ENZYMES, OXIDATIVE STRESS AND WHITE BLOOD CELL DIFFERENTIALS IN CCl_4-INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT
Paullinia Pinnata is a plant introduced to tropical Africa and has been reported to be useful in folkloric medicine. The current study investigated the effects of methanolic extract of the leaf of *Paullinia Pinnata* against CCl4 – induced hepatotoxicity, oxidative stress and white blood cell differentials in Wistar rats. Twenty Five (25) rats arbitrarily appropriated into five groups (1-5). Group 1 served as control. Groups 2, 3, 4 and 5 were were separately actuated intraperitoneally with single organization of 2 ml/kg (b. wt) CCl4. Twenty Four hours after induction, the rats in groups 3, 4 and 5 were orally treated with 50mg/kg, 100mg/kg and 150 mg/kg b. wt. of Methanol extract from *Paullinia. pinnata* respectively, once daily for 28 days while the rats in group 2 were left untreated. Levels of liver function indices and the activities of antioxidant enzymes were determined. Administration of methanolic extract from *Paullinia Pinnata* significantly ($p < 0.05$) ameliorated CCl4- induced hepatotoxicity with respect to liver function indices as ALP, ALT, AST and GGT reduces in a dose-dependent manner and oxidative stress markers such as SOD, GPX and CAT increases but that of MDA reduces. There was also significant increase in total white blood cell and differential white blood cell counts. The study suggests that methanolic extract of *Paullinia Pinnata* leaf is a potential hepatoprotective agent against CCl4-mediated hepatic injury through fortification of antioxidant defense mechanisms and increased immune response.

1. INTRODUCTION
The liver assumes an imperative part in the digestion, detoxification, and expulsion of various harmful synthetic compounds from the body. The guideline of various physiochemical capacities, like oxidation, decrease, hydroxylation, hydrolysis, formation, sulfation, and acetylation, occurs in the liver. Presently, liver sicknesses present genuine medical problems. Natural toxins, irresistible specialists, and hepatotoxins (carbon tetrachloride) are accounted for to cause liver wounds.[1,2] Artificially ready or ordinary medications utilized for the avoidance and treatment of liver wounds may be deficient and can cause genuine aftereffects. Because of these reasons, countless individuals all throughout the planet like to devour natural plants for the treatment of liver infections. Subsequently, track down elective medications from regular plants with less poisonousness and high adequacy for the treatment of liver issues.[3]

White Blood Cells (WBC), or leucocytes are cells of the immune system involved in defending the body against both infections, disease and foreign materials.[3]

White blood cells are the effector cells of the immune system and circulate throughout the bloodstream and lymphatic system. An infection or a physical injury results in an inflammatory response, which induces increased production of WBCs for resolving the injury or infection.

2. MATERIALS AND METHODS
Identification of Plant
Fresh leaves of *Paullinia Pinnata were* harvested from a nearby bush behind the Faculty of Basic Medical Sciences of the University of Port Harcourt, Rivers state, Nigeria. The leaf was sent to Plant Science and Biotechnology Department of the University of Port Harcourt for identification and authentication, with Herbarium Number **(UPH/P/236).**

Preparation of the *Paullinia Pinnata* Leaf Extract
The fresh leaves of *Paullinia Pinnata was* dried under room temperature for two weeks. The dried samples were grinded into powder form using grinding machine. Soxhlet method was used for extraction. 2kg of the

leaves was gotten after grinding and the powdered leaves sample was dissolved in 1600ml of 100% methanol for 72 hours. *Paullinia Pinnata was* strain using filter paper N0.1001125 to take apart the remains from residue, filter paper was folded into four portions placed in a funnel and the funnel is placed into a beaker, the filtrate containing the extract was cautiously poured into the funnel which filtered into the beaker through the filter paper. The filtration was repeated three times to achieve a clear filtrate, the filtrate is then transferred into a rotary evaporator to remove the methanol from extract, the semi-liquid was poured into an evaporating dish and placed on a steam bath for complete drying at a temperature of 45°C to 50°C. The drying was monitored until it turned into a paste form. The percentage yield of *Paullinia Pinnata was* estimated. The extracts were moved to air-tight bottles and maintained at 4°C, away from light.

Experimental Animals

A total of 25 Wistar rats weighing between 120–140g were acquired from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt were used for this experiments. After weaning, animals received standard laboratory rat feeds and water *ad libitum*. Rats were housed in approved cages and kept on a regular 12 hours dark/light cycle. All animals received care in accordance with the Nigerian law on experimentation with laboratory animals which is based on the US National Institutes of Health guidelines. Animals were acclimatized for 2weeks.

Research Design

GROUPS	DIVISION	TREATMENT	PROCEDURE AND DURATION
Group 1	Control (5 rats)	Distilled water	Stress free throughout the experiment
Group 2	CCl_4 induced hepatotoxicity (2mL/Kg) (5 rats)	Untreated	The rats were left untreated to serve as negative control.
Group 3	CCl_4 induced hepatotoxicity (2mL/Kg) (5 rats)	50mg/kg of *Paullinia Pinnata* extract	The rats were treated with 50mg/kg of *Paullinia Pinnata* extract for 3 weeks
Group 4	CCl_4 induced hepatotoxicity (2mL/Kg) (5 rats)	100mg/kg of *Paullinia Pinnata* extract	The rats were treated with 100mg/kg of *Paullinia Pinnata* extract for 3 weeks
Group 5	CCl_4 induced hepatotoxicity (5 rats)	150mg/kg of *Paullinia Pinnata* extract	The rats were treated with 150mg/kg of *Paullinia Pinnata* extract for 3 weeks

Oral LD_{50} values of methanolic extract of *Paullinia Pinnata* in animals range from 200 mg/kg to 400 mg/kg.

Administration of Extract

Administration of the extract was given orally once a day (4pm-5pm) for ten days using an oral gavage for 21days. All animals were weighed again before they were anesthetized using di-ethyl ether and subsequently euthanized.

Chemicals

Carbon Tetrachloride (CCl_4) was procured from AXO Industry SA, Chaussee de Louvain 249, 1300 Wavre, Belgium.

Collection of samples/preparation of mixture homogenates

The rats were anesthetized in a di-ethyl ether saturated chamber. The rats were then dissected and blood from the jugular vein was collected and liver tissues were harvested. Tissues were flushed in super cold PBS (0.02 mol/L, pH 7.0-7.2) to eliminate abundance blood altogether and gauged prior to homogenization. The tissues were then minced into little pieces and homogenized in 5mL of PBS with a glass homogenizer on ice. The subsequent suspension was exposed to two freeze-defrost cycles to additionally break the cell layers. From that point forward, the homogenates were centrifuged for 5 minutes at 5,000 x g. the supernatant was eliminated and assay was done as quickly as possible.

Assay for Liver Enzymes

Aspartate Aminotransferase (AST)

It is intended to be used for quantitative in vitro evaluation of Aspartate Aminotransferase (AST) in serum.

Principle

α - oxoglutarate + L -glutamate + Oxaloacetate

AST is determined by monitoring the concentration of oxaloacetate hydrazone produced with 2,4-dinitrophenylhydrazine. Serum is the sample material.

Transaminase action in some sera are excited by concentration of aldehydes, ketones, or oxo acids, so measuring a serum blank (Procedure 2) instead of a reagent blank (Procedure 1) completely eliminates that.

Procedure

Wavelength	Hg 546nm
Cuvette	1cm light path
Temperature (incubation)	37°C

Evaluation against Reagent Blank
AST Analysis

Pipette inside test tubes:

	Blank Reagent	Sample
Samples for analysis	-	0.1 ml
Reagent 1	0.5 ml	0.5 ml
Distilled water	0.1ml	-

Mix, store for exactly 30 min. at 37°C

Reagent 2	0.5 ml	0.5 ml

Mix well, leave to stand for exactly 20 min. at 20 °C to 25°C

Sodium Hydroxide	5.0 ml	5.0 ml

Mix, read the absorbance of sample (A_{sample}) against the reagent blank after 5 minutes.

Measurement against Sample Blank
Pipette reagent into test tubes

	Sample Blank	Sample
Sample	-	0.1 ml
Reagent 1	0.5 ml	0.5 ml

Mix well, store for exactly 30 min. at 37°C

Reagent 2	0.5 ml	0.5 ml
Sample	0.1 ml	--

Mix, allow to stand for exactly 20 min. at 20 - 25°C

Sodium Hydroxide	5.0 ml	5.0 ml

Mix, match the absorbance of the sample (A_{sample}) against the sample blank after 5 minutes.
Normal Value in serum- 12 U/I

Alkaline Phosphatase (ALP)
Alkaline Phosphatase is an enzyme whose concentration may be altered by various diseases. It is used for in vitro evaluationof Alkaline Phosphatase in serum or plasma.

Principle
Serum alkaline phosphatase hydrolyzes a substrate (colourless) of phenolphthalein monophosphate bringing about the colour of pink from phosphoric acid and phenolphlein.

Procedure
The reagent and analyzer is brought to a temperature of 37 °C

Technique	Sample ml	Standard ml
Deionized water	1.0	1.0
Substrate	1 drop	1 drop

Mix, and store for 5 min at 37 °C.

Standard	--	0.1
Sample	0.1	--

Mix and start the chronometer, incubate 20 min at 37°C

Colour developer	5.0	5.0

The reference values are:
Adults: 9 - 35 U/L
Children: 40 - 90 U/L

Alanine Aminotransferase (ALT)
The intended use of Amino Transferase is for quantitative in vitro evaluation of Alanine Aminotransferase (ALT) in serum.

Principle
α-oxoglutarate + L-alanine — L - glutamate + pyruvate
Alanine Aminotransferase is determined by checking the concentration of pyruvate hydrazone generated with 2,4-dinitrophenylhydrazine.

Transaminase actions in some sera are excited by concentration of aldehydes, ketones, or oxo acids, against a serum blank (Procedure 2) rather than a reagent blank (Procedure 1) completely eliminates that.

Procedure

Wavelength	Hg 546nm
Cuvette	1cm light path
Incubation Temperature	37°C

Evaluate against Reagent Blank

Pipette inside test tubes

	Reagent Blank	Sample
Sample	--	0.1 ml
Solution R1	0.5 ml	0.5 ml
Distilled water	0.1 ml	--

Mix well, store for exactly 30 min. at 37°C

Solution R2	0.5 ml	0.5 ml

Mix, allow to stand for exactly 20 min. at 20 to 25°C

Sodium Hydroxide	5.0 ml	5.0 ml

Mix, read the absorbance of sample (A_{sample}) against the reagent blank after 5 minutes.
Quantifying against Sample Blank

Pipette inside test tubes

	Sample Blank	Sample
Sample	--	0.1 ml
Solution R1	0.5 ml	0.5 ml

Mix well, store for precisely 30min, at 37°C

Solution R2	0.5 ml	0.5 ml
Sample	0.1 ml	--

Mix thoroughly, leave to stand for exactly 20 min at 20 °C to 25°C

Sodium Hydroxide	5.0 ml	5.0 ml

Mix, read the absorbance of the sample (A_{sample}) against the sample blank after 5 minutes.

The normal value for ALT is Serum up to 12 U/I.

Statistical Analysis

Data were analysed using one way analysis of variance (ANOVA). Thereafter, the post-hoc test of multiple comparisons (Newman Keuls test) was used to test the individual groups against each other. Confidence level was set at 95% and P-value <0.05 was considered significant.

3. RESULTS AND DISCUSSION

Results

Table 1: Assessment of Liver enzymes across the groups.

Groups	Aspartate transaminase (AST) IU/L	Alanine transaminase (ALT) IU/L	Alkaline phosphatase (ALP) IU/L	Gamma-glutamyltransferase (GGT) IU/L
Group (Control)	22.80 ± 0.73	9.42 ± 0. 83	22.80 ± 1.46	3.10 ± 0.33
Group 2 (Induced CCl_4)	27.80^{a} ± 1.35	13.10^{a} ± 0.67	27.80^{a} ± 2.63	5.96^{a} ± 0.11
Group 3 (CCl_4 + 50mg/Kg)	24.40^{ab} ± 1.86	11.48 ± 0.31	23.40^{b} ± 2.90	4.20 ± 0.45
Group 4 (CCl_4 + 100mg/Kg)	22.60^{b} ± 1.22	11.38 ± 0.49	22.40^{b} ± 3.10	3.80^{b} ± 0.15
Group 5 (CCl_4 + 150mg/Kg)	22.30^{b} ± 1.36	9.92^{b} ± 0.78	22.01^{b} ± 1.20	3.90^{b} ± 0.91

Data were shown as mean ± sem. [a] means values were statistically significant when compared to the normal control value. [b] means values are significant when compared to the induced group.

Table 1 showed the overview of the result of the Liver enzymes across the groups. The result revealed significant changes across the groups when compared to the induced control and the negative control as the value of AST in the control group was 22.80±0.73 while induced group had 27.80±1.35 and the extract goups 3, 4 and 5 had 24.40, 22.60±1.22 and 22.30±1.36 respectively.

ALT control group had 9.42±0.83 while induced group without treatment had 13.10±0.67 and subsequent treated groups 3, 4 and 5 had 11.48±0.31, 11.38±0.49 and 9.92±0.78 respectively.

ALP treated group 3,4 and 5 had 23.40±2.90, 22.40±3.10 and 22.01±1.20 respectively as against the induced group which had 27.80±2.63.

Furthermore, GGT had 5.96±0.11 in the induced group with 4.20±0.45, 3.80±0.90 and 3.90±0.91 in group 3, 4 and 5 respectively.

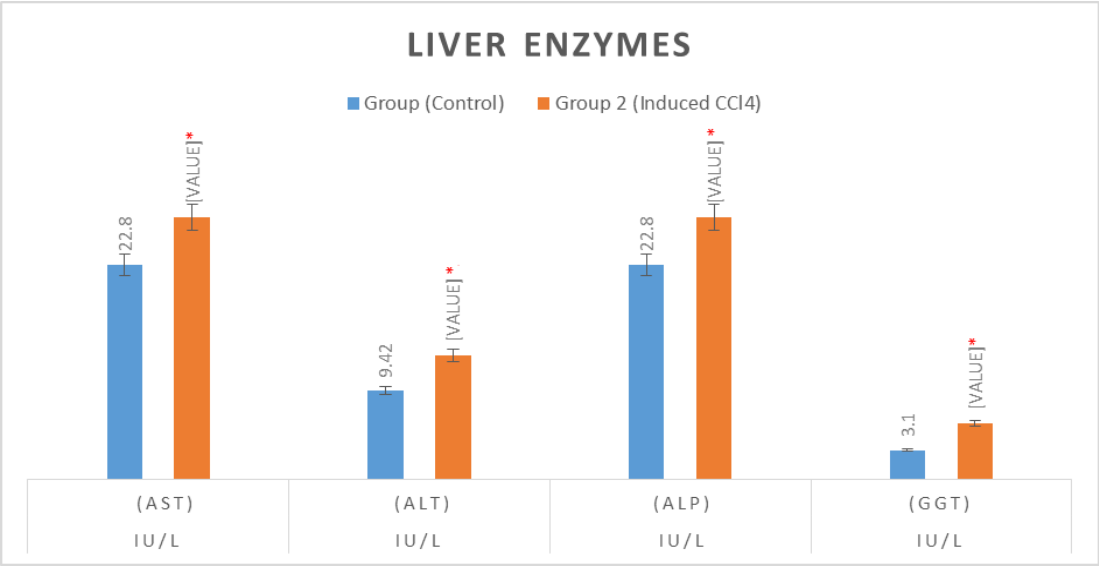

Fig. 1: Liver enzymes level in Normal control group and Induced control.

Figure 1 above revealed the level of various liver enzymes in negative control group and positive control group. The result revealed significant increase in the induced group when compared to the negative control.

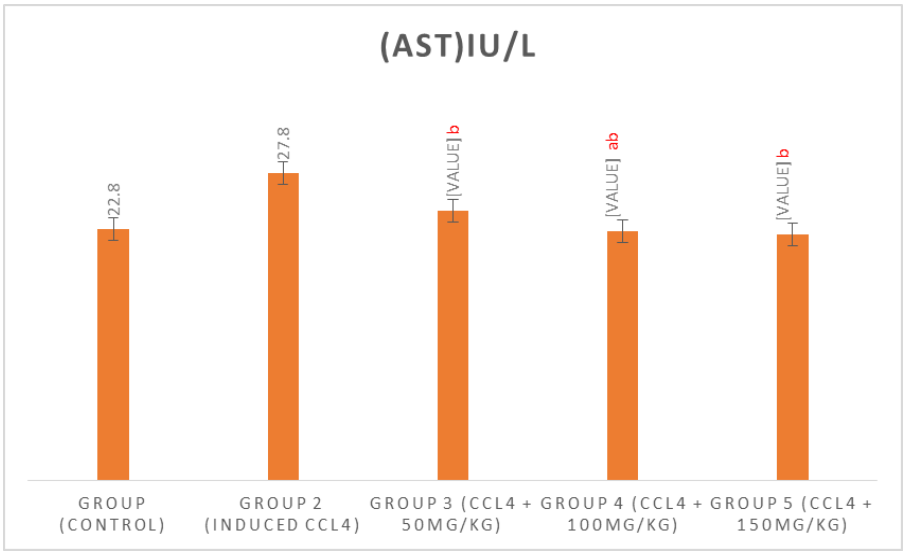

Figure 2: AST level in the test groups compared to negative control and induced control.

Fig. 2 revealed the mean value of AST following induction of rats with CCl4 and subsequent administration of graded doses of the extract. The mean value of the treated groups 3, 4 and 5 were 24.4, 22.6 and 22.3 respectively as compared to the induced group which had 27.8.

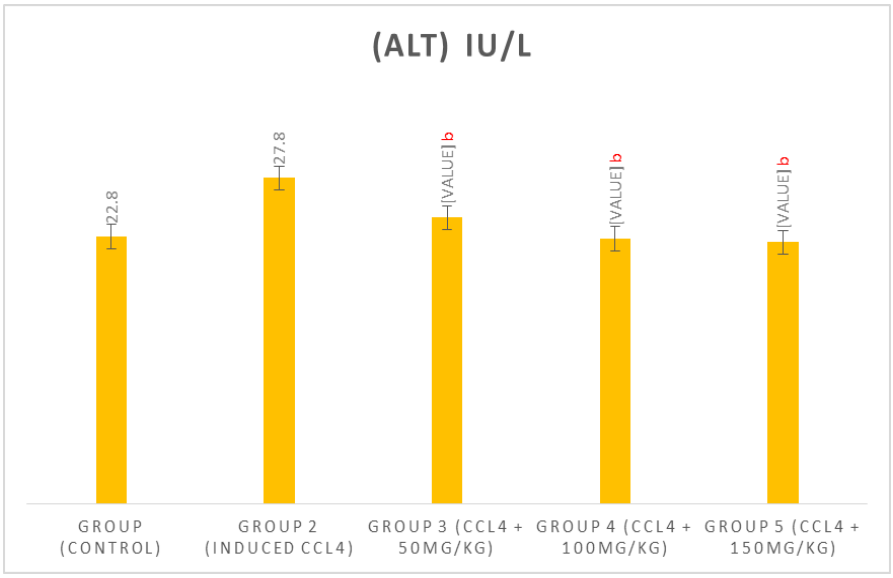

Figure 3: ALT level in the test groups compared to negative control and induced control.

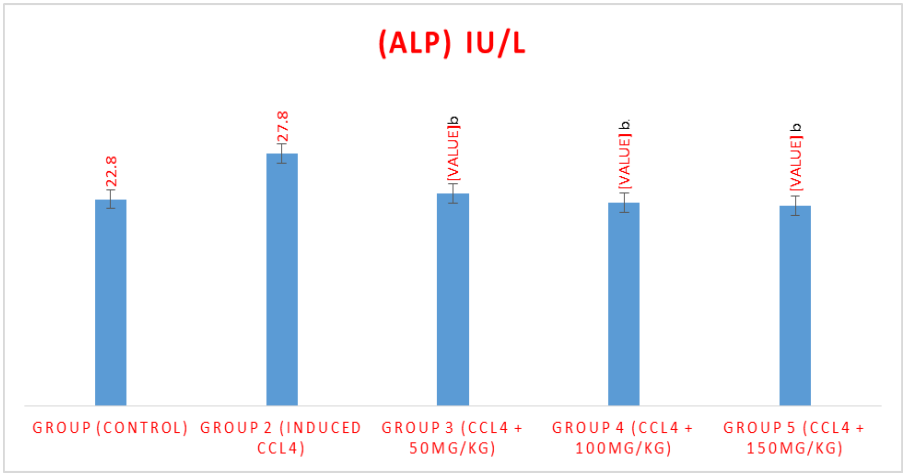

Figure 4: ALP level in the test groups compared to negative control and induced control.

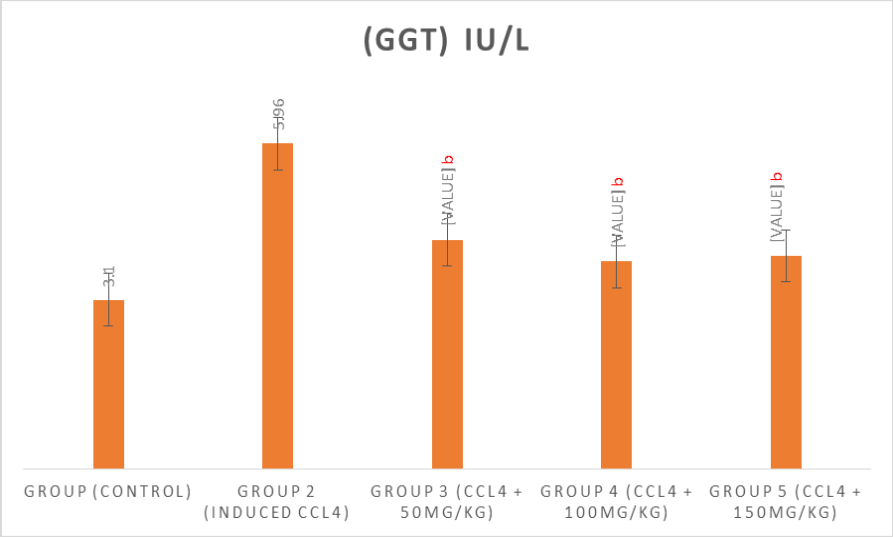

Figure 5: GGT level in the test groups compared to negative control and induced control.

Table 2: Assessment of Oxidative stress markers across the groups.

Groups	MDA (mmo/l)	GPX (ug/ml)	CAT (mmo/l)	SOD (mmo/l)
Group (Control)	0.43 ± 0.02	12.42 ± 0. 83	5.04 ± 0.16	0.04 ± 0.01
Group 2 (Induced CCl_4)	0.48^{a} ± 0.02	9.10^{a} ± 0.67	3.30^{a} ± 0.06	0.02^{a} ± 0.01
Group 3 (CCl_4 + 50mg/Kg)	0.43^{ab} ± 0.37	11.48^{b} ± 0.31	5.46^{b} ± 0.09	0.06^{b} ± 0.01
Group 4 (CCl_4 + 100mg/Kg)	0.27^{ab} ± 0.01	11.38^{b} ± 0.49	5.51^{b} ± 0.05	0.08^{ab} ± 0.01
Group 5 (CCl_4 + 150mg/Kg)	0.35^{ab} ± 0.05	11.92^{b} ± 0.78	5.25^{b} ± 0.20	0.08^{ba} ± 0.01

Data were shown as mean ± sem. [a] means values were statistically significant when compared to the normal control value. [b] means values are significant when compared to the induced group.

Table 2 revealed the mean value of oxidation stress markers MDA, GPX, CAT and SOD.

The result revealed significant increase in MDA in the induced group, while the extract group showed reduced level of MDA when compared to the induced group. GPX, CAT and SOD were significantly reduced in induced group while extract reconsolidated the effect of CCl4.

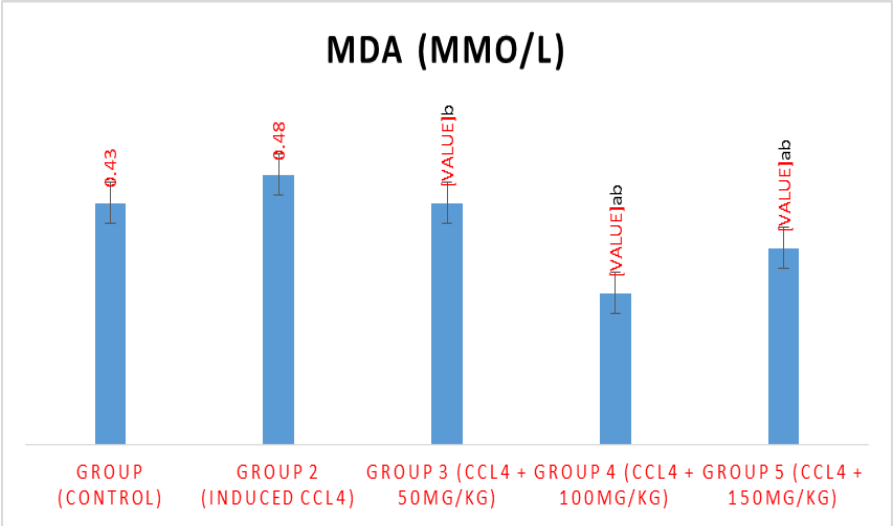

Figure 6: Level of MDA in the test groups compared to negative control and induced control.

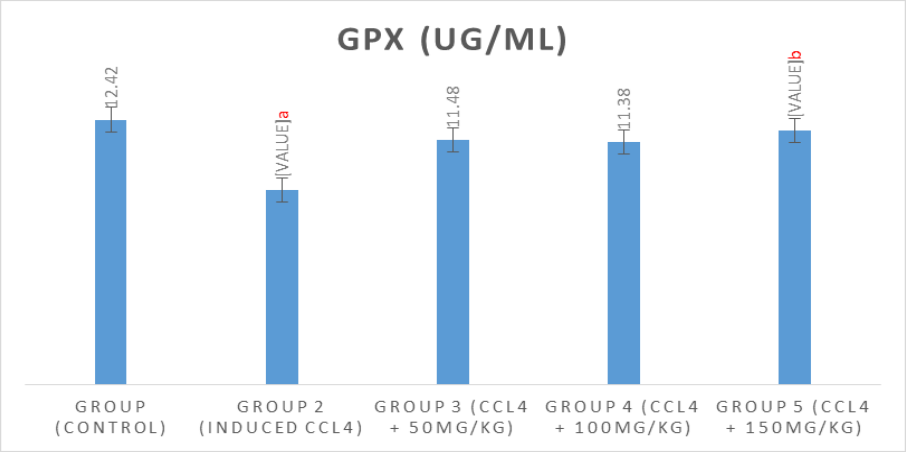

Figure 7: Level of GPX in the test groups compared to negative control and induced control.

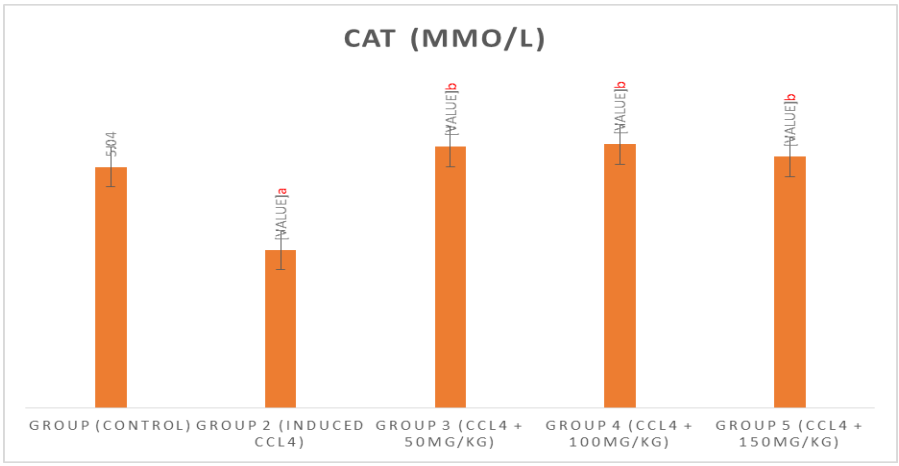

Figure 8: Level of CAT in the test groups compared to negative control and induced control.

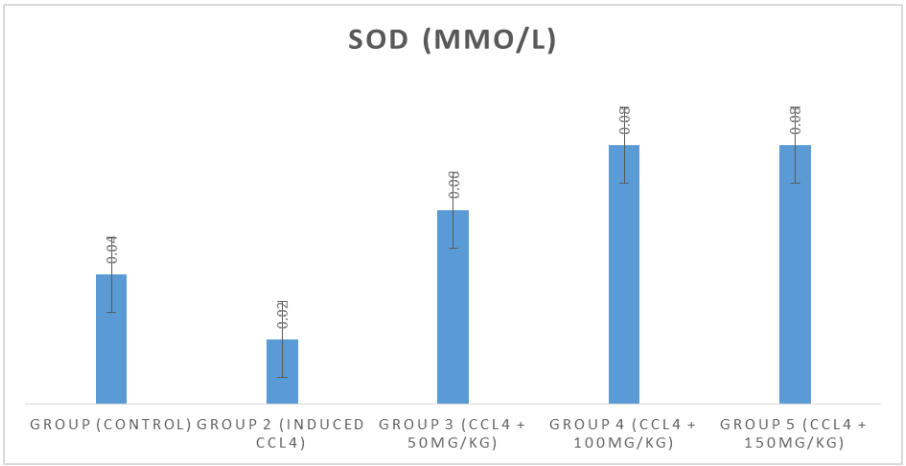

Figure 9: Level of SOD in the test groups compared to negative control and induced control.

Table 3: White blood cell counts in.

Group	WBC (x103 /µL)
Group (Control)	9.98± 2.67
Group 2 (Induced CCl_4)	17.20^{a}± 0.4
Group 3 (CCl_4 + 50mg/Kg)	14.60^{ab}± 0.18
Group 4 (CCl_4 + 100mg/Kg)	27.20^{ab}± 3.8
Group 5 (CCl_4 + 150mg/Kg)	16.00^{ab}± 1.8

Values are presented in mean ± sem, n= 5. [a] means values are statistically significant when compared to the normal control. [b] means values are statistically significant when compared to the induced control.

Table 4. White blood cells differentials.

Groups	Neutrophils	Lymphocytes	Eosinophil's	Monocyte
Group (Control)	26.40± 1.12	66.00 ± 1.87	2.60 ± 0.24	5.00 ± 0.54
Group 2 (Induced CCl_4)	32.80[a]± 0.96	56.80[a] ± 1.71	4.20 ± 0.37	9.20 ± 0.37
Group 3 (CCl_4 + 50mg/Kg)	35.60ab± 3.42	52.80[ab] ± 4.35	3.40 ± 0.24	8.20 ± 0.91
Group 4 (CCl_4 + 100mg/Kg)	23.40[ab]± 2.27	65.80[b] ± 2.69	3.80 ± 0.20	7.00 ± 0.44
Group 5 (CCl_4 + 150mg/Kg)	31.60ab± 2.01	59.00 ± 1.87	3.40 ± 0.40	6.00 ± 0.44

Values are presented in mean ± sem, n= 5. [a] means values are statistically significant when compared to the normal control. [b] means values are statistically significant when compared to the induced control

Discussion of Findings

The organ (liver) is where maximum metabolic reactions take place. It acts in energy metabolism by providing enough fuel to the brain, muscle and other peripheral organs. The integrity of the liver is compromised if it is impaired or damaged because the liver serves as the centre for metabolism.

Table 1 shows the results of the biochemical parameters investigated in this study. There is a significant difference in all the liver enzymes across the groups. These marker enzymes are cytoplasmic in origin and are discharged to the flow after cellular damage.[4]

However, Fig. 1 shows that there is a significant increase in the levels of AST, ALT, ALP and GGT in group 2 (induced group) when compared to that of group 1 which is the normal control group. This confirmed that hepatotoxicity was truly induced in all the rats.

Fig. 2 shows the result for AST across all the groups. AST is a liver enzyme seen majorly in the heart muscles, liver cells, skeletal muscles, and kidney. This enzyme is discharged into the blood stream when harm to the tissue occurs. Elevated levels are seen in myocardial infarction, cirrhosis and hepatitis.

The result from this research showed significant decrease ($P<0.05$) in the level of AST in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf reduces AST level in a dose-dependent manner.

Fig 3 reveals ALT levels across the groups. The result from this research showed significant decrease ($P<0.05$) in the level of ALT in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf reduces ALT level in a dose-dependent manner.

ALP transports metabolites across membrane, production of certain enzymes, protein synthesis, secretory activities and glycogen metabolism. However, the rise in this enzyme actions may not be unconnected with a disturbance in the movement of metabolites or alteration in some enzymes as in other hepatotoxic conditions.[5]

Fig. 4 reveals ALP levels across the groups. The result from this research showed significant decrease ($P<0.05$) in the level of ALP in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf reduces ALP level in a dose-dependent manner.

Fig 5 reveals GGT levels across the groups. The result from this research showed significant decrease ($P<0.05$) in the level of GGT in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf reduces GGT level in a dose-dependent manner.

Raised transaminase, like ALT and AST, exercises are known marker for hepatocyte injury interceded by ROS by means of oxidative pressure. GGT is a particular biomarker of hepatobiliary injury, particularly cholestasis and biliary impacts, while ALP is a marker catalyst for the plasma layer and endoplasmic reticulum. The raised levels of these biochemical boundaries are an immediate impression of adjustments in the hepatic underlying uprightness.[6]

The current perceptions agree with the prior reports of Adewale[7] where lessening of bilirubin level was seen in hepatotoxic rodents following organization of Paullina Pinnata remove.

Table 2 revealed the mean value of oxidation stress markers MDA, GPX, CAT and SOD.

Fig 6 reveals MDA levels across the groups. The result from this research showed significant decrease ($P<0.05$) in the level of MDA in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). It was also seen that the MDA level in group 4 and 5 which were administered 100mg/kg and 150mg/kg respectively were lower than the negative control group (group 1).

Fig 7 reveals GPX levels across the groups. The result from this research showed significant increase ($P<0.05$) in the level of GPX in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg

respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf increases GPX level in a dose-dependent manner.

Fig 8 reveals CAT levels across the groups. The result from this research showed significant increase ($P<0.05$) in the level of CAT in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf increases CAT level in a dose-dependent manner.

Fig 9 reveals SOD levels across the groups. The result from this research showed significant increase ($P<0.05$) in the level of SOD in group 3, 4 and 5 which were administered 50mg/kg, 100mg/kg and 150mg/kg respectively when compared to that of the induced group (group 2). This means that the methanolic extract of *Paullinia Pinnata* leaf increases SOD level in a dose-dependent manner.

Free radicals scavenging enzymes are first line cell protection against oxidative pressure, dispersing O2 and H2O2 before their cooperation to shape hazardous hydroxyl (OH·) revolutionary which can prompt cell death.[8] Hence, the decrease in the movement of these catalysts might bring about various injurious impacts because of the aggregation of superoxide revolutionaries and hydrogen peroxide. The noticed abatement in cancer prevention agent compounds (CAT and SOD) following exposure to CCl4 might be because of enlistment of the atomic component kappa B (NF-κB) initiation and its movement, which are answerable for tweak of liver injury.[9] It might likewise be because of raised nitric oxide (NO) through initiation of iNOS. This finding is steady with the report of Wu[10] where a comparative decrease in cancer prevention agent catalysts was noticed after the organization of CCl4. Notwithstanding, the inversion in CCl4 instigated oxidative pressure as clear in the height of SOD and CAT following organization of Paullina Pinnata is implicative of its antioxidative capacity, which can be credited to the capacity of the concentrate to search ROS in the liver. Lipid peroxidation items are framed when receptive oxygen species assault polyunsaturated unsaturated fats, prompting loss of film trustworthiness opposite primary capacities.[11] In this way, the huge expansion in MDA saw in the hepatoxic rodent might portray erratic oxidative surges of CCl4 on film bound lipids which may thusly, upset the layer uprightness as far as ease and design. The rebuilding of the film respectability through constriction of CCl4-induced peroxidative materials by Paullina Pinnata is demonstrative of the huge degree of insurance on the layer lipids. This might be ascribed to capacity PP to advance detoxification of responsive metabolites, which may have incited and empowered peroxidation of polyunsaturated lipids of the hepatoxic layer.[12]

Methanolic concentrate of *Paulina Pinnata* actuated changes altogether and differential WBC includes in induced hepatotoxicity in rats as found in the tables above. 21 days after administration of the concentrates at the portion levels of 50 mg/kgbw, there was significant increase in degrees of absolute WBC, neutrophils, lymphocytes and monocytes counts. the aftereffect of white platelet count across the treatment gatherings and the benchmark groups. The outcomes uncovered huge expansion in Group 2 which was incited with ccl4 (2ml/Kg) when contrasted with the typical benchmark group. Gathering 3 which was directed with 50mg/kg of the concentrate showed critical decrease in white platelet when contrasted with the prompted control (Group 2). Anyway the concentrate at this level couldn't enhance the impact back to the level of the ordinary control. Gathering 4 and 5 which were managed with 100mg/kg and 150mg/kg separately showed a decrease which were not genuinely huge.

The result of the white blood cell differential as seen in table 4 Neutrophils, Lymphocytes, Eosinophils, Monocytes were assessed. The results showed significant increase in neutrophils of the induced group when compared to the normal control group. There was no significant difference in group 3 and 5 which were induced and treated with 50 mg/kg and 150 mg/kg of the extract respectively when compared to the induced group but significantly higher when compared to the normal control group. However group 4 which received 100 mg per kg of the extract showed significant reduction in neutrophils when compared to the induced control and also to the normal control group. Lymphocyte in the induced control group showed significant reduction when compared to the normal control group. Group 4 and 5 showed significant increase in lymphocyte when compared to the induced group. Eosinophils significantly ($p<0.05$) increased in the induced group when compared to the normal control group. The treated groups showed a reduction in Eosinophils across the groups. Monocytes on the other hand increased in level significantly in Group 2 when compared to the normal control, however there was a significant reduction of monocytes across the treatment groups.

4. CONCLUSION

The present data showed that the methanol extract of *Paullinia Pinnata* leaf elicits effective protection against CCl4 induced hepatic damage by attenuation of oxidative stress and liver damage which thus lend credence to the potential of methanolic extract of *Paullinia Pinnata leaf* in amelioration of hepatic damage caused by CCl4 intoxication. The concentrate likewise came about to a huge improvement in resistant reaction. The critical expansion in complete white blood and differential white blood includes in actuated rats after oral organization of the concentrate shows that the plants might advance and further develop the insusceptible stimulatory exercises hence can be pursued for their clinical relevance in management of immunity dependent disorders.

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