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Methotrexate-Induced Tissue Toxicity and Cancer Herbal Management: Anti Oxidative and Protective Roles of Extract of *Lannae Egregia* Leaf

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Abstract: Tissue toxicity have been linked to oxidative damage elicited by orthodox anti-cancer agents. Bioactive components from plants are used locally for treatment of cancer. This study evaluated the roles of ethyl acetate extract of *Lannae egregia* (EELE) leaf, a local anticancer plant of West Africa following methotrexate administration in rats. Thirty-two male Wistar rats averagely weighing 150g used for this study were acclimatized and randomly selected into four groups, A-D and treated: Group A (Control), Group B (Methotrexate only,2.5mg/kg.bw at 3 days interval for 21 days), group C (pre-treated extract at 100mg/kg.bw. daily for 14 days, challenged with 2.5mg/kg.bw of Methotrexate for 21 days at 3 days interval), group D (extract only at100mg/kg.bw daily), all carried out orally in 0.1 ml solution for 35days.Antioxidants, lipids profile, liver and kidney indices were determined using standard methods. Results showed that methotrexate significantly ($p<0.05$) decreases liver and kidney Superoxide dismutase, Catalase and Glutathione peroxidase activities, reduced glutathione and total protein levels with corresponding significant ($p<0.05$) increases in malondialdehyde concentrations and percentage fragmented DNA. In contrast, combined treatment with extract (group C) and extract alone (group D) showed improved metabolic alterations as these were reversed comparably with control. Results of lipid profile, liver and kidney indices showed elevated levels of plasma total triglyceride and cholesterol accompanied with attenuated level of high density lipoprotein cholesterol (HDL-c)while activities of Gamma-glutamyltransferase (γ-GT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) , as well as levels of bilirubin , plasma creatinine and urea were significantly ($p<.05$) increased in group B,which were reversed by group C. Results are suggestive of toxic effect of methotrexate via induction of oxidative stress while extract exhibited antioxidative and tissue protective potentials suggestive of its rich-bioactive contents and thus validating its medicinal values and as potential adjunct in cancer management.

Keywords: *Methotrexate; Redox Imbalance; Antioxidative; Lannae Egregia; Cancer Management; Tissue Protective.*

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I. INTRODUCTION

Altered cellular function to biological molecules such as deoxyribonucleic acid (DNA), proteins, lipids, nucleic acids, enzymes and other macromolecules have been implicated in a spontaneous and accumulative oxidative damage to cellular components via an unregulated influxes and effluxes of free radicals also known as reactive oxygen species [1,2]. More so, several indications support the hypothesis that oxidative damage to tissues and cellular molecules in humans are major events in diseases and infections [3].

Although, ROS plays significant roles in normal cell signalling and homeostasis [4], beyond the normal physiological thresholds, oxidative stress ensue. Accumulating evidences have linked drug-induced oxidative stress as a mechanism of toxicity to many organs such as liver and kidney [2]. The scientifically characterized drugs associated with myriads of toxicity are connected to certain therapies such as cancer, non-steroidal anti-inflammatory conditions, antiretroviral agents, antipsychotics as well as analgesics agent [5]. It has been reported that the biotransformation of some drugs and their metabolites during metabolism generate reactive intermediate species that can reduce molecular oxygen directly to generate ROS [1]. Furthermore, for other drugs, evidence of elevation in cellular ROS in response to drug exposure generates ROS and implicates oxidative stress in toxicity even if the mechanisms by which ROS are produced are enigmatic [6].

Methotrexate, a known folic acid antagonist and substrate analogue of dihydrofolate reductase have been widely used as a cytotoxic chemotherapeutic agent in the treatment of various stages of malignancies and inflammatory diseases [7,8]. The efficacy of this agent is often limited because of its association with liver cirrhosis, fibrosis of the liver, hypertrophy of the hepatocytes, hepatitis, necrosis and death [9,10]. Majorly, the toxicity of methotrexate in accelerating the rate of cellular damage is linked to its ability to increase the amount of hydrogen peroxide levels [11], as well as other free radicals released by stimulated polymorphonuclear neutrophils (PMNs), in-addition to reducing the levels of 5-methyltetrahydrofolate availability which occur via reduction in the levels of homocysteine, S-adenosylmethionine (SAM) necessary for methylation reaction in DNA synthesis [12]. Furthermore, methotrexate has been shown to cause a reduction in methionine synthesis, as well as several antioxidant enzymes [13,14].

The alterations in antioxidant enzymes status and deficiency of SAM may probably be the reason for increased ROS and redox imbalance. However, intracellular antioxidant defence complex (such as reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase) [4], and antioxidants from extracellular sources could react directly with the oxidizing radicals to augment the reactions of intracellular antioxidant essential to produce a non-radical product [15]. It is believed that natural products from plant contain several variety of bioactive components with antioxidant capabilities [16], that may defend against noxious and detrimental effects of free radicals and have shown broad range of pharmacological consequences against mutagenesis, allergy, diabetes and cancer [17,18].

Lannea egregia Engl belong to the family of plants known as *Anacardiaceae [19]*. Species of this class are geographically distributed in both tropical and sub-tropical African. It is identified as false marula and known locally as Ekudan in Nigeria by the Yorubas of the south west [20]. The stem bark of *Lannae egregia* has been used extensively and externally for the treatment of ulcers, sores and leprosy [21], as well as a decoction drunk against gastric pains, diarrhoea, oedema, paralysis, epilepsy and madness. Nigerian and the Central African Republic (CAR) use the stem bark decoction as stomachic to boost haemoglobin level and added as part of vermifuge medicine [22]. The drenched root is used in poultice for the treatment of wounds [23,19]. The leaves of *Lannea egregia* are also used in folkloric medicine as an anticancer herb [20]. However with limited scientific data on the medical claims of *Lannae egregia* leaves and its usage traditionally for the treatment of cancer and several ailments, it is imperative to evaluate these claims, hence this study investigated the roles of ethyl acetate extract of *Lannae egregia* leaves following methotrexate treatment, an anti-cancer agent in male Wistar rats.

II. MATERIALS AND METHODS

- *Materials*

Materials used in this study includes; electronic weighing balance, measuring cylinders, syringes and needles, beakers, test tubes, conical flasks, plasma bottles, water bath, pH meter, spatula, centrifuge, thermometer, disposable gloves, tissue papers, micropipette, washing brushes, detergents, separating funnels, refrigerator, spectrophotometer, dissecting sets, mortar and pestle, stopwatch and test tube racks.

- *Reagents*

All the reagents used were of good and high analytical grade mostly obtained from Sigma USA. These include: Trisbuffer, Phosphate buffer, Carbonate buffer, Sodium azide, Hydrogen peroxide, Potassium chloride, sodium hydroxide, adrenaline, Trichloroacetic acid (TCA), Tris-hydrochloric acid, Ellman's reagent (5,5-thiobis-2-nitrobenzoic acid, DTNB), Tris-EDTA glutathione, thiobarbituric acid (TBA), Tris(hydroxylmethyl)aminomethane, Diphenylamine, Triton X-100 distilled water, chloroform, ethyl-acetate, washing buffer and homogenizing buffer. Methotrexate a product of EBEWE Pharma Gen. m. b. H. Nfg.KG, A-4866 Unterach, Austria was purchased from an authorized pharmaceutical store in Agege, Lagos State Nigeria.

- *Plant Material and Preparation of Extract*

Lanna eegregia leaves was collected at Igbeti area of Oyo State and identified at the Botany Unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso with herbarium voucher number LHO 520 deposited. The plant was air dried in the laboratory and powdered after dryness with 1500g of the powdered

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leaves soaked in 5000ml ethyl acetate for 72 hours and was filtered using filtered litmus paper after this period. The filtrate of the extract was concentrated to dryness between 30-35^{0}C to obtain dried ethyl acetate extract residue inside a 1000ml measuring beaker [19].

➢ *Experimental Animals and Groupings*

Male Wistar rats used for this study were obtained from the animal house of Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The animals were handled and treated based on our institutions guidelines on ethics and conducts for handling experimental animals which conform with the international standards. They were acclimatized in the laboratory for two weeks before any experimental work was undertaken. Animal were fed with dietary pellets and water ad-libitum and their weights were monitored during this period. The animals were randomly selected into four groups; A-D, with eight animals in each group and treated as shown below.

- Group A: Control (treated with normal saline)
- Group B: Methotrexate only (2.5 mg/kg body weight) made into 0.1 mL of normal saline and administered orally by intubation for 21 days at 7 days interval.
- Group C: Pre-treated daily with extract (100 mg/kg body weight) made into 0.1 mL of corn oil for 14 days and exposed to methotrexate (2.5 mg/kg body weight) orally by intubation for 21 days at 7 days interval.
- Group D: Administered only with 100 mg/kg body weight of the extract daily as positive control.

➢ *Preparation of Plasma*

The animals were sacrificed using mild anaesthetic chloroform. Blood was obtained through cardiac puncture from the jugular vein using a 5 ml syringe and needle. The obtained blood was transferred into an EDTA sample bottle and centrifuged (model 3538) at 4000 rpm for 10 minutes. The plasma (supernatant) was extracted into plasma bottle, covered and stored at 4ºC inside refrigerator [24].

➢ *Preparation of Liver and Kidney Homogenates*

The experimental animals were euthanized at the end of administration using mild anaesthetic chloroform. The animals were carefully open using dissecting set and tissue homogenates (liver and kidney) were prepared as they were excised and placed in a pre-weighed beaker containing 5ml of washing buffer. They were thoroughly washed in cold washing buffer to remove haemoglobin which may inhibit the activity of the enzymes. The washed tissues were weighed while 1g of each of the tissues were transferred to a beaker containing 4 ml of homogenising buffer and homogenised to prepare the homogenates.

➢ *Biochemical Indices Studied*

The following indices were assayed using liver and kidney homogenates ;Malondialdehyde (MDA) concentrations, estimated spectrophotometrically by thiobarbituric acid- reacting substances (TBARS) as described by [25],reduced glutathione (GSH) concentration was evaluated using the method described by [26], glutathione peroxidase activity determined by the method of [27],while superoxide dismutase (SOD) and catalase (CAT) activities were determined by the methods of [28],respectively. Plasma and tissue total protein were determined by Biuret method as described by [29]. The percentage fragmented DNA was determined spectrophotometrically according to the method of [30].

Lipid profile indices were determined as total plasma cholesterol was measured spectrophotometrically by the enzyme hydrolysis of cholesteryl esters [31], triglycerides concentration was determined by enzymatic colorimetric method as described by [32]. The quantitative determination of high-density lipoprotein (HDL-C) cholesterol was based on HDL-cholesterol (HDL-C) precipitating method of [31]. Furthermore, gamma glutamyltransferase(γGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST)and alkaline Phosphatase (ALP) were determined by the methods of [33]; [34]);[35] and [36], respectively. In addition, plasma urea, bilirubin and creatinine concentrations were determined according to the methods described by [31], [37] and [38].

➢ *Statistical Analysis*

The results were reported as means ± SD from eight repeated determinations and evaluated with data obtained and analysed using analysis of Variance (ANOVA). Value of $p<0.05$ was considered statistically significant.

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III. RESULTS

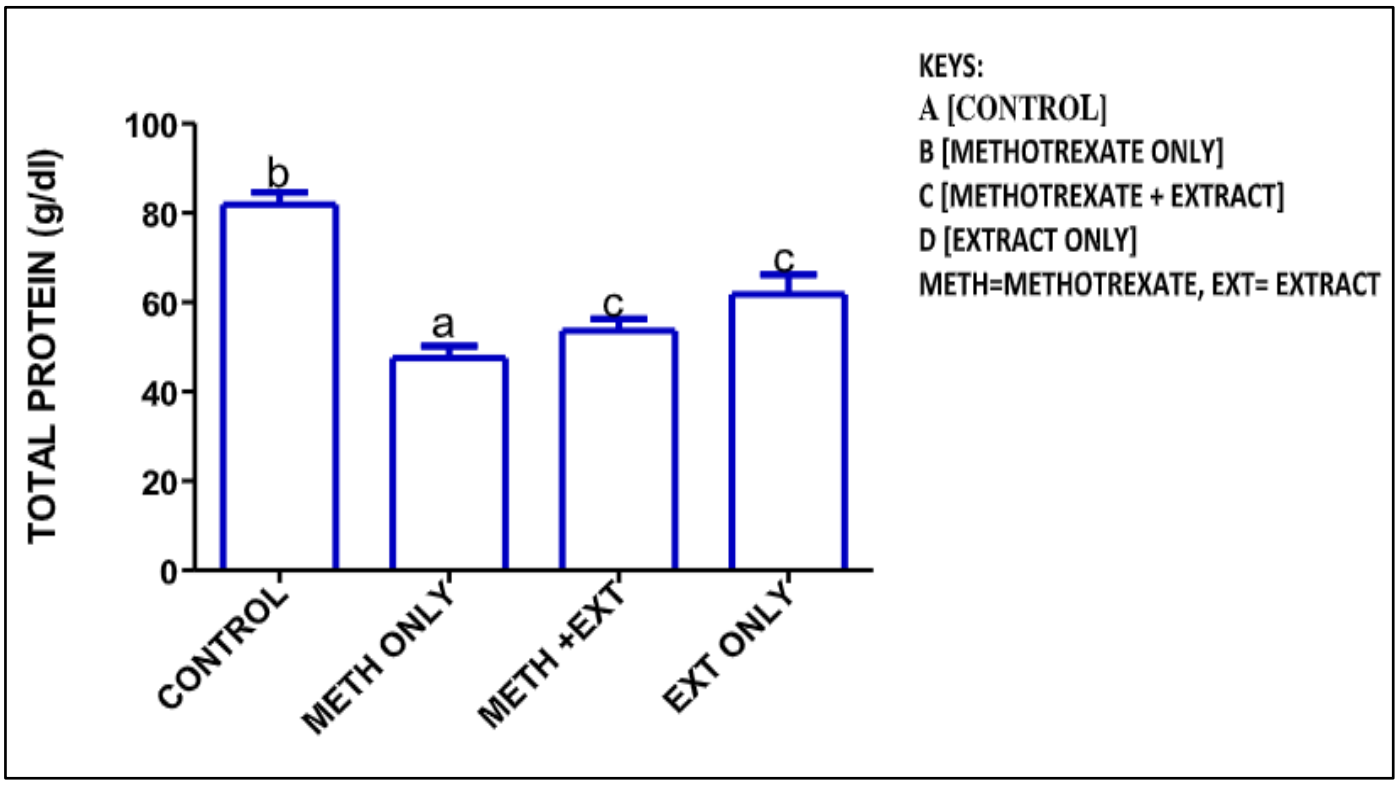

Fig 1 Plasma Total Protein Concentrations in Different Treatment Groups Each Value Represents Mean ± SD for 8 Animals in Each Group. Bars With Different Superscripts are Significantly Different from Each Other at $p<0.05$

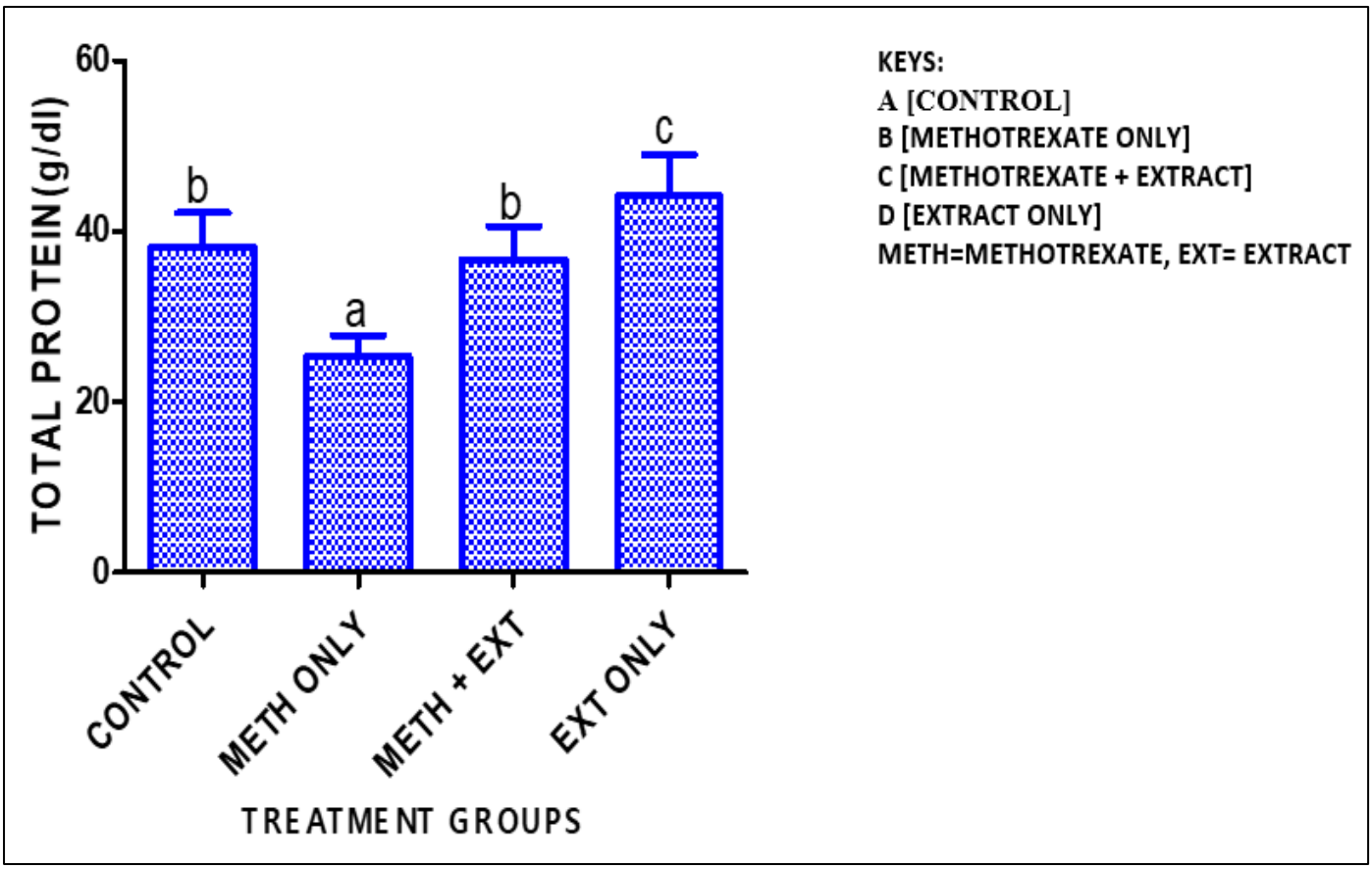

Fig 2 Total Protein Concentration in the Liver of Various Treatment Group. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

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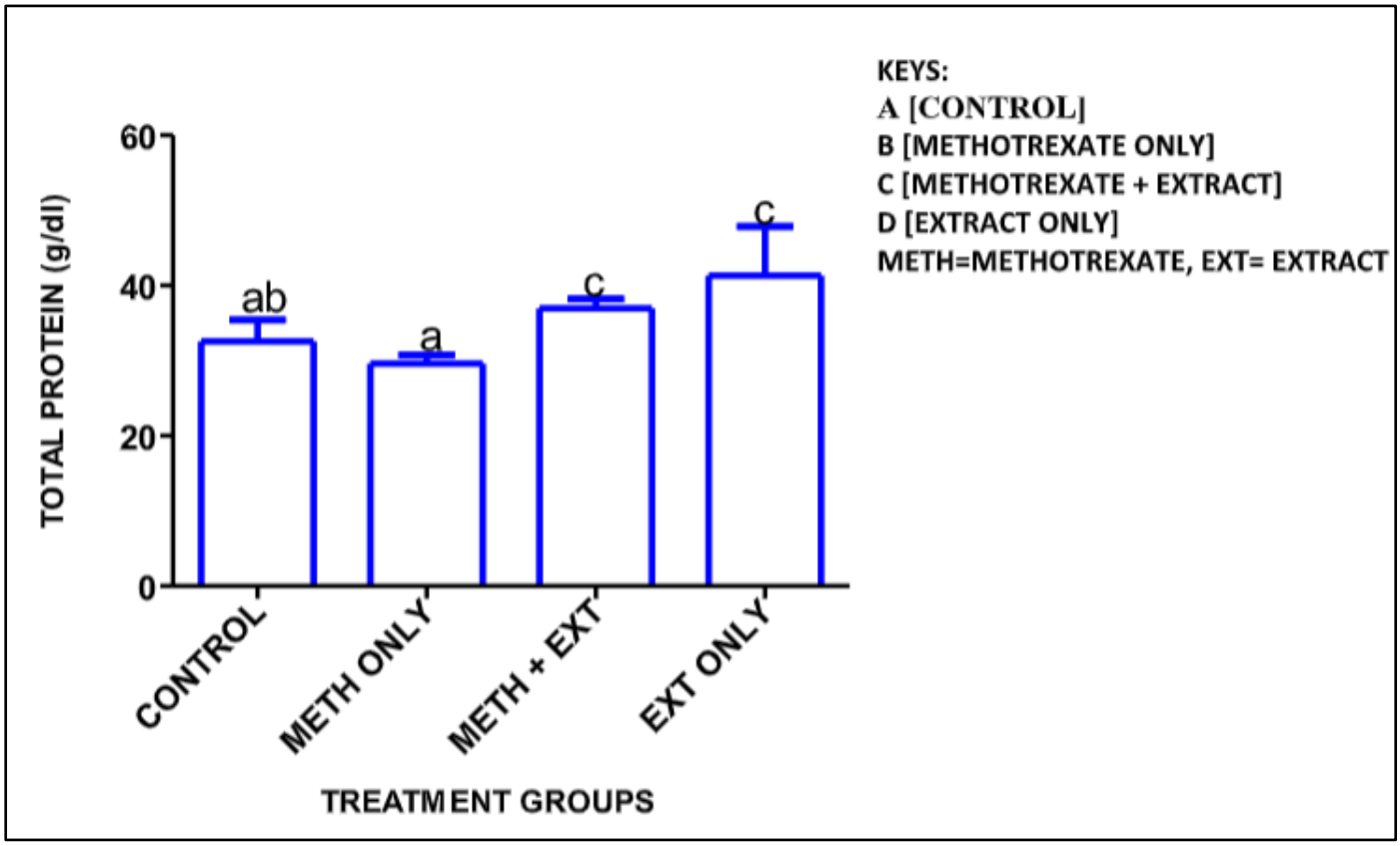

Fig 3 Total Protein Concentration in the Kidney of Various Treatment Groups Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

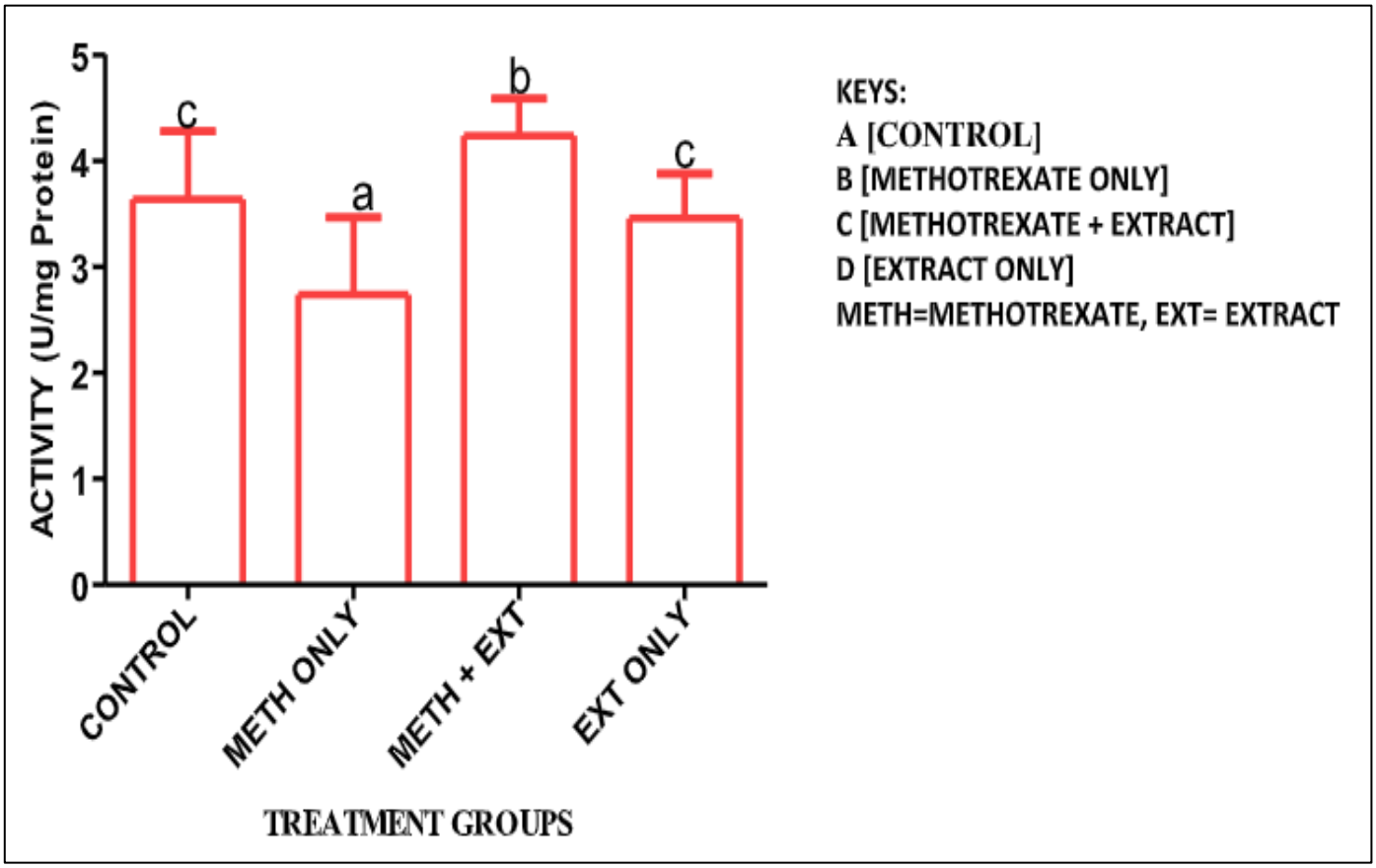

Fig 4 Superoxide Dismutase Activity in the Liver Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

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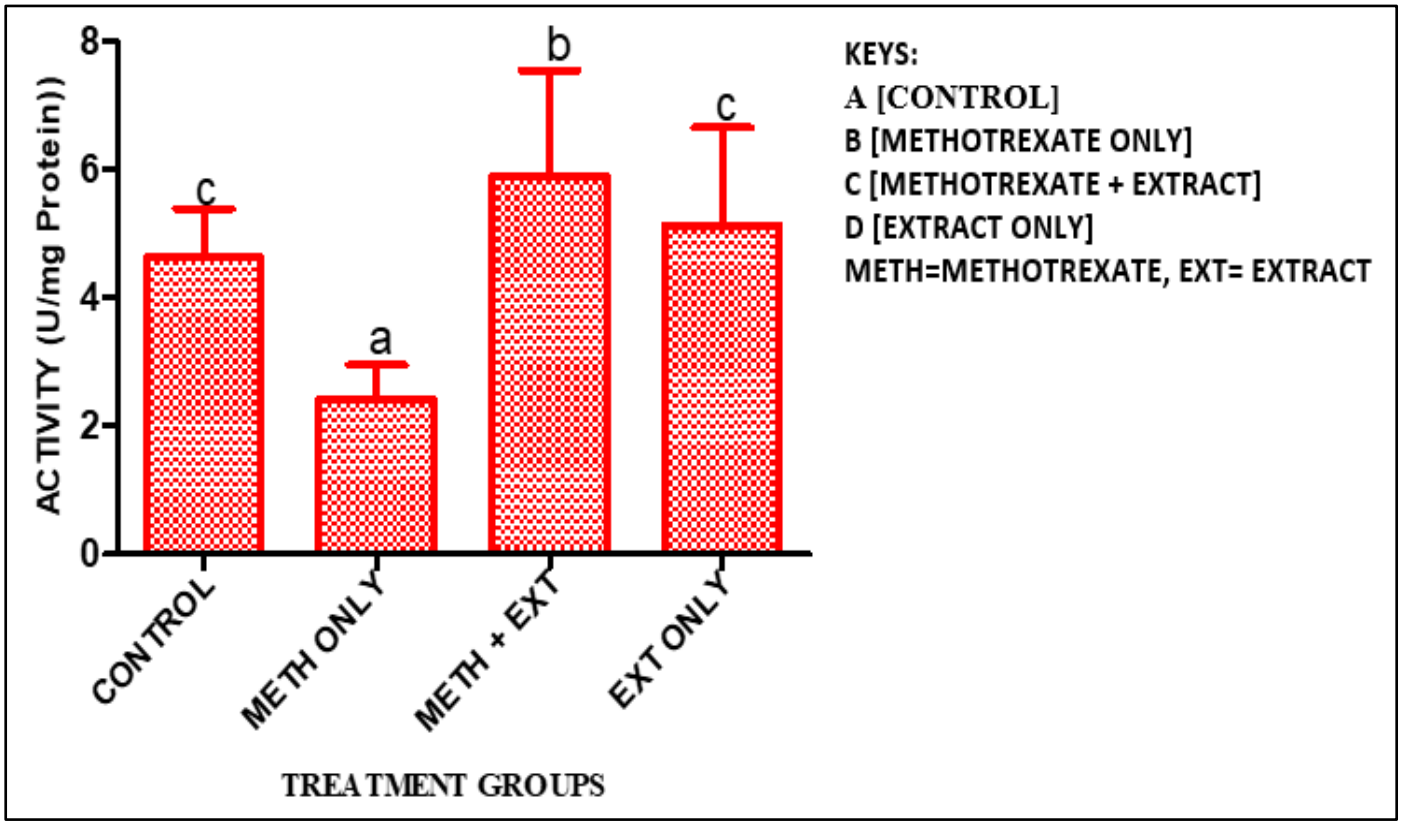

Fig 5 Superoxide Dismutase Activity in the Kidney Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

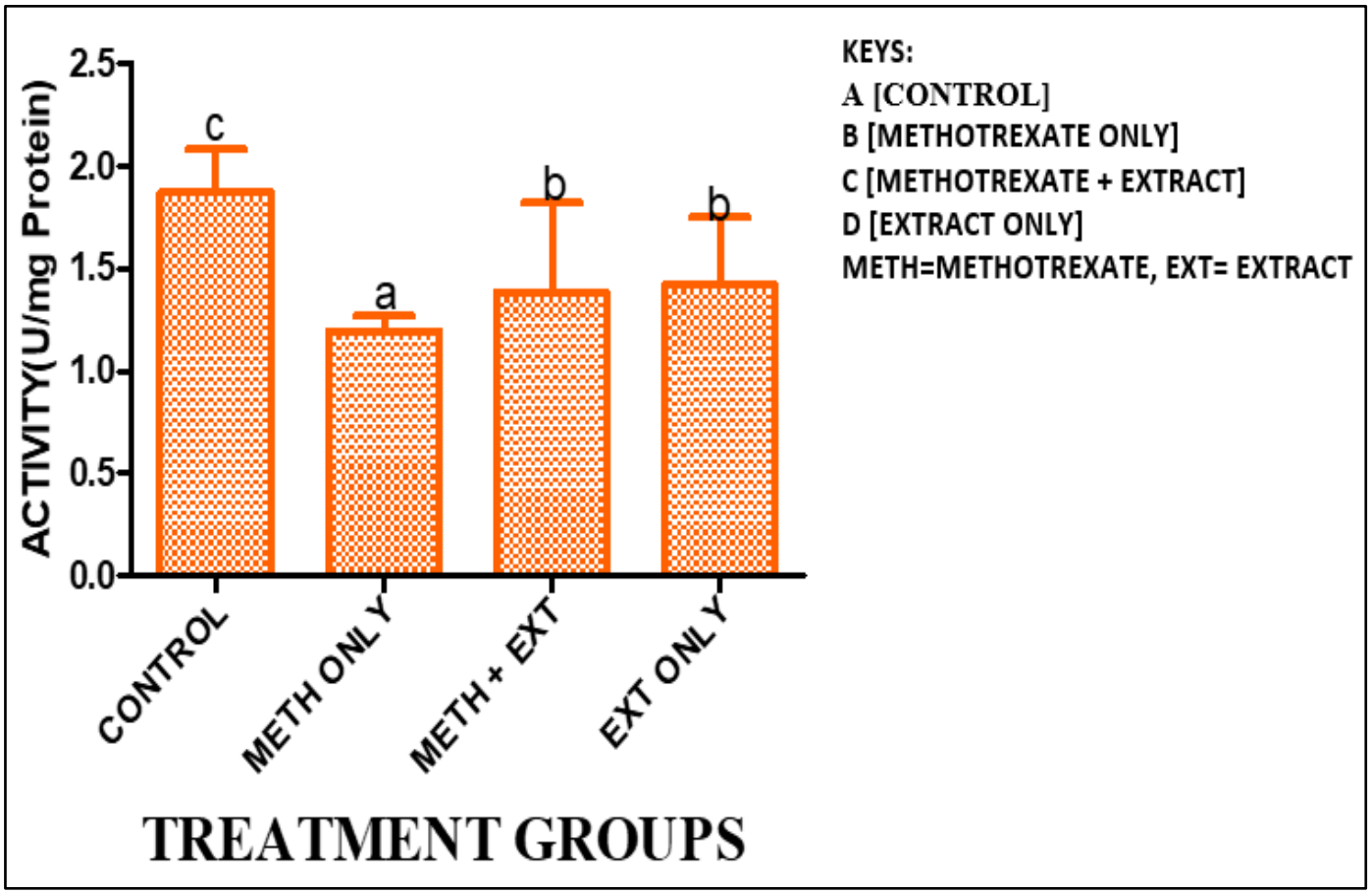

Fig 6 Catalase Activity in the Liver Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

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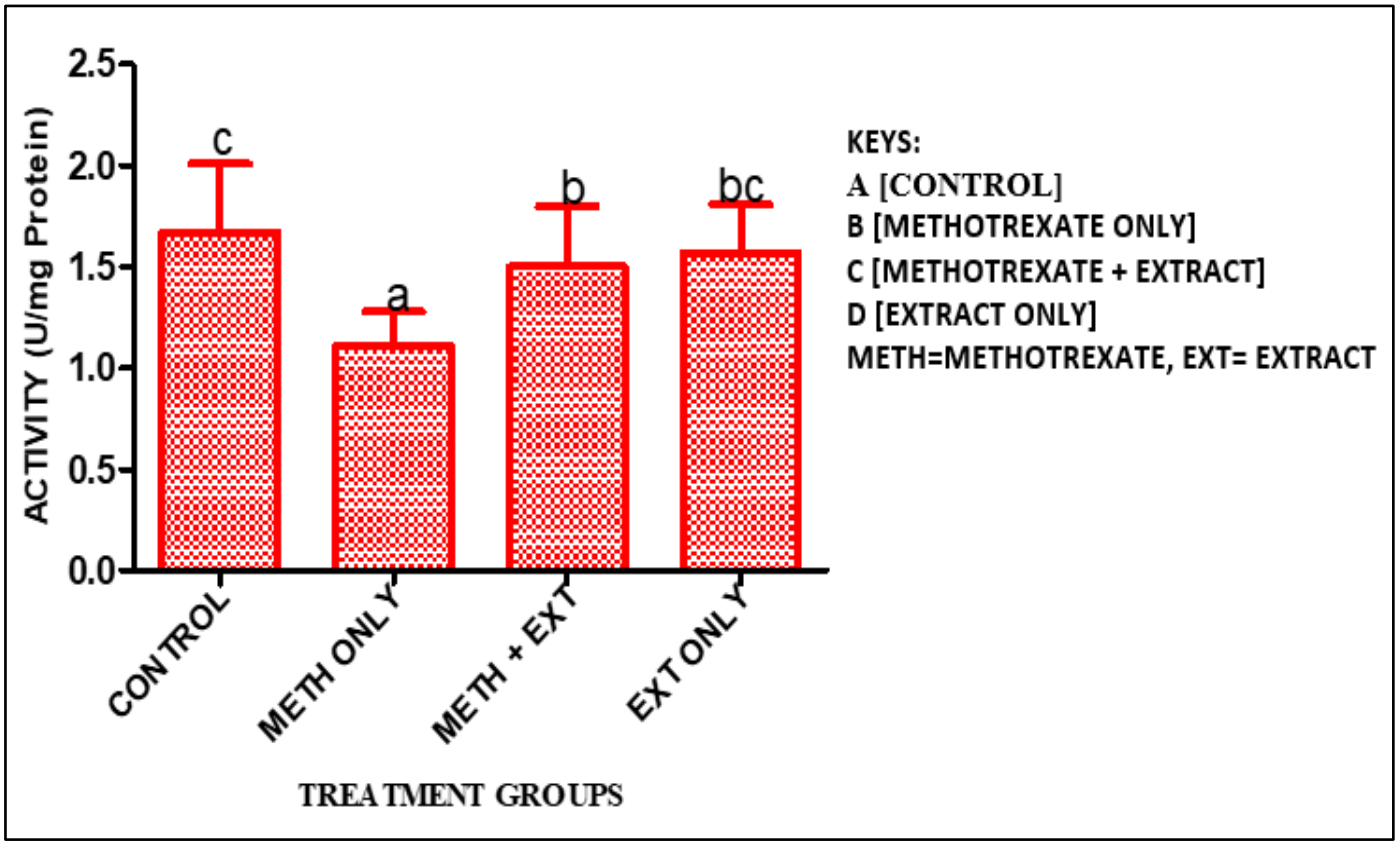

Fig 7 Catalase Activity in the Kidney Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

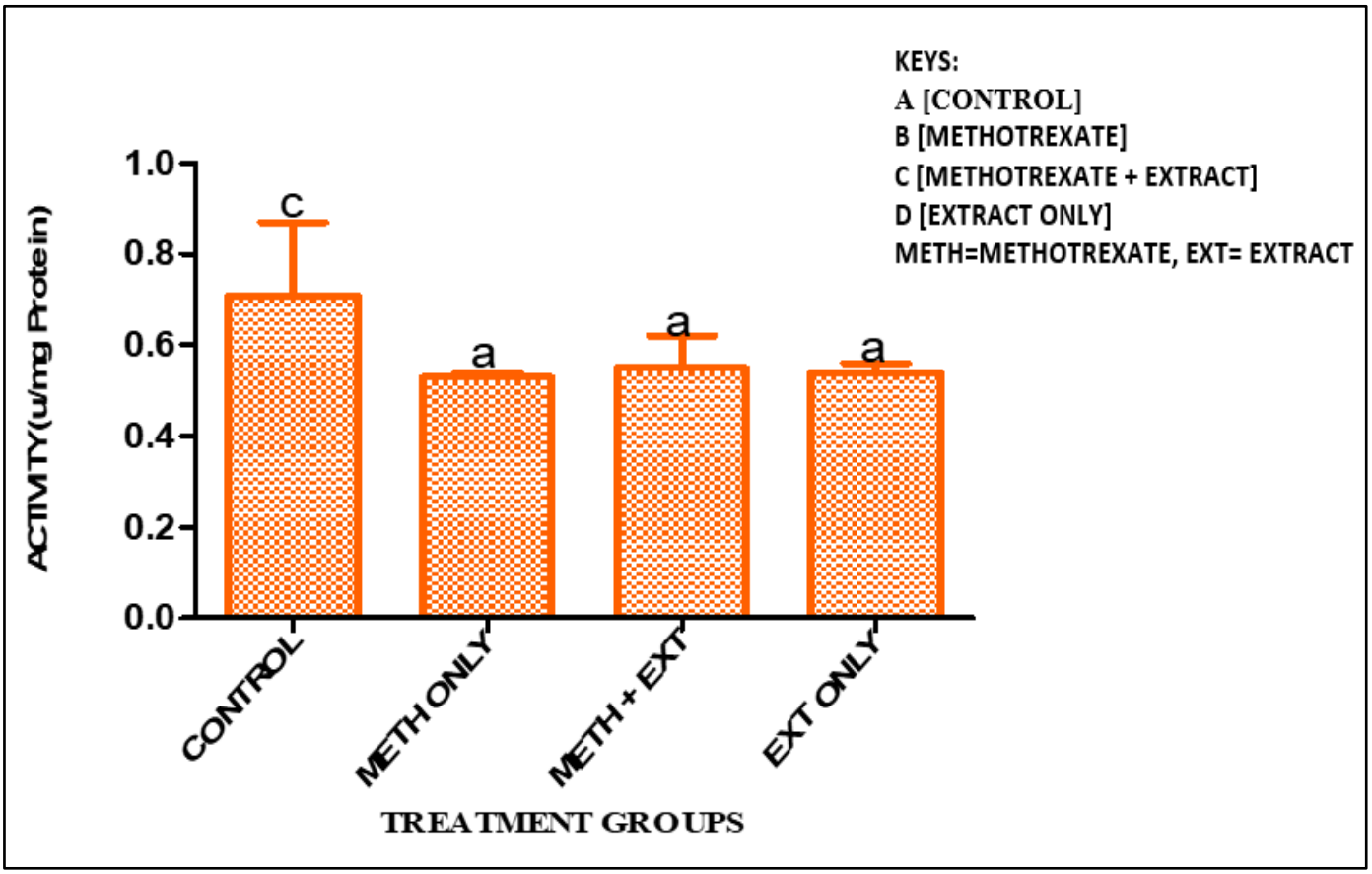

Fig 8 Glutathione Peroxidase Activity in the Liver Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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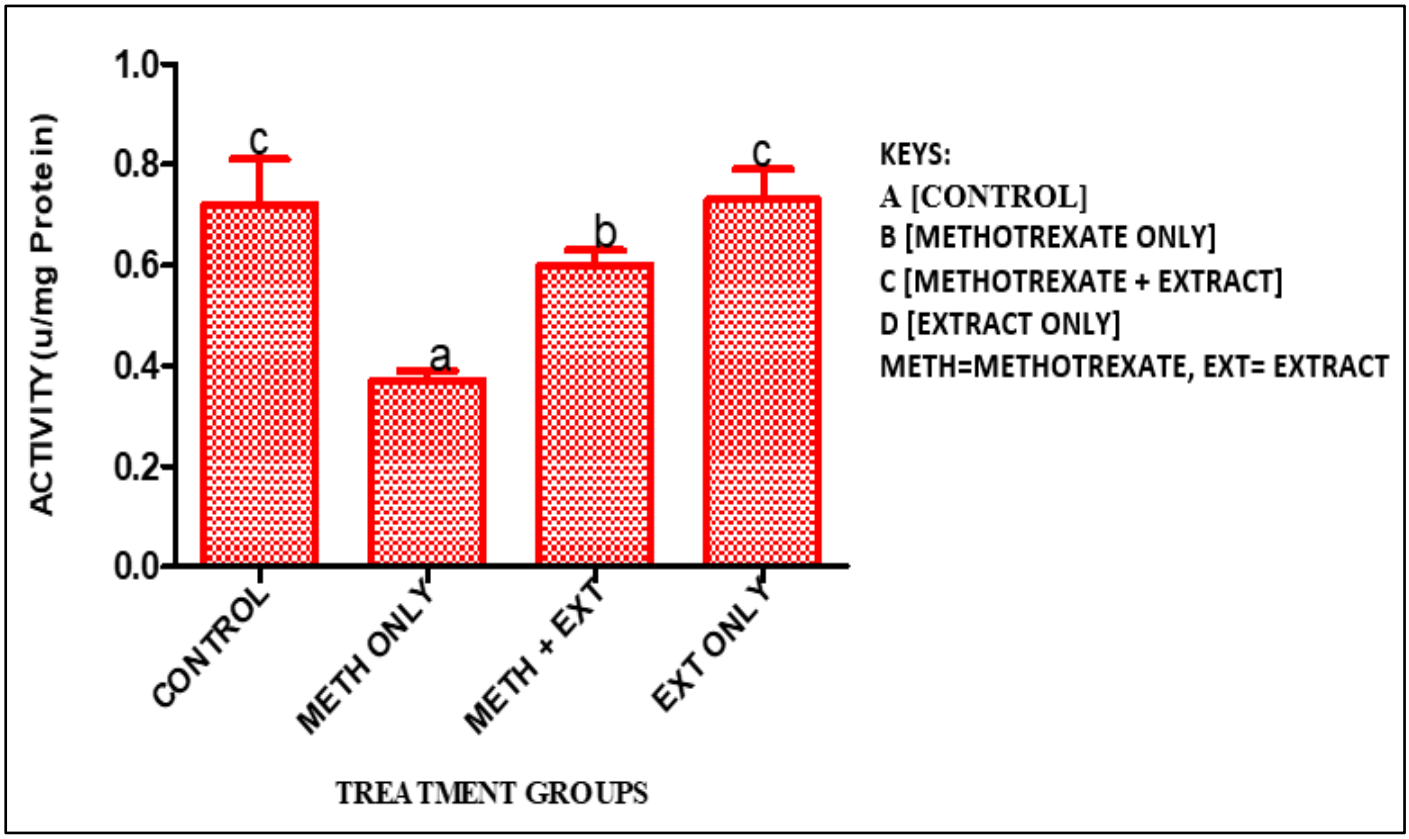

Fig 9 Glutathione Peroxidase Activity in the Kidney Homogenate of Various Treatment Groups. Each value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

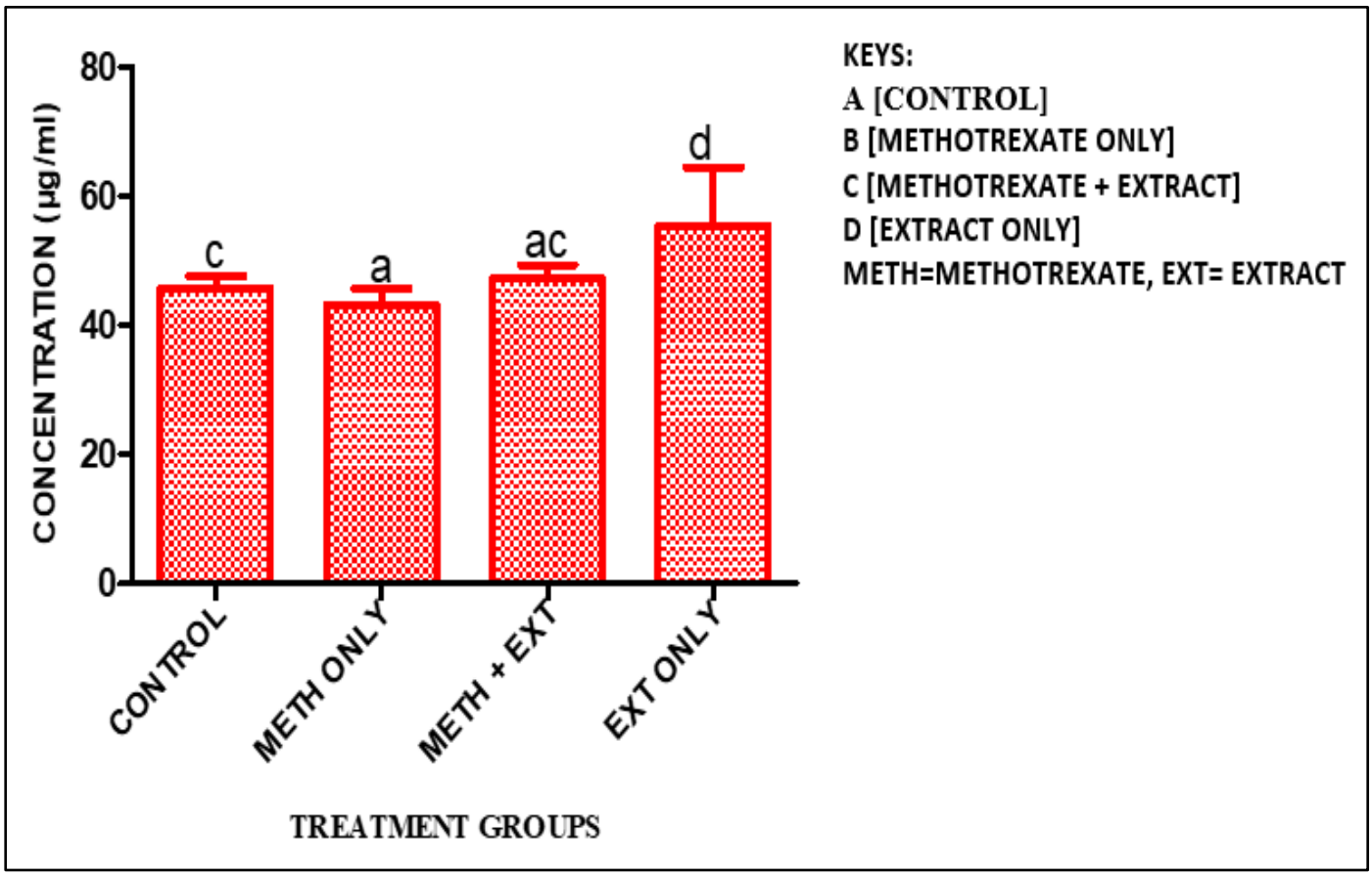

Fig 10 Reduced Glutathione Concentrations in Liver Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

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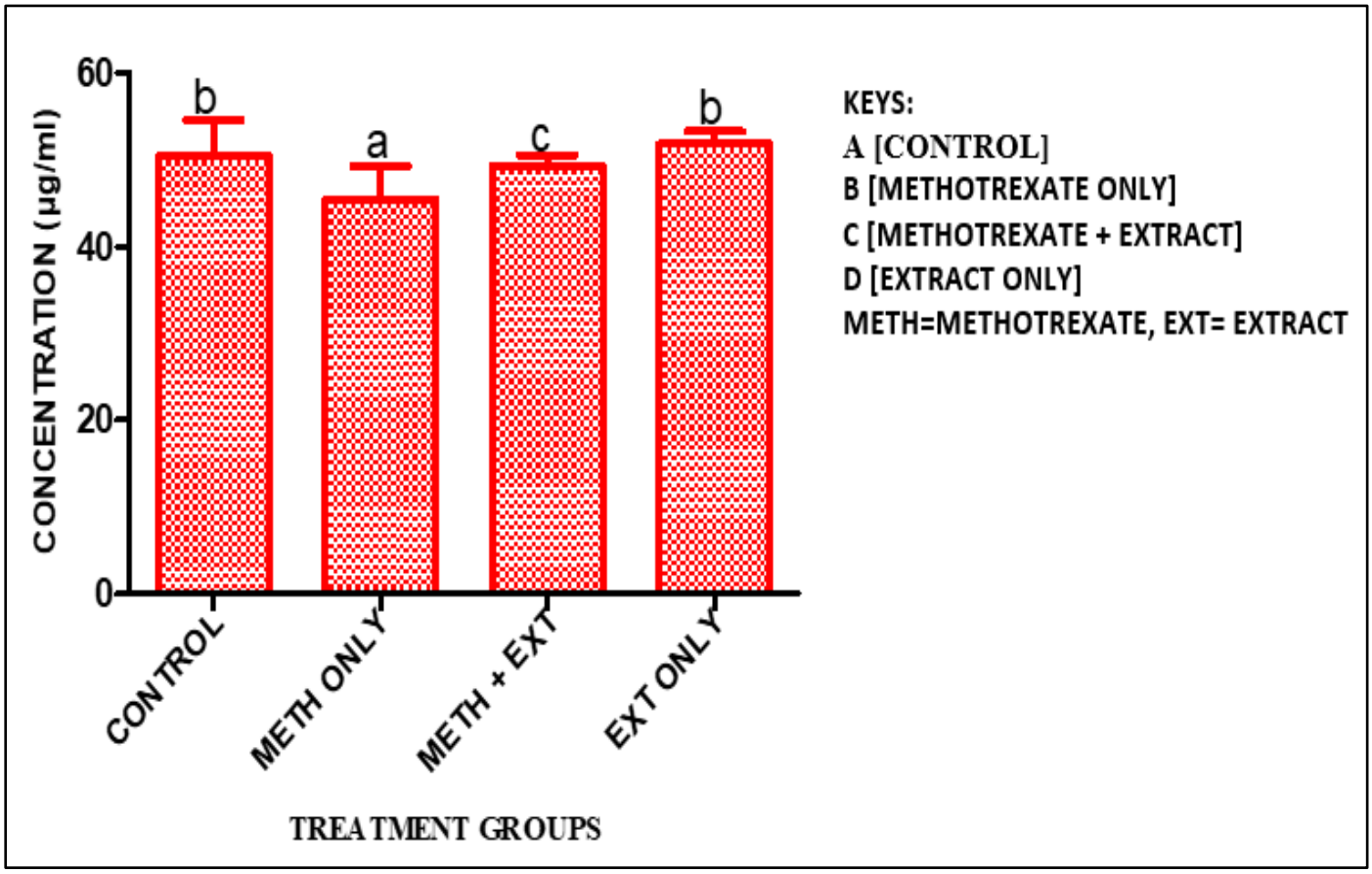

Fig 11 Reduced Glutathione Concentrations in Kidney Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

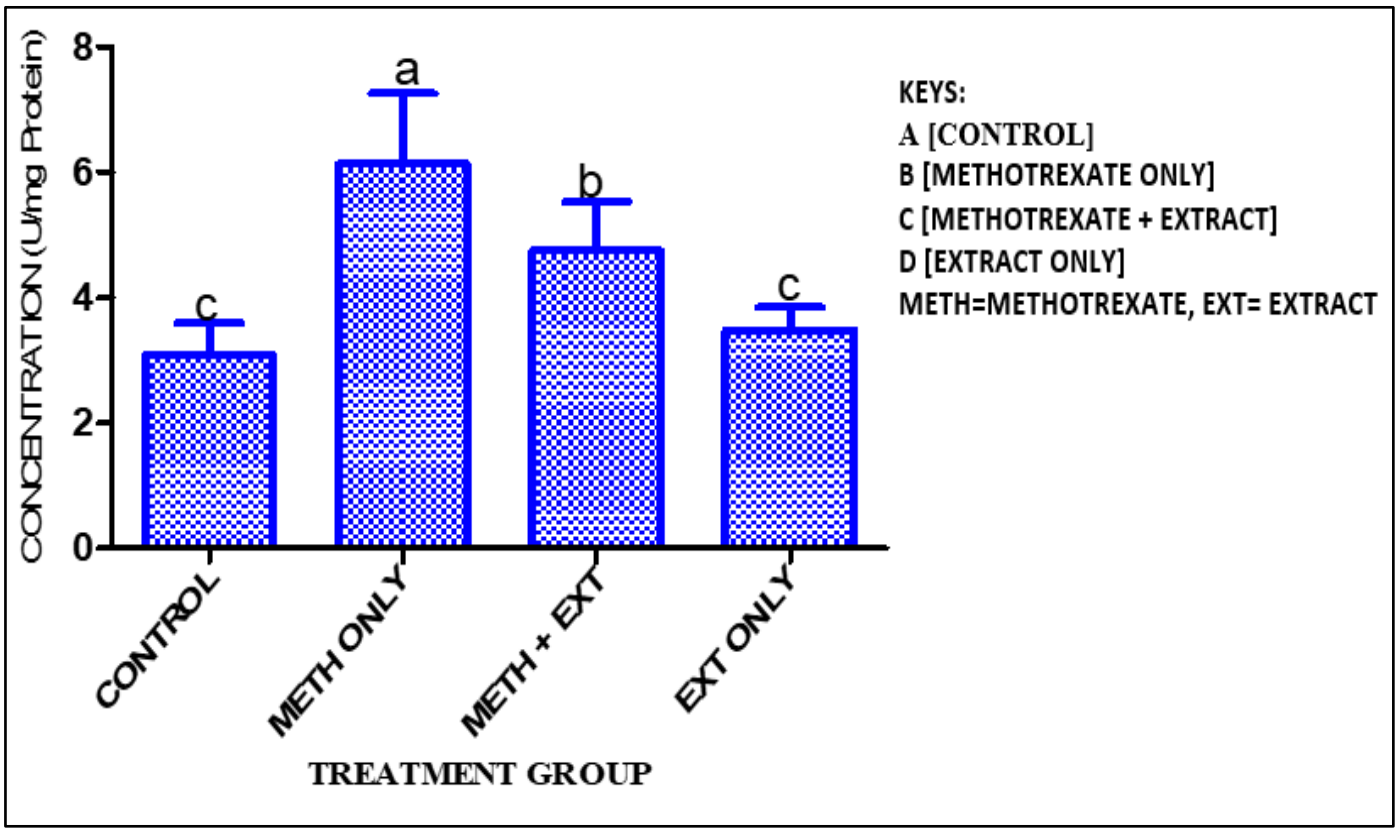

Fig 12 Malondialdehyde Concentrations in Liver Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

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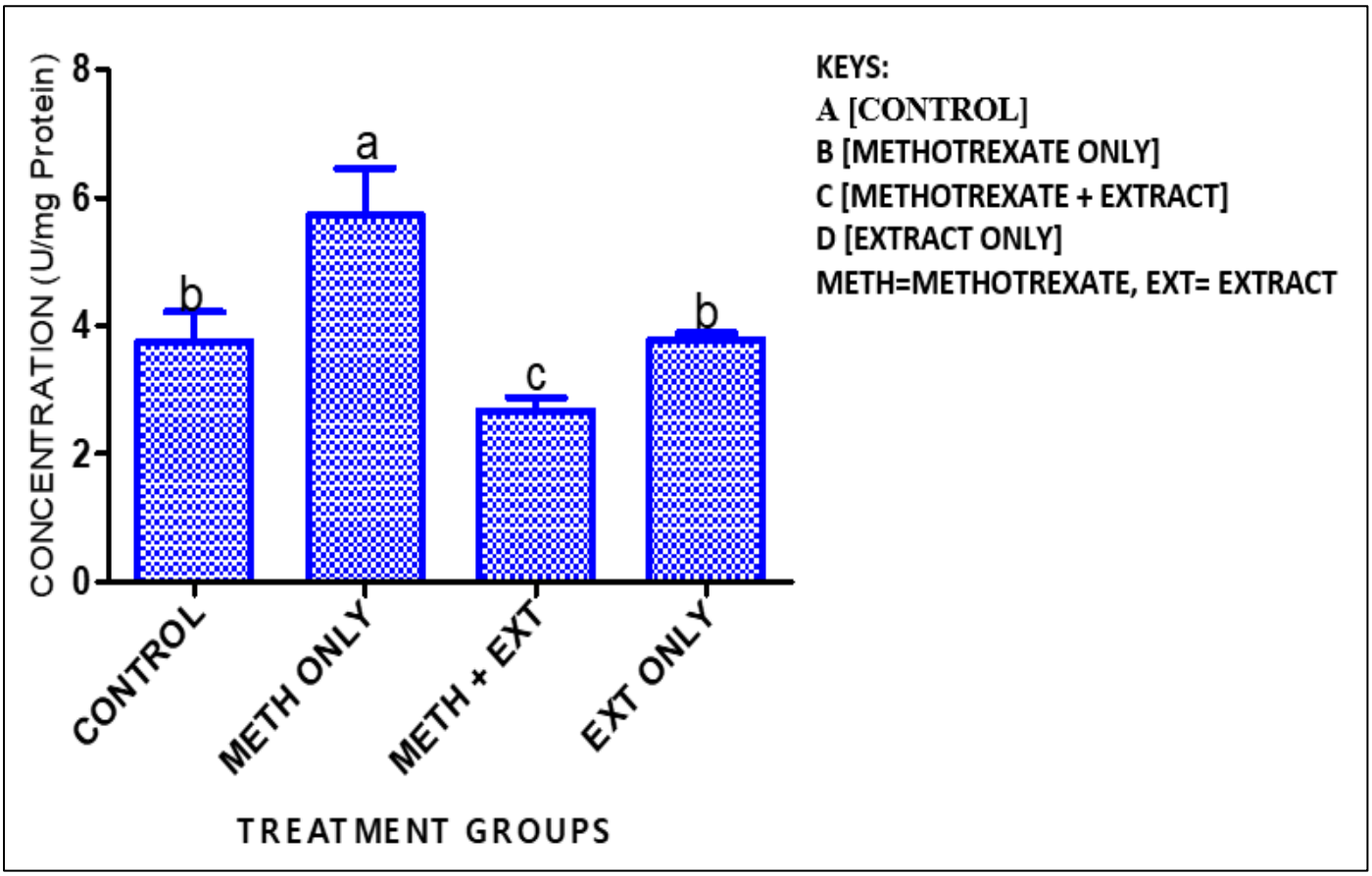

Fig 13 Malondialdehyde Concentrations in Kidney Homogenate of Various Treatment Groups. Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

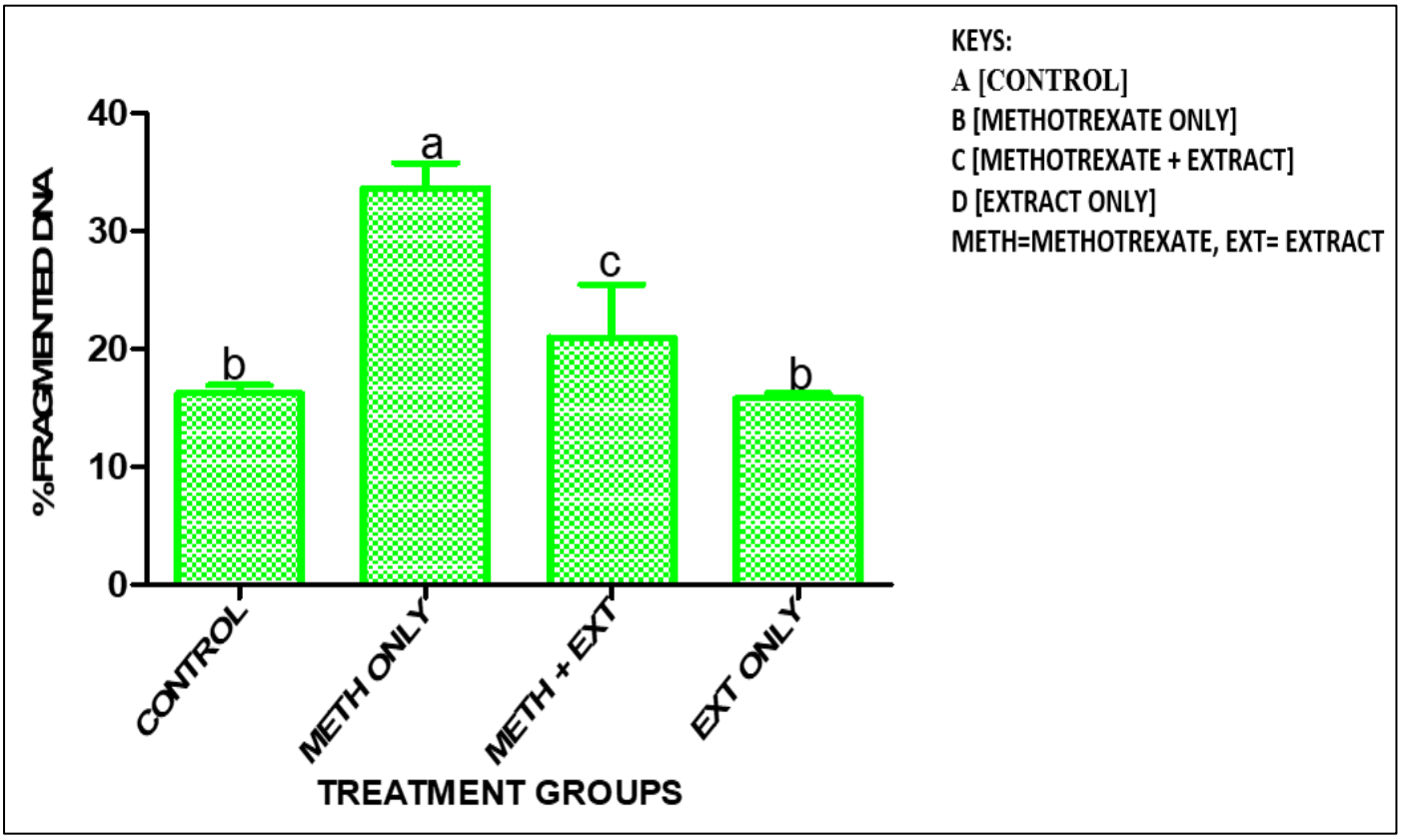

Fig 14 Percentage Fragmented DNA in Liver of Rats in Various Treatment Groups Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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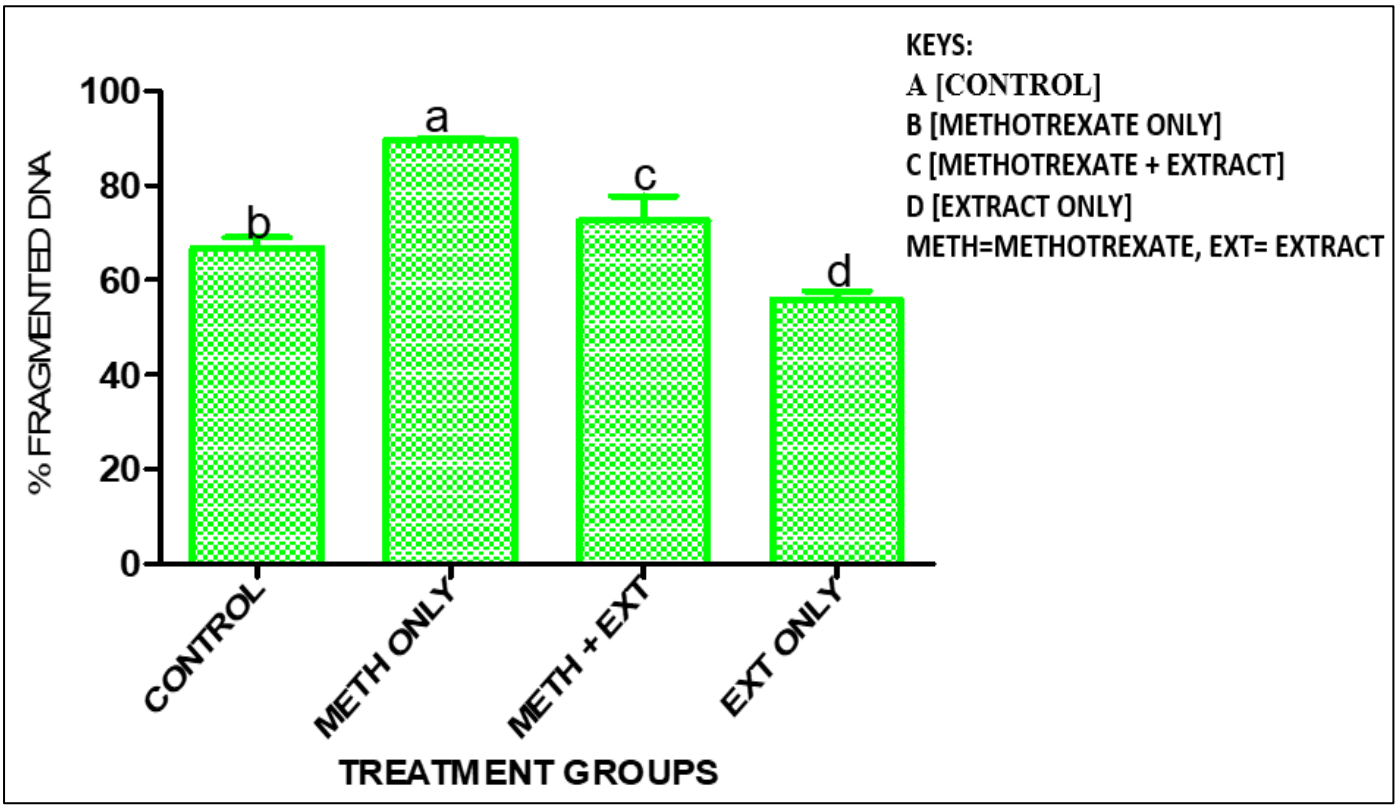

Fig 15 Percentage Fragmented DNA in Kidney of Rats in Various Treatment Groups Each Value Represent Mean ± SD for 8 Animals in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at p<0.05

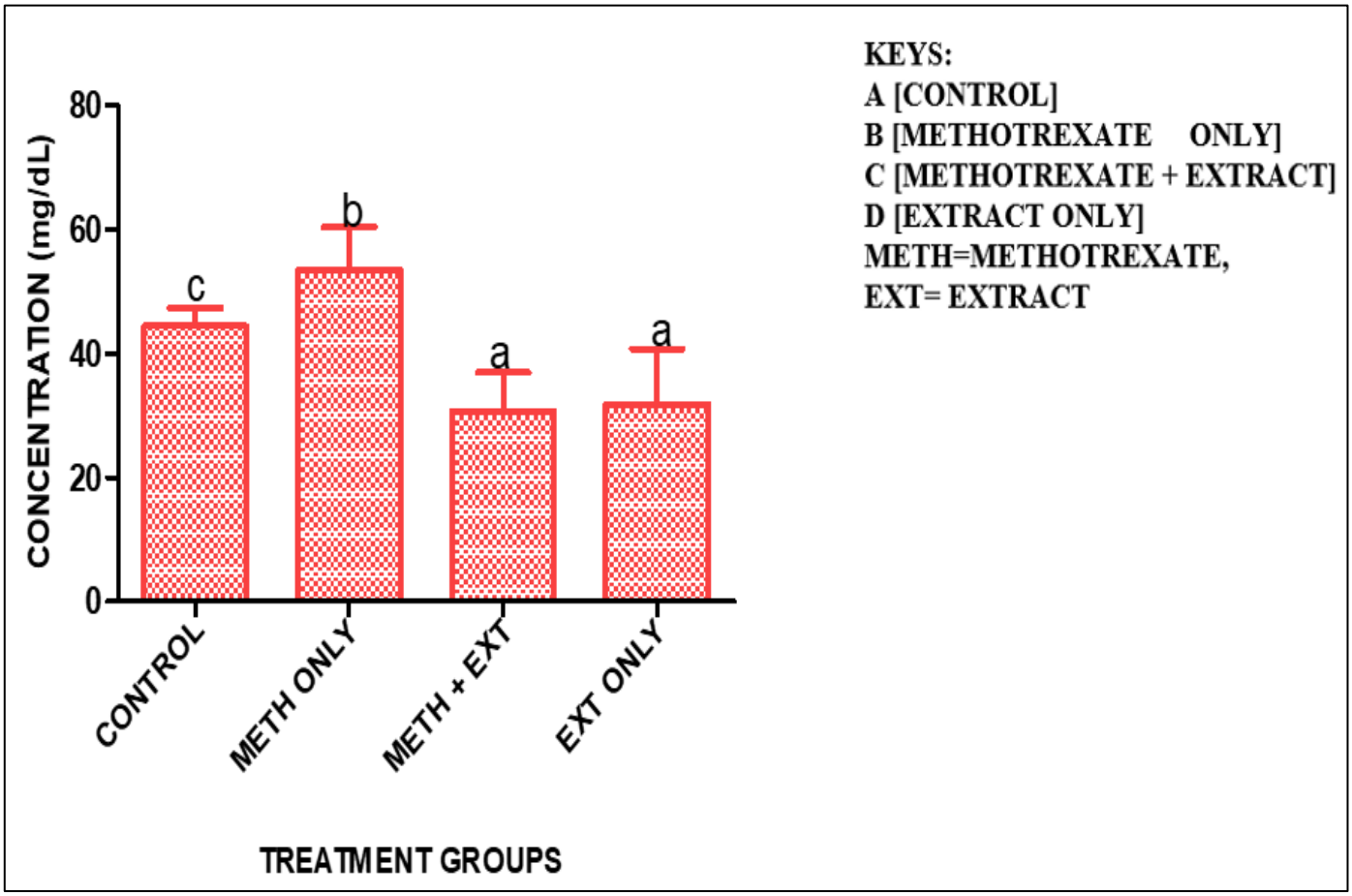

Fig 16 Triglycerides Concentrations in the Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at p<0.05

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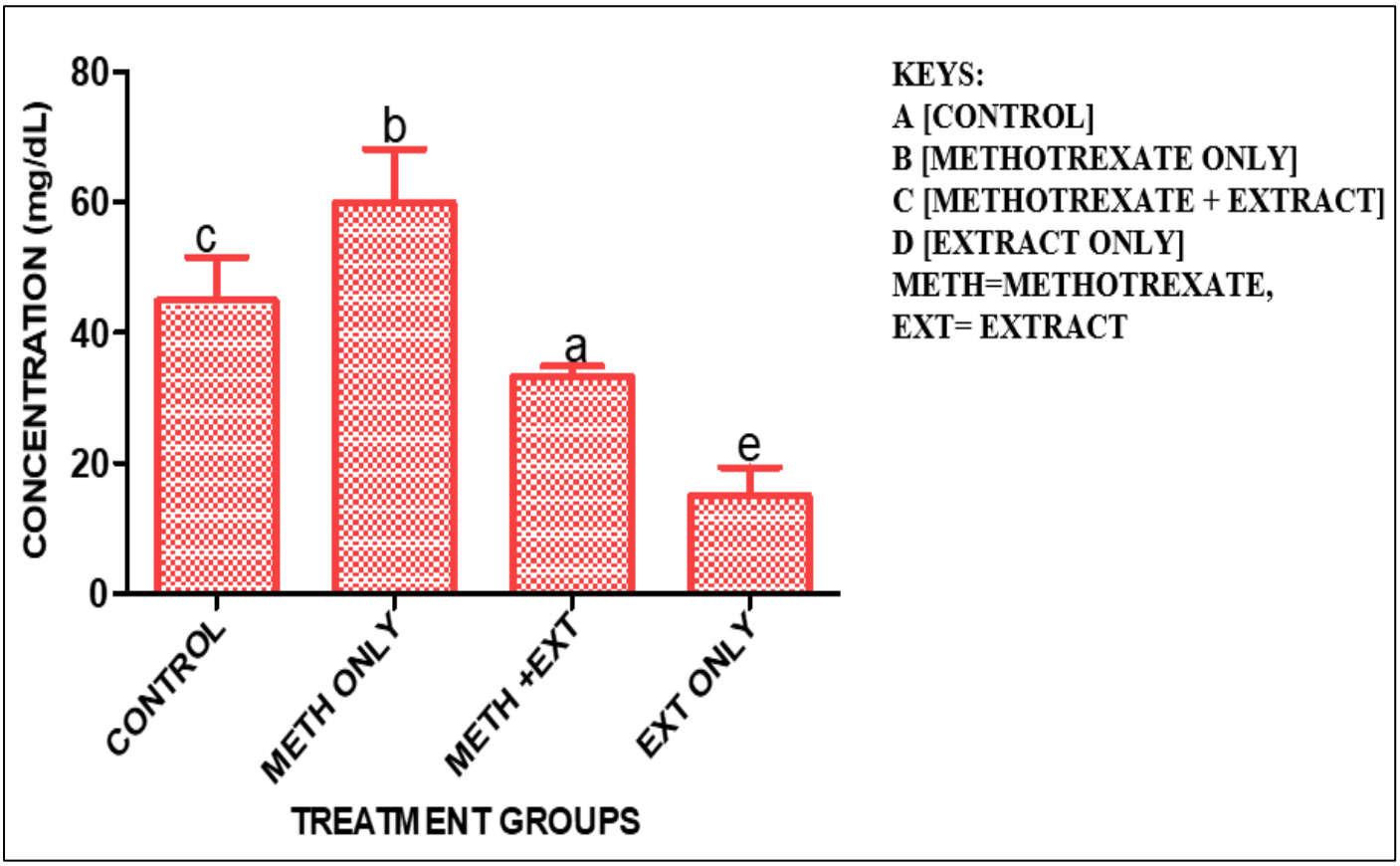

Fig 17 Cholesterol Concentrations in the Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

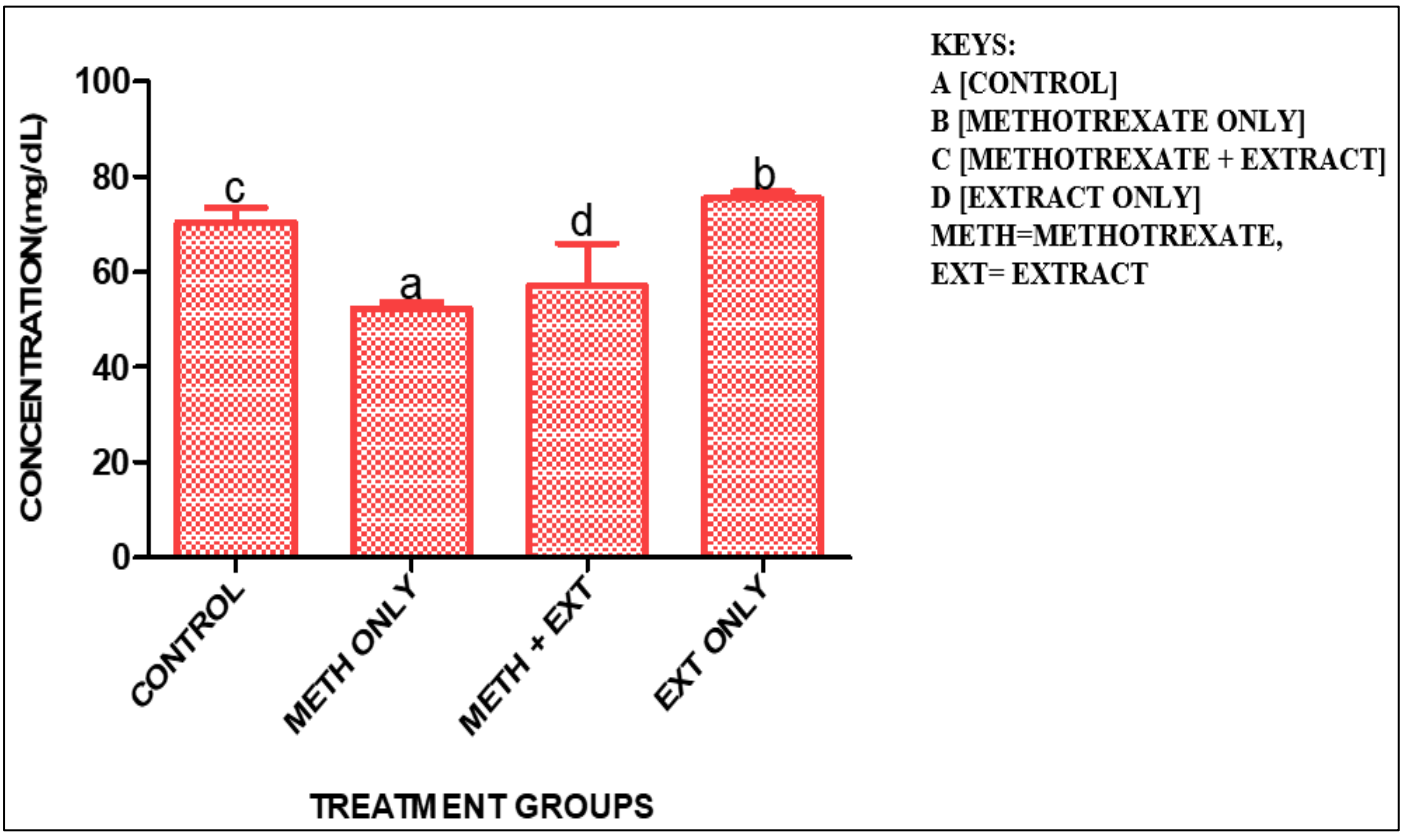

Fig 18 High Density Lipoprotein Cholesterol (HDL-c) Concentrations in Plasma of Rats in Different Treatment Groups Each Value Represent Mean ± SD for 8 Rats in Each Sgroup. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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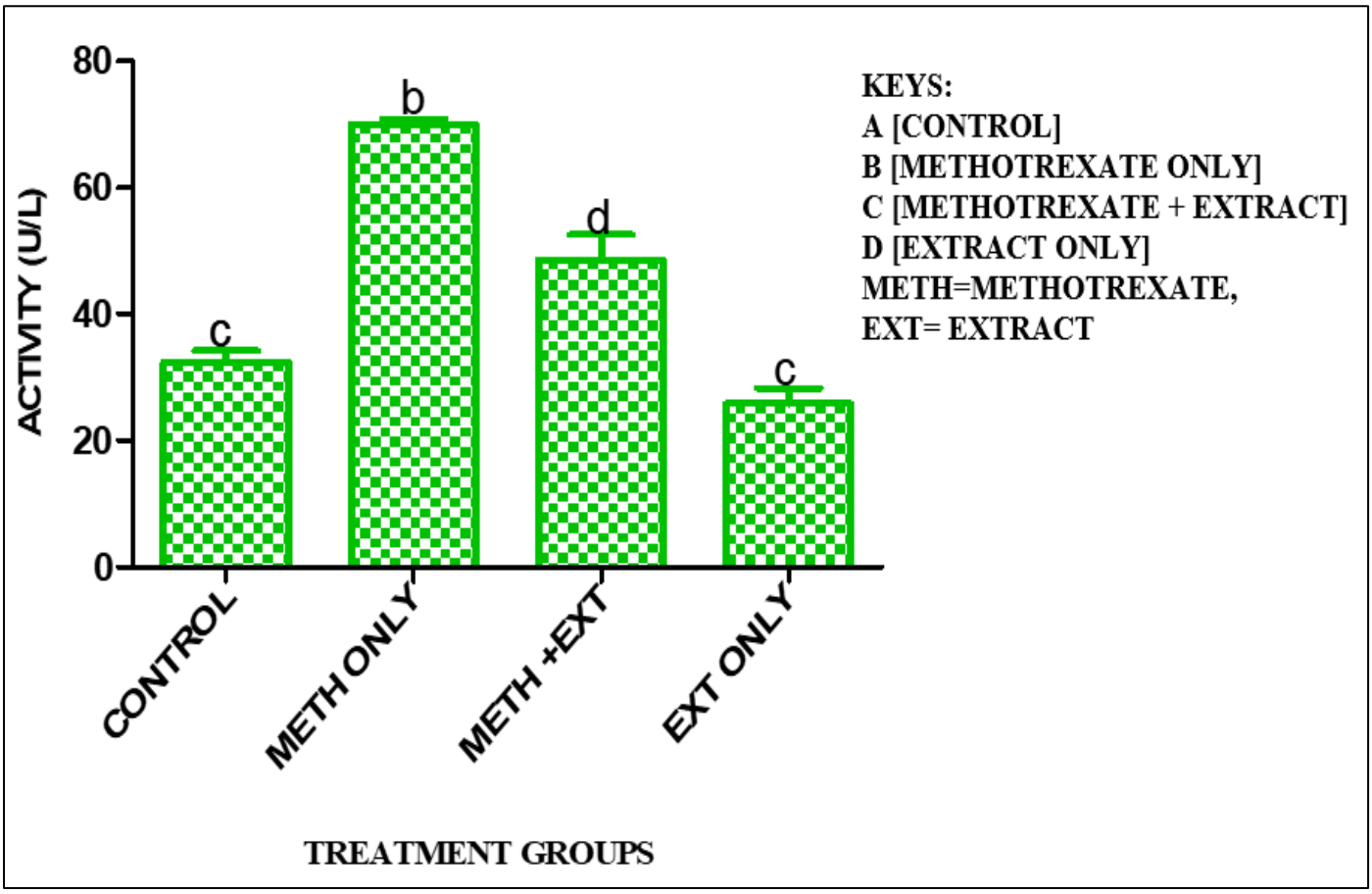

Fig 19 Gamma Glutamyl Transferase (GGT) Activity in Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

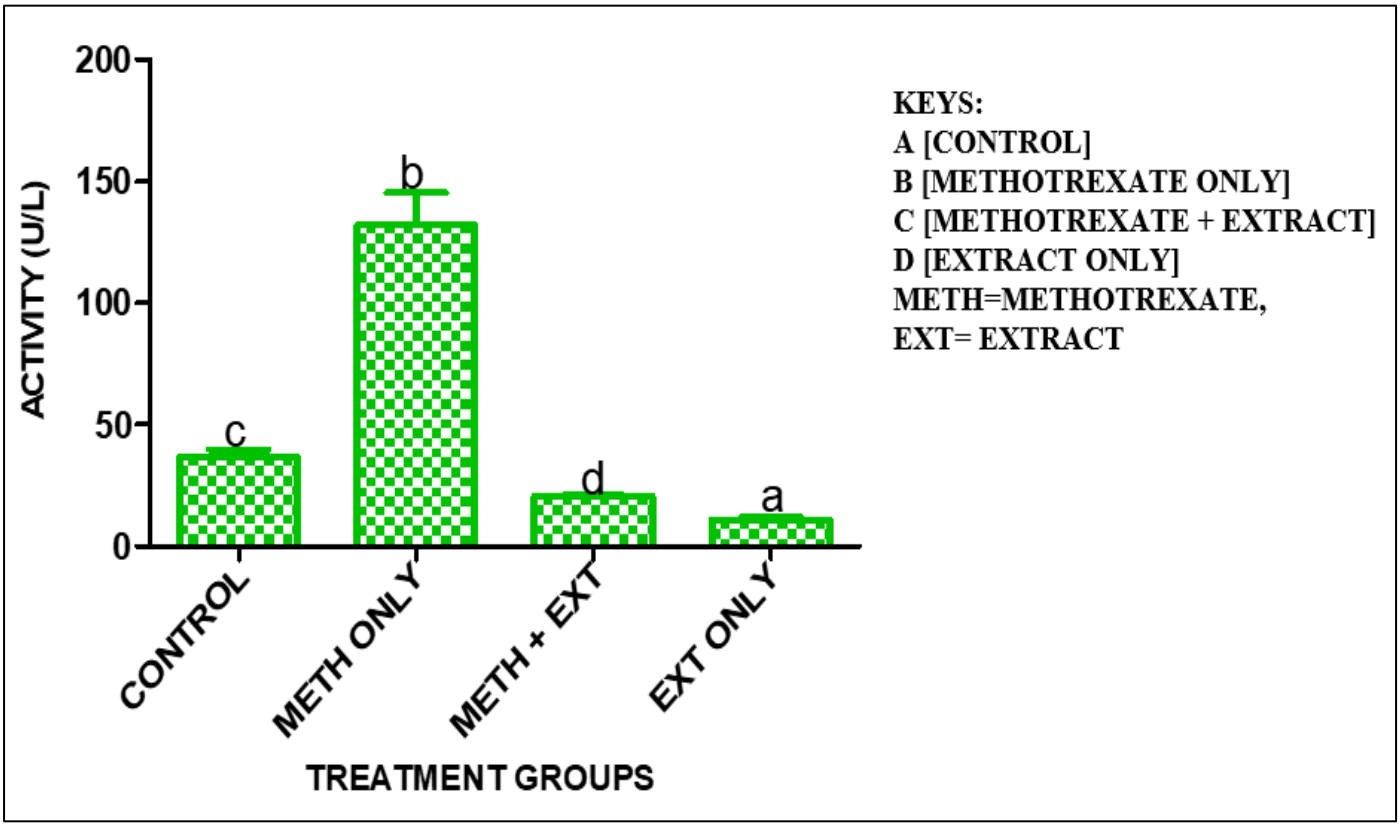

Fig 20 Alanine Amino Transferase (ALT) Activity in Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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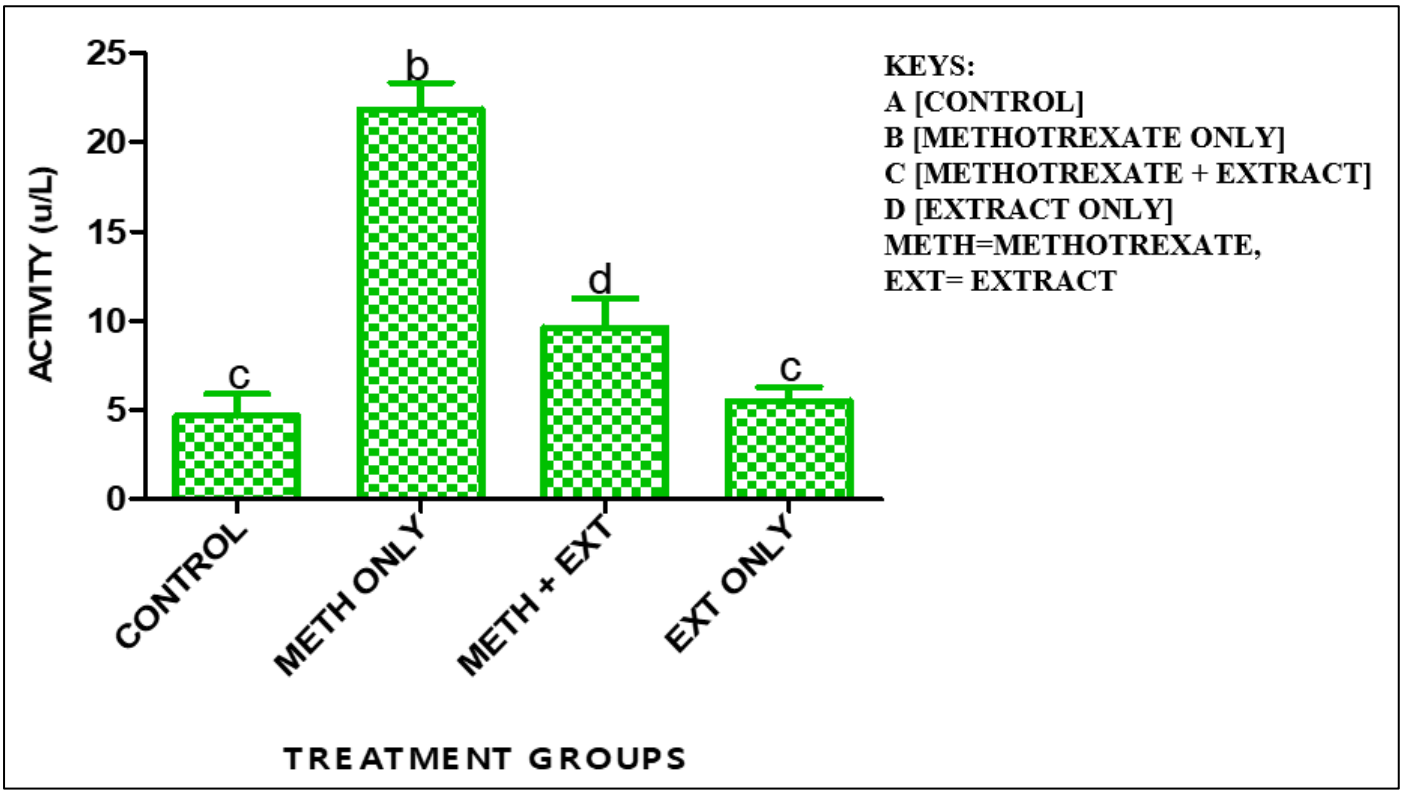

Fig 21 Aspartate Amino Transferase (AST) Activity in Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$.

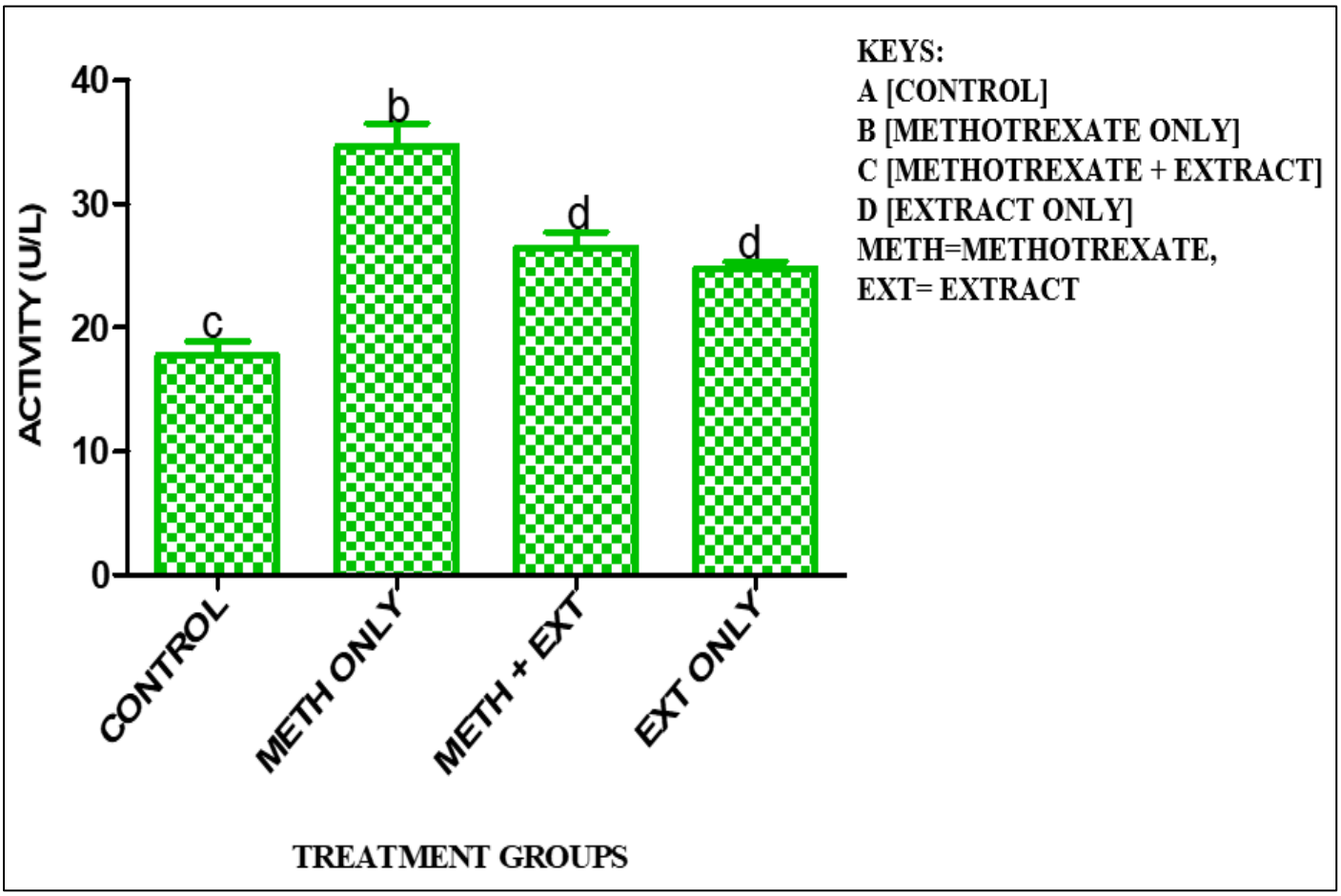

Fig 22 Alkaline Phosphatase (ALP) Activity in Plasma of Rats in Different Treatment Groups. Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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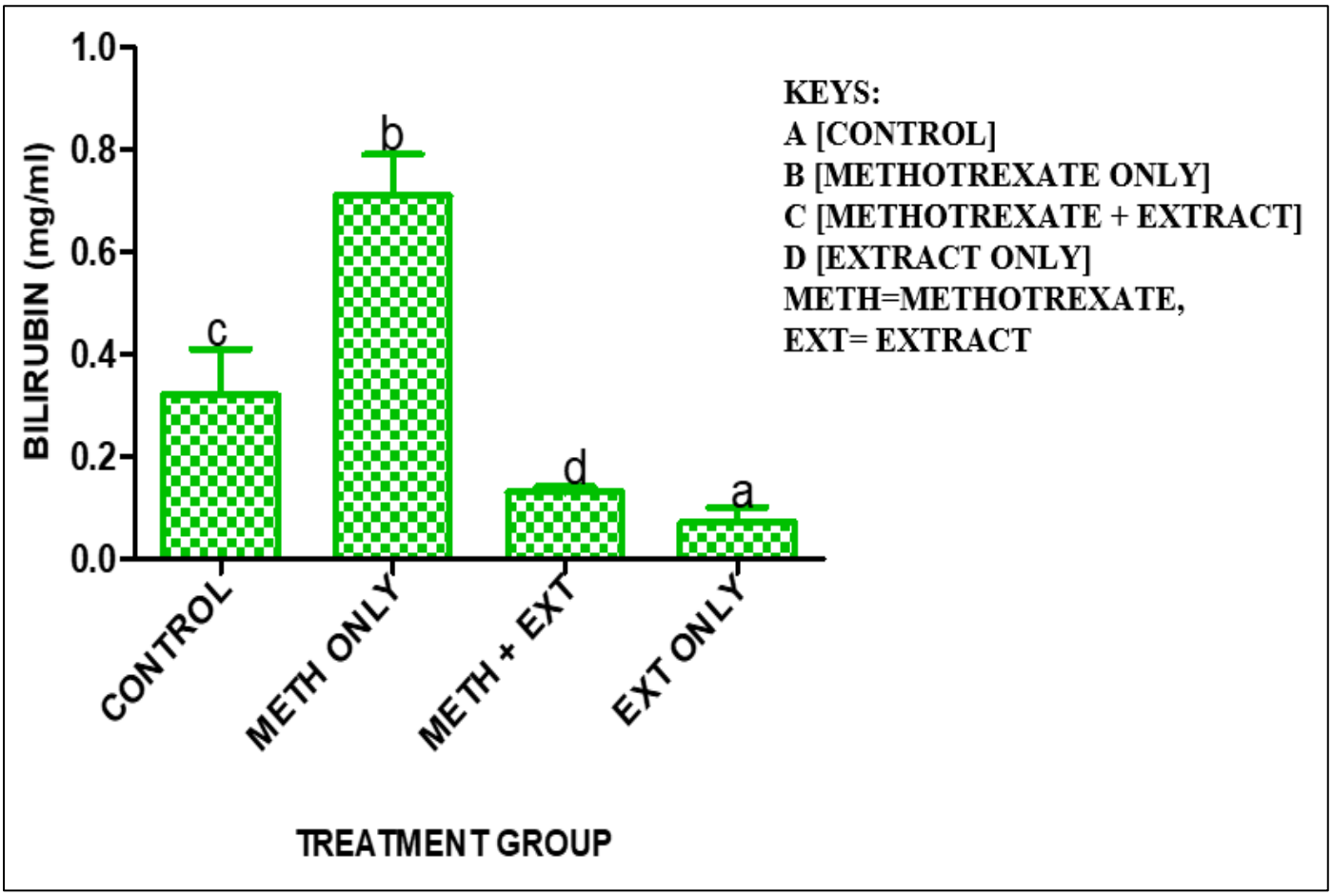

Fig 23 Bilirubin (BIL) Concentration in Plasma of Rats in Different Treatment Groups Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

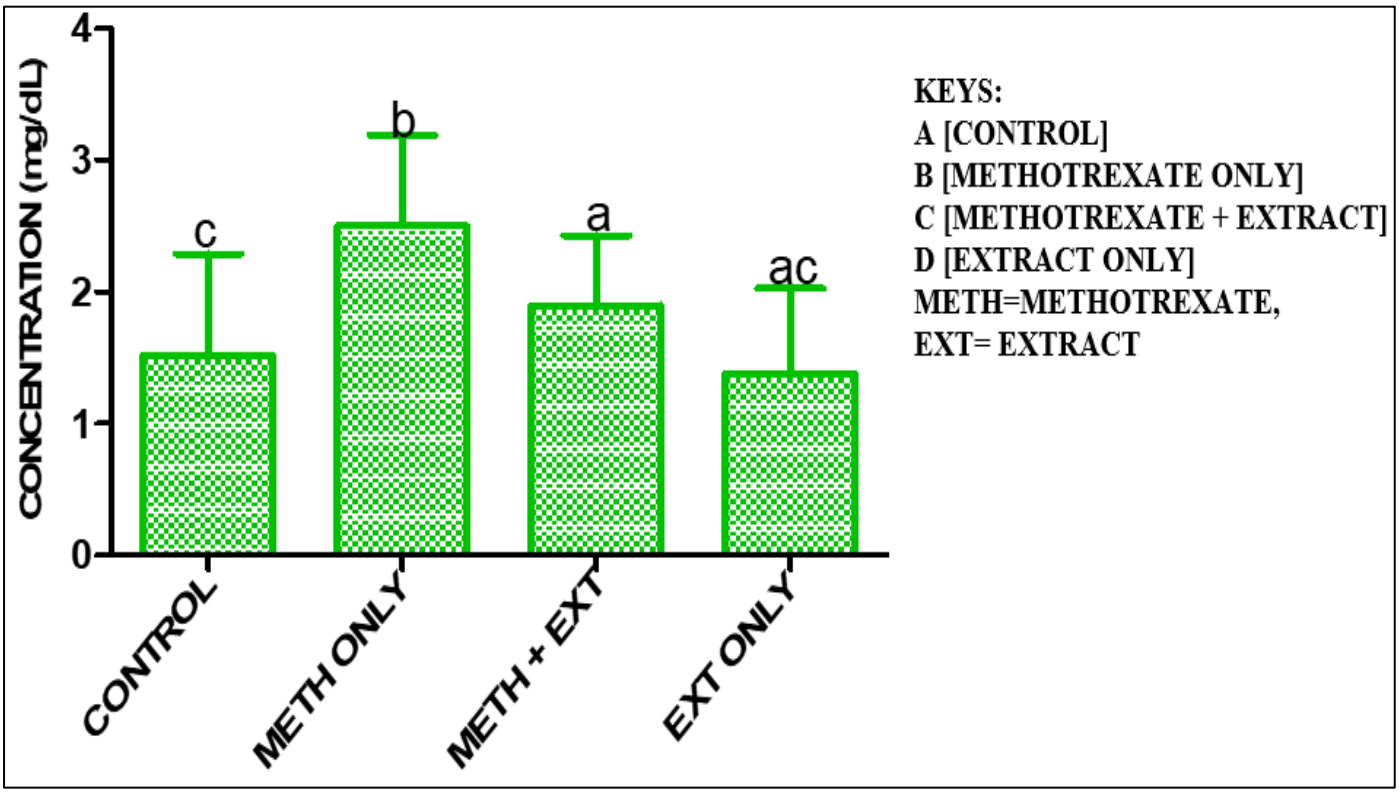

Fig 24 Creatinine Concentration in Plasma of Rats in Different Treatment Groups Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

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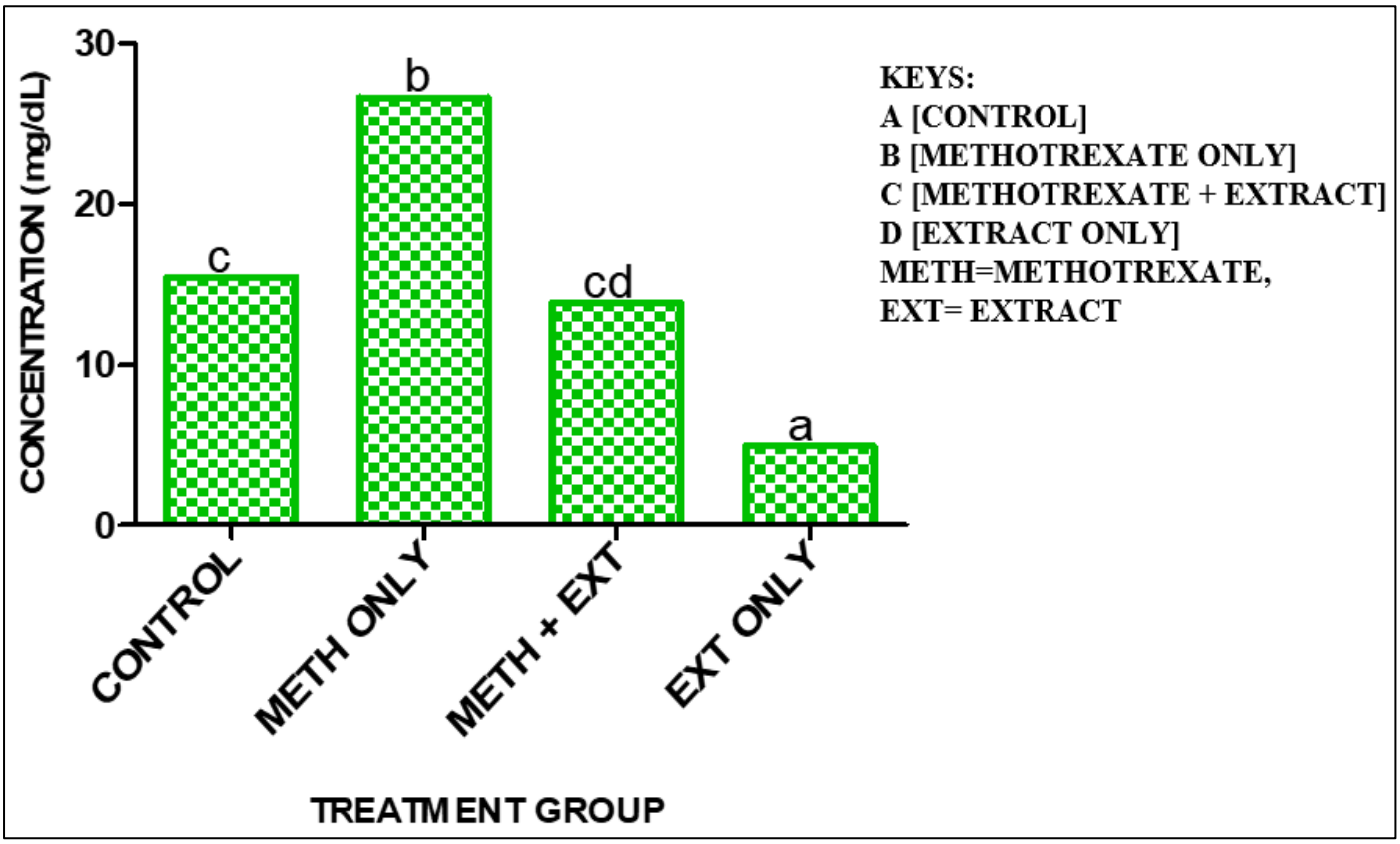

Fig 25 Urea Concentration in Plasma of Rats in Different Treatment Groups Each Value Represent Mean ± SD for 8 Rats in Each Group. Bars with Different Superscripts are Significantly Different from Each Other at $p<0.05$

IV. DISCUSSION

Complications arising from chemotherapy are major concern for cancer patients undergoing treatment [39]. Till date several disease modifying anticancer drugs were discovered whose mechanism of actions remain unclear. Methotrexate, is one of those potent anticancer agents employed therapeutically for the treatment of certain types and stages of cancer[40]. However, a factor limiting its use is its associated toxicity at multiple sites [41]. Therefore further elucidation of its accompanied toxicity may be helpful in the development of an improved efficacy with less to nothing toxicity profiles since this toxicity are known to perturb antioxidant status and induces apoptosis. From the results obtained in figure 1, 2 and 3, it was observed that group B (Animals exposed to methotrexate only) elicits a significant ($p<0.05$) decrease in the levels of plasma, liver and kidney total proteins compared with other treated groups. However, the combined treatment group C, (Extract and Methotrexate) showed significant ($p<0.05$) increases in these protein levels comparably with group B. Moreover, group D treated rats (Extract only) showed the highest increase in proteins comparably with controls. The decrease observed in total protein concentration in the liver may probably suggest the result of the toxic effect of methotrexate which makes more protein to be released into the blood from the liver, since these proteins serves as a major constituents of membranes which became a core targets of oxidative attack by ROS especially the OH^- and nitrogen –reactive radicals that could predominantly cause protein damage[41].Interestingly, the ability of both combined treatment (group C) and extract only (group D) to reverse this trends may be suggestive of their potentials to increase protein synthesis by reduction in the reactive oxygen species(ROS) levels on membranes exposed to oxidative damage via the drug metabolites and an indication of their potentials in ameliorating drug-induced toxicity [42,43].The decrease in the tissue protein concentration equally may be as a result of the toxic effect of the drug (methotrexate) on the liver which may encourage protein hydrolysis and its subsequent release into the blood.

Furthermore, results of antioxidant indices are used as reliable tools in ascertaining specific health and pathological conditions [44]. The nutritional and antioxidant status of individuals also determine the extent of tissue oxidative stress status[45]. In the same vein, animals treated with methotrexate only (group B) showed significant decreases ($p<0.05$) in activities of Superoxide dismutase (figure 4 and 5), Catalase (figure 6 and 7) and Glutathione peroxidase (figure 8 and 9) in the liver and kidney, respectively when compared with the control group. However, co-administration of animals with methotrexate and extract (group C),elicit significant ($p<0.05$) increases in the activities of these enzymes compared with group B (methotrexate treated animals only). Treatment with extract only (group D) showed no significant ($p>0.05$) difference in the activity of these enzymes in the liver and kidney of rats when compared with the control. Observations from this study showed that methotrexate administration exhibited alterations in tissue antioxidant enzymes activities in liver and kidney

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respectively. The decreased activities of different free radical scavenging enzymes in animals treated with methotrexate only (group B) were possibly attributed to feedback inhibition or oxidative inactivation of enzyme proteins due to an excessive increase reactive oxygen species (ROS) generation via the drug possible mode of action [46] and [47]. Conversely, the ethyl acetate extract supplement raised the levels of activities of SOD, CAT and GPx in the treatment group. The observed increases in the activities of these enzymes administered methotrexate and extract in both liver and kidney were suggestive of ameliorative effect of ethyl acetate extract to reduce redox imbalances and oxidative stress in these tissues[48]. Administration of methotrexate reduced the activity of antioxidant enzymes in these tissues and a pointer to higher intracellular concentrations of oxidants and free radicals due to its action, while the concomitant co-administration of methotrexate with extract (group C) modulates the oxidant/antioxidant balance as reflected by the stimulations of the antioxidants enzymes activities in these tissues and a pointer to its protective potentials.

Glutathione is an important low molecular weight tripeptide molecule obtained in all living cells, forming an important substrate for GPxand several other enzymes [49]. Reduced glutathione (GSH) plays an important role in antioxidation and drug metabolism. Elevated GSH levels minimise damage and enhance better resistance under conditions of oxidative stress [49], as it constitutes the first line of defence against peroxidative damage by reactive species. In this study, the hepatic and renal GSH concentrations in methotrexate treated animals were significantly ($p<0.05$) reduced than that of the controls in figure 10 and 11, respectively. In contrast, the combined treatment (group C) showed insignificant ($p>0.05$) increase in GSH concentrations when compared with rats treated with methotrexate (group B). However, animals fed with extract only elicits significant ($p<0.05$) increase in GSH level in liver and kidney when compared with the control.

The decrease observed in reduced glutathione concentrations in tissues attest to challenge on the body's antioxidant status. However, the behaviour of methotrexate treatment with ethylacetate extract is an indication of the protective effects of the bioactive components of the extract in the tissues exposed to methotrexate. Furthermore, the combined treatment in group C ameliorates methotrexate-induced free radical oxidative damage as the result showed an increased renal and hepatic GSH concentrations compared with group B, probably boosting the redox potentials of the animals, while treatment with extract only showed its ability to boost the antioxidant status with elevated levels of GSH.

Moreover, free radicals scavenging antioxidants enzymes and low molecular weight known antioxidant molecules have been shown to protect the cell against lipid peroxidation or inflammation in this manner preventing the occurrence of tissue damage[50,8].

Malondialdehyde (MDA), a unique biomarker of lipid peroxidation is generated by degradation of lipid components of most membranes and forms wide array of primary oxidation products (including conju-dienes or lipid hydro peroxides) due to free radical-induced oxidative damage to biological membranes. In this study, methotrexate administration in rats (group B) induced significant increases in the level of liver and kidney MDA as shown in figure 12 and 13. Co-administration of methotrexate with extract (group C) in rats restores the level of liver and kidney MDA concentrations back to normal when compared with the control ($p<0.05$). Furthermore, administration of extract only (group D) showed no significant difference ($p>0.05$) when compared with the control as MDA levels were restored in both tissues.

The increased MDA levels in methotrexate treated rats as observed in liver and kidney is an indication of increased free radical generations induced by methotrexate which attacks membrane structures, suggestive of lipid peroxidation, while reduction in MDA levels exhibited by ethylacetate extract is an indication of a possible chain breaking antioxidant capacity of the bioactive components of the extract which protect the membranes from oxidative damage induced by presence of oxidants elicited by methotrexate [51,52,] and [53]. In addition, previous study had attributed methotrexate induced MDA generation to increased level of hydrogen peroxide and activation of apoptotic pathway as a means for cancer management and its possible mode of action [43].

The fragmentation of DNA is a distinctive feature and an hallmark of morphological and biochemical changes, associated with programmed cell death (Apoptosis). These processes involve the breaking down of DNA strands into pieces [54], suggestive of pathological apoptosis. In the same vein, Methotrexate administration in animals induced a significant ($p<0.05$) increase in percentage fragmented DNA in the liver and kidney (Figure 14 and 15) of rats when compared with the control while co-administration of animals with methotrexate and extract (group C) altered these effects in the liver and the kidney. The elevated level of fragmented DNA induced by methotrexate administration in kidney and liver in this study is an indication of tissue toxicity which have been linked to free radical induced peroxidative damage exerted on rate limiting enzymes dihydrofolate reductase and thymidine synthase [55]. Hence, observation from the present study possibly showed that methotrexate affects thymidine nucleotide and purine synthesis leading to the observed fragmented DNA. Ethyl acetate extract, however with methotrexate (group C), showed modulatorypotentials as it ameliorated methotrexate toxicity from the results obtained [56] and [57].

Further investigation of the Plasma Lipid profile in various treatment groups as an important index in pathological conditions showed increases in the levels of total triglycerides and total cholesterol in methotrexate treated rats (group B), compared with control and other treatment groups. The elevated levels in total triglyceride and cholesterol were accompanied with decrease in the level of high-density lipoprotein cholesterol (HDL-c), (Figures 16,17 and 18), which may suggest methotrexate-induced alterations in lipid

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metabolism and may attest to increased hepatic fatty acid synthesis linked with rise in key enzyme activities associated with lipid metabolism due to oxidative stress induced by methotrexate to the membranes [58]. However, the observed significant ($p<0.05$) decrease in the levels of total triglycerides and total cholesterol as well as the increase in HDL-c levels triggered with the administration of the extract (group D) and in combination with methotrexate (group C) showed the extract's anti-oxidant potential via possible scavenging of induced free radicals and ROS produced by methotrexate metabolites and consequent inhibition of membrane lipid peroxidation .Also, the reverse lipid-lowering effect of extract may be linked to impeded hepatic fatty acid synthesis via reductions in key enzyme activities supplying the required substrates for the pathway [59].

In several biochemical pathways some liver enzymes catalyses the transfer of specific α-amino group to an intermediate such as alpha-ketoglutarate which are vital reactions in amino acid catabolism. These are liver transaminases which are intracellular enzymes and specific indicators of hepatic injury, damage and necrosis that are often released into the circulation after alterations in hepatocellular functions [2]. Evaluation of these enzymes in this study (figure 19, 20, 21and 22) showed that methotrexate elicit significant ($p<.05$) increases in the activities of plasma Gamma-glutamyltransferase (γ-GT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP), (group B),compared with other treatment groups.Also, the extract singly (group D) as well as its combinative treatment with methotrexate (group C), significantly ($p<.05$) decrease the activities of these enzymes compared with controls and methotrexate treated group (group B). Evidence have shown that prolonged unregulated exposure to high concentrations of methotrexate can result in its precipitation in the liver which could in turn causes alterations in the liver functions leading to upregulations of these enzymes activities. The current study however showed significant alterations in the activity of GGT, ALT, AST and ALP in the blood of methotrexate-treated rats which may be linked to impaired liver function and possible disorder in the biosynthesis of these enzymes with possible modulation in the permeability of the liver membrane [60]. On the other hand, treatment of rats with extract alongside methotrexate-mediated toxicity (group C) causes significant ($p<0.05$) reductions in the liver enzymes activities attributable to extract ability to probably stabilize hepatocytes plasma membrane and abridged transmission of these enzymes into the extracellular fluid via alteration or inhibition of methotrexate-related oxidative stress induced liver damage [61].

Evaluation of bilirubin as an index of liver function test in this study showed that albumin conjugates with methotrexate to enhance its transport to target tissues and stimulate its anti-tumour effects through endocytosis. The conjugative effect decreases methotrexate clearance and prolonged methotrexate retention in the target tissue have been linked to hypo albuminemia with concomitant use of nephrotoxic agents ([40]. Interestingly results from this study showed that animals administered methotrexate only (Group B) elicits significant ($p<0.05$) increase in plasma bilirubin concentration compared to control and other treatment groups (Figure 23). This is in agreement with the observation of [40], where increase in bilirubin concentration was accompanied by prolonged methotrexate retention. Methotrexate retention burden may cause impaired liver function; a factor that could contribute to bilirubin over-secretion associated with reduced hepatocyte uptake as observed in group B. However, the treatment of rats with extract and methotrexate (group C) showed significant ($p<0.05$) decrease in bilirubin level compared with methotrexate only treated animals and control (Figure 23) respectively, suggestive of the ameliorative and protective effect of the extract against methotrexate-mediated adverse effect on the liver.

Also, Kidney function indices observed in this study showed that plasma creatinine and urea concentration (Figure 24 and 25) respectively, were significantly ($p<0.05$) increased in animals exposed to methotrexate only when compared with the control. However, significant ($P<0.05$) decrease were observed in these parameters in combined treatment (group C) compared with the intoxicated group with methotrexate (Group B), an indication that methotrexate induces elevations in plasma urea and creatinine levels with concomitant decrease in creatinine clearance. However, significant ($p<0.05$) decrease in urea concentration (Figure 25) in animals treated with extract and methotrexate (group C) compared with the control group were observed. Chronic exposures to methotrexate in rats have shown nephrotoxicity and glomerular tuft [40], while these adverse effects may have been linked to methotrexate precipitation or its secondary metabolites in the renal tubules; which is known to be the primary sites of renal damage. The effects may lead to renal obstruction and diminished renal clearance with prolonged methotrexate exposure. In this study, increased plasma urea and creatinine concentration is an indicator of prolonged critical accumulation of methotrexate in the kidney capable of causing renal failure as reported by [62]. Similarly, methotrexate precipitation could also acts as a direct toxin on the tubular epithelium [63]and may actuate vasoconstriction of the afferent arteriole. However the ameliorative effect by the extract administration may probably be done possibly by enhancing the structural integrity of the kidney via reduction in redox imbalance facilitated by the mode of action of methotrexate.

V. CONCLUSION

The results obtained revealed methotrexate potential to induce redox imbalance, alter antioxidant status, caused oxidative stress and tissue toxicity. However, the ethyl acetate extract of *lannae egregia* leaves showed possible indication of its rich relevant bioactive components with antioxidative property, tissue protective effect and a possible ameliorative potential against these effects. Hence, the use of the plant in folkloric medicine in Western Nigeria, as a supplement with orthodox medicines and as a possible template for drug discovery in cancer management could be encouraged.

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