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HEPATOPROTECTIVE POTENTIALS OF METHANOL EXTRACTS OF *GOSSWEILERODENDRON BALSAMIFARUM* AND LIPID PROFILE OF ALBINO RATS.

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ABSTRACT

Methanol extract of *Gossweilerodendron balsamifarum* leaves at 250mg/kg and 500mg/kg was evaluated for hepatoprotective potentials using female albino rats. The lipid profile of the same rats were also evaluated. The plant extract (250 and 500 mg/kg), however showed no remarkable hepatoprotective and antioxidant activity against chloroform/ hexane-induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. Chloroform/hexane-induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). Treatment of rats with different doses of the plant extract (250 and 500 mg/kg), however, could not reverse the condition. Also lipid profiles of the control group and that of the extract were found not to differ. This was ascertained by comparing the levels of high density lipoprotein, (HDL), low density lipoprotein, (LDL), total cholesterol and triacylglycerol, (TAG), in the two groups.

KEYWORDS: *Gossweilerodendron balsamifarum* leaves, hepatoprotective potentials.

INTRODUCTION

Gossweilerodendron balsamifarum (**Agba**, **Tola**) is a tall forest tree in the family Fabaceae. It is native to lowland tropical rainforest in west Africa, from Nigeria southwards to the Congo basin in Angola, Cameroon, Republic of the Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon, scattered or in local pockets, favouring deep soil and plenty of moisture. It is threatened by habitat loss and over-cutting for timber. (International legume database and information). It is a large to very large tree growing to 60 m tall, with a trunk 70–180 cm diameter with resinous bark. The leaves are pinnate, with 6–10 alternately-arranged leaflets 4–9 cm long and 2–4 cm broad. The flowers are small, with four (rarely five) white sepals 2 mm long and no petals; they are produced in panicles. The pod is 10–14 cm long and 3.5–4.5 cm broad, superficially resembling a maple Samara with a single 2–3 cm seed at one end, with the rest of the pod modified into a wing. It has high spruce gum content. (Watson and Dallwitz, 2005)

Other names for this tree are *Achi, Egba, Emongi* (Nigeria), *Tola blanc* (Congo-Brazzaville), *Tola branca* (Angola), *N'Tola* (Zaire).

PHYTOCHEMICALS PRESENT IN *G. BALSAMIFARUM* WOOD

The wood of *G. balsamifarum* has five diterpenes. These are;

- Hardwickiic acid (Carman and Marty. 1969)
- Agbaninol (Pratt, 1951)
- Agbanindiols A (Cambie and Franich, 1969)
- Agbanindiols B
- Monomethyl ester known as kolavic acid.

OBJECTIVES OF THE STUDY

The purpose of the study includes;

- To evaluate the lipid profile of albino rats treated with methanol leave extract of *Gossweilerodendron balsamifarum*
- To evaluate the hepatoprotective effects of *G. balsamifarum* against chloroform / hexane-induced liver injury in rats.

To the best of our knowledge, no scientific data regarding the hepatoprotection/ antioxidant potentials of *G. balsamifarum* leaves are available. Thus, the present study was undertaken to evaluate the lipid profile, and hepatoprotectiveness of *G. balsamifarum* leaves in chloroform/ hexane-induced hepatocyte damaged rats.

BACKGROUND INFORMATION

The liver is the largest internal organ. It is reddish brown, weighs approximately three pounds (in the adult male) and is about the size of a football. It is located behind the ribcage on the upper right side of the abdomen. The liver has the unique ability to regenerate its own tissue as much as three quarters of the liver can be lost, and the organ can grow back or expand to its original size within several weeks. This allows people who need transplants to receive part of the liver of living donor. The liver is divided into four lobes; these are in turn composed of multiple lobules, which contain the hepatocytes, or working liver cells. It has an extensive blood supply about one half quarts of blood flow through it every minute. It receives oxygen rich blood from the hepatic artery. The portal vein delivers blood containing nutrients, toxins and other substances absorbed from the intestines to the liver. The liver filters this blood, then sends it on the heart via the hepatic vein.

The liver has well over 500 functions and is known as the laboratory of the human body. The body is tied to all bodily processes because it is responsible for filtration of all incoming foods and fluids. The body relies upon the liver to remove toxins so that nutrients supplied to the body are pure and capable of providing nourishment. Many scientists believe the liver is connected to, or at least aware, of every disease or dysfunction that is happening inside the body.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances. They can be dietary, endogenous or metal binding proteins.

SIGNIFICANCE OF THE STUDY

Liver is an important organ in the body that helps to carry out different body functions. These numerous activities of the liver lead to generation of free radicals. It is therefore important to protect the liver from toxicity and thus this work is aimed at establishing relationship between hepatoprotection and the leave extract of gossweilerodendron balsamiferum as well as the lipid profile of the plant extract.

MATERIALS

PLANT MATERIAL: *Gossweilerodendron balsamifarum*

EXPERIMENTAL ANIMALS

Adult albino rats of wistar strain (95-145)g of female sex were procured and housed in animal house of college of natural sciences, Michael Okpara University of Agriculture Umudike. They were exposed to equal amount of light and dark cycles and feed with vital growers feed for the whole period of the experiment. The animals were then acclimatized for three weeks after which, administration of sample commenced. The animals were also randomized to ensure equal distribution.

PLACE OF WORK

The whole experimental work was carried out at the college of natural sciences, Michael Okpara University of Agriculture Umudike.

PLANT COLLECTION AND IDENTIFICATION

Leaves of *Gossweilerodendron balsamifarum* were collected in and around local forest area of Umudike and authenticated by Dr. Omosun Garuba, Department of Botany, College of natural science MOUAU. 100g of the fresh leave of G. balsamifarum was grinded and soaked with methanol for 24 hours under room temperature and was occasionally stirred. The extract was filtered and concentrated to dryness. A known volume of extracts was suspended in deionized water and orally administered to the animals using syringe

EXPERIMENTAL DESIGN

The rats were divided into four (4) groups of four rats each. Group I- VI

Group II- VI were given chloroform and hexane at ratio of 6:4.

Group I; received distilled water and food

Group II; also received normal food and distilled water

Group III: this group was given 250mg of the leave extract of *G.balsamifarum*

Group IV: this group also received the extract of *G. balsamifarum* but at the dose of 500mg.

The animals were treated for eight days and then sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifugation at 2500 rpm for 15minutes and analyzed for various biochemical parameters.

BIOCHEMICAL ANALYSIS

CHOLESTEROL DETERMINATION

The method of (Abell et al., 1952) was followed.

Test procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added (10ul) distilled H_2O, 10ul standard specimen to the standard test tube and 10ul sample (serum) to the sample test tube. To each of these test tubes was added 1000ul of the cholesterol reagent. It was thoroughly mixed and incubated for 10minutes at room temperature (20-25^oC). The absorbance of the sample (A_{sample}) against the blank was taken within 60 minutes at 500nm.

LOW DENSITY LIPOPROTEIN (LDL)

Procedure

The serum samples were kept at 2-8^0C. The precipitant solution (0.1ml) was added to 0.2ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined. The concentration of the serum total cholesterol as described by (Kameswara et al., 1999) was used.

Calculation

LDL-C (mmol/L) = Total Cholesterol (mmol/L) – 1.5 x Supernatant Cholesterol (mmol/L).

HIGH DENSITY LIPOPROTEIN (HDL)

Procedure

The precipitant solution 0.1ml was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined.

TRIACYLGLYCEROL

Clinical significance

Triacylglycerols measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g diabetes mellitus, nephrosis and liver obstruction.

Method

A quantity of the sample (0.1 ml) was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 minutes. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown below

S/N	Blank	Standard	Sample
1. Distilled water	0.5	-	-
2. Standard solution (ml)	-	0.5	-
3. TCA (ml)	0.5	0.5	-
4. Supernatant (ml)	-	-	1.0
5. Reagent mixture (ml)	1.0	1.0	1.0

The mixtures were allowed to stand for 20 minutes at 25 ^{0}C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

DETERMINATION OF LIPID PEROXIDATION (MALONDIALDEHYDE)

Lipid peroxidation was determined by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by (Wallin et al., 1993).

Procedure

A volume, 0.1ml of the serum was mixed with 0.9ml of H_2O in a beaker. A volume, 0.5ml of 25% TCA (trichloroacetic acid) and 0.5ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 minutes in water-bath and then cooled in cold water. Then 0.1ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532nm and 600 nm against a blank.

$$\% \text{ TBARS} = \frac{\text{A532- A600 x 100}}{0.5271 \text{x} 0.1} \quad \text{(mg/dl)}$$

TOTAL SERUM PROTEINS

The principle lies on the fact that at alkaline pH 7.0, proteins form a stable complex with Cu^{2+}, which is photometrically measured.

Procedure

Three test tubes, blank, standard and sample were labeled and to the sample tubes were added 0.02ml of serum, to the standard test tube, a volume, 0.02ml of protein standard was added and 0.02ml water to the blank test tube. One mililitre of the protein reagent was added to the test tubes each. This was mixed well and left to stand for 25mins at room temperature (20-25^{o}C). The absorbance was taken at 540nm.

$$\text{Total serum Proteins (in g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5 = \text{g of protein/dl}$$

LIVER FUNCTION TEST

DETERMINATION OF ALKALINE PHOSPHATASE (ALP)

Method

The blank and sample test tubes were set up in duplicates and 0.05ml of sample was pipetted into the sample test tubes. 0.05ml of distilled water was pipetted into the blank tube. Three mililitres (3.0ml) of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one minute intervals.

Calculation: alkaline phosphatase activity was calculated as follows

$$\text{Activity of ALP (in U/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3300$$

DETERMINATION OF ALANINE AMINOTRANSFERASE (ALT)

Method: The blank and sample test tubes were set up in duplicates. 0.1ml of serum was pipetted into the sample tubes. To these were added 0.5ml buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37 ^{0}C ml and pH 7.4. A volume, 0.5ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to both tubes while 0.1ml of sample was added to sample blank tube. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25 ^{0} C. Five militres of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 540nm.

DETERMINATION OF ASPARTATE AMINOTRANSFERASE (AST)

Method: The blank and sample test tubes were set up in duplicates. A volume, 0.1ml of serum was pipetted into the sample tubes and 0.5ml of reagent 1 was pipette into both sample and blank tubes. The solutions were thoroughly mixed and incubated for exactly 30 minutes at 37 ^{0}C ml and pH 7.4. 0.5ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added into all the test tubes followed by 0.1ml of sample into the blank tubes. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25 ^{0} C and 5.0ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 546nm.

ANTIOXIDANT ENZYMES ASSAY

SUPEROXIDE DISMUTASE (SOD) ASSAY

This was determined using the method of (Xin et al., 1991).

Procedure

Adrenalin (0.01g) was dissolved in 17ml of distilled water and 0.1ml of serum and 0.9ml of phosphate buffer (pH 7.8) were taken in triplicates in 2.5ml buffer. A volume, (0.3ml) adrenaline solution was added and mixed inside the cuvette. The absorbance was taken at 480nm at 30 seconds interval for five (5) times. The changing rate of absorbance was used to determine superoxide dismutase activity.

CATALASE ASSAY

This was done according to the method of (Aebi, 1983).

Procedure

Two mililitres of hydrogen peroxide and 2.5ml of phosphate buffer were added to a beaker. Adequately, 0.5mls of the sample was also added and mixed. One mililitre (1ml) portion of the reaction mixture was added to 2ml of dichromate acetic acid reagent. The absorbance was read at 540nm (at a minute interval in tripricates). Catalase activity was calculated using the following equation:

$$\text{Catalytic concentration (unit/L)} = \frac{\log\ [\text{abs } 1/\text{abs } 2] \times 0.23}{0.00693}$$

GLUTATHIONE ESTIMATION

This was determined according to the method of (King and Wootton (1959).

Procedure

A volume of the sample (0.1ml) was mixed with 0.9ml of distilled water in a beaker. Sodium sulphate (0.02ml) was also added, shaken and allowed to stand for 2mins at room temperature. A volume, 0.02ml of Lithium Sulphate (20%), 0.2ml of 20% $NaCO_3$ and 0.2ml of phosphor- 18-tungstic acid were also added to the beaker, it was shaken and allowed to stand for 4mins while observing for maximum colour development. A volume, 2.5ml of 2% sodium sulphite was also added and the absorbance was taken at 680nm within 10mins-A blank (0.1m H_2O) was also set up. Glutathione concentration was calculated from a standard cystein curve.

ESTIMATION OF VITAMIN E (ALPHA TOCOPHEROL)

Vitamin E content was estimated by the method of (Palan et al., 1973). This method involves the conversion of ferric ions to ferrous ions by tocopherol and the formation of red coloured complex with 2,2 dipyridyl. Absorbance of chromophore was measured at 520 nm in the spectrophotometer.

The requirements are the following

1. 2% 2,2 dipyridyl solution
2. 5 % ferric chloride solution
3. Standard : 100 mg of tocopherol in 0.1% ethanol
4. n- Butanol

Procedure

To 0.5 ml of serum, 1.5 ml of ethanol was added, mixed and

centrifuged. The supernatant was dried at 80C for 3 hours. To this 0.2 ml of 2, 2 dipyridyl solution and 0.2 ml of ferric chloride solution were added, mixed well and 4 ml of butanol was added. The colour developed was read at 520 nm in the spectrophotometer. Values were read as mg/dl of serum.

VITAMIN C

This was done according to the method of caraway

Procedure

1.0ml of plasma was placed into a small test tube. 1.0ml of 10 percent trichloroacetic acid was added and then 0.5ml of chloroform. It was stoppered, shaken vigorously for 15 seconds

and centrifuged at 3,000rpm. 1.0ml of clear supernatant was placed in a test tube. Blank and standard was prepared by adding 0.5ml of 10% trichloroacetic acid to 0.5ml of water and working standard reagent. To each tube was added 0.4ml of freshly prepared combined color reagent. The tube were stopped and placed in a water-bath at 56% for one hour. They were cooled in an ice bath for about 5 minutes. To each tube was added, slowly with mixing 2.0ml of ice cold 85% sulphuric acid. The tube were left at room temperature for 30minutes, then mixed and the optical density (OD) read in the colourimeter at 490mm using blank to zero the instrument. The OD of the standard was also read.The normal range of plasma Ascorbic acid is 0.5 to 1.5mg/100ml (0.03 to 0.9mmol/L) for these methods.

RESULT

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on AST and ALT

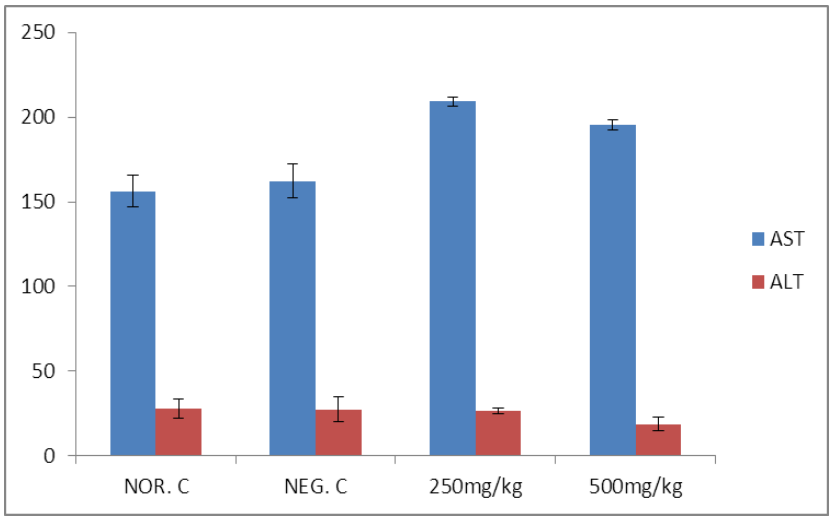

Fig. 4.1 MEAN OF AST, ALT

The mean concentration of AST and ALT (IU/L). The mean serum of AST (IU/L) is significantly ($P<0.05$) lower in the control group (NOR. C AND NEG. C) than animals treated with methanol extract of *G. balsamifarum* (250mg/kg 500mg/kg). However, there is no significant ($P>0.05$) difference in the mean value of ALT (IU/L) in control group and test group, at 95 percent confidence.

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on MDA, T. Protein and ALB

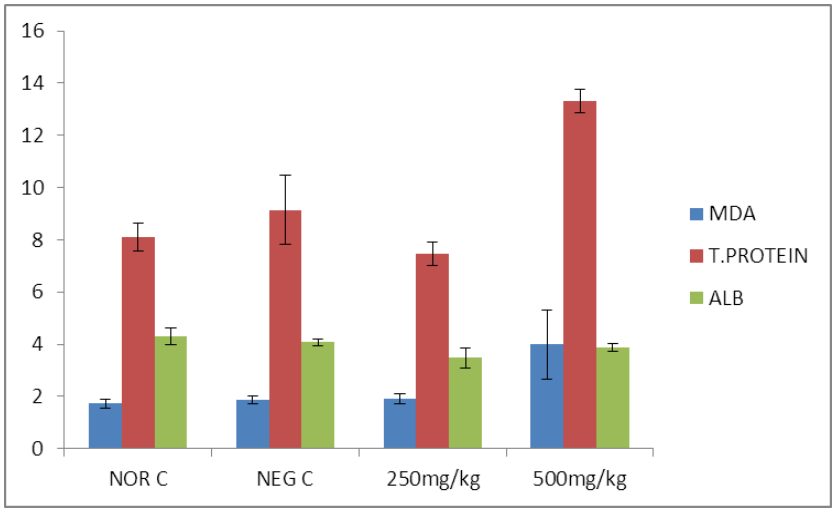

FIG. 4.2 Mean Of Mda, Total Protein And Albumin

Mean difference of MDA in control group is significantly (P<0.05) lower than that of the test group. There was no significant (P>0.05) difference between the mean serum total protein in control groups animals and that of the test group (*G. balsamifarum* 250mg/kg). However, serum mean total protein of *G. balsamifarum* (500mg/kg) was significantly (P<0.05) higher I than that in control group at 95 percent confidence interval.

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on GSH, CATALASE and SOD

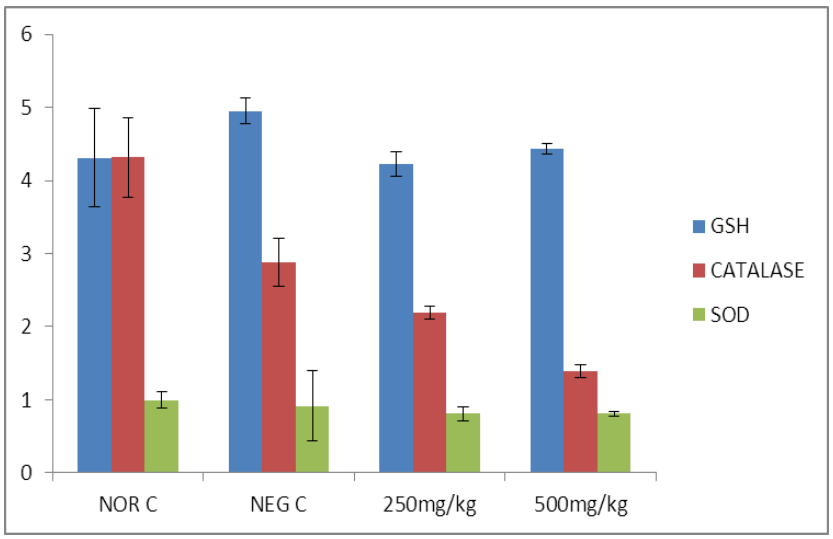

Fig. 4.3 Mean Of Gsh, Catalase And Sod

There was no significant difference between the mean serum level of GSH in normal control and that of the test group (250mg/kg and 500mg/kg). But serum mean is significantly (P<0.05) higher in negative control group than that of the test group (250mg/kg and 500mg/kg). Mean serum level of catalase is significantly (P< 0.05) in the control group than that of the test group of *G. balsamifarum.* Mean serum level of SOD is significantly (P< 0.05) higher in control group than in test group of 500mg/kg. However there is no significant difference between that of the control group and test group.

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on TAG and T. Cholesterol

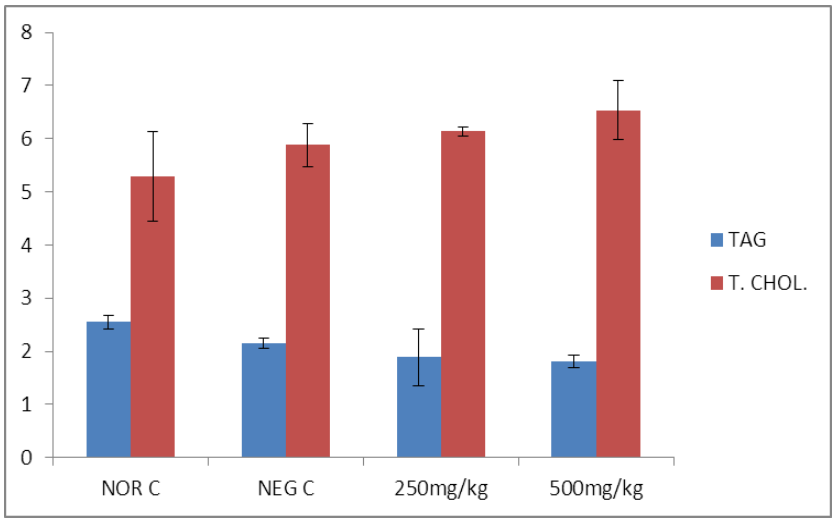

Fig. 4.4 Mean Of Tag And T. Cholesterol

Serum mean TAG was found to be significantly higher in control groups than that of the test groups (both 250mg/kg and 500mg/kg). There was no significant difference between the mean serum of total cholesterol in control and test group of *G. balsamifarum.*

There was no significant difference (P>0.05) in mean serum of both LDL and HDL, of control group and test group animals.

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on HDL and LDL

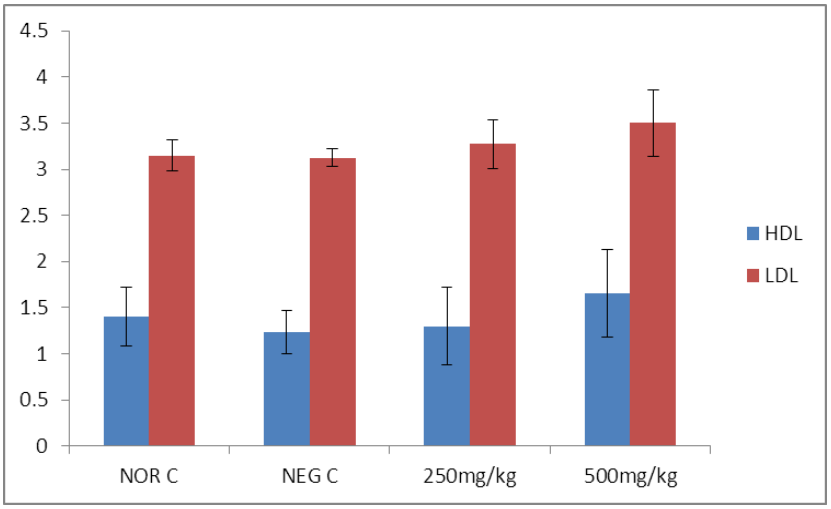

Fig. 4.5 Mean Of Hdl And Ldl

Effect of methanol extract of *G. balsamifarum* (250mg/kg and 500mg/kg) on VIT. C and VIT. E

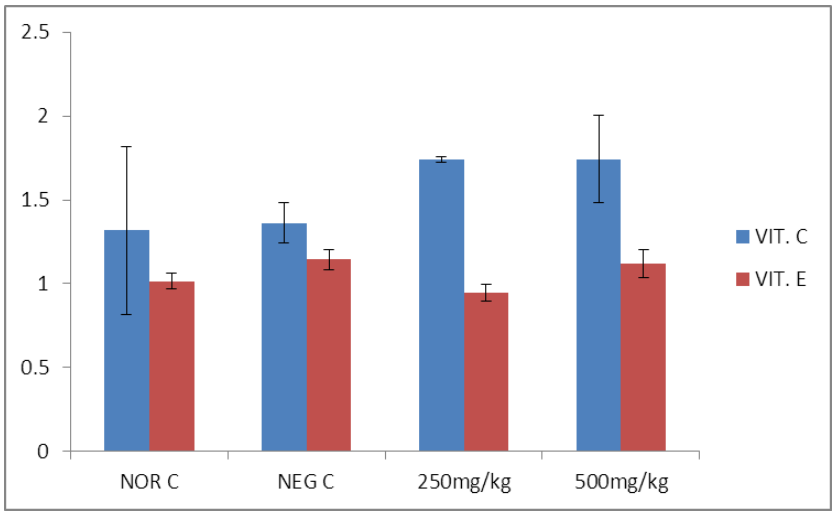

Fig. 4.6 Mean Of Vit. C And Vit. E

There was no significant difference (P>0.05), in serum mean of VIT. C, of control and test group as well as that of serum mean of VIT. E.

STATISTICAL ANALYSIS

The data are expressed as quadruplet mean±standard deviation using bar charts. Comparisons were made between the chloroform/ hexane treated rats (negative control) and *G.*

balsamifarum rats (250mg/kg nd 500mg/kg), as well as normal rats and the two groups treated with different doses of the extract. These were performed using paired t test. The significance was accepted at $p < 0.05$.

DISCUSION

Metabolism of chloroform occurs primarily by cytochrome P 450 dependent pathways with CYP2EI (ethanol inducible) being the primary isozyme responsible (Wang, et al; 1994). The initial reaction results in the formation of a reactive intermediate, which gives off hydrochloric acid to form phosgene, which is then free to react with cellular macromolecules (including GSH, proteins and nucleic acid) or conjugate with water to form carbohydrate and hydrochloric acid. (Ade, et al., 1994). Workers exposed to 14- 400ppm chloroform for 1- 6 months developed toxic hepatitis and other effects including jaundice, nausea and vomiting without fever (phoon, et al., 2001). Toxic hepatitis with hepatomegaly, enhanced serum glutamic pyruvic transaminases (SGPT) and serum glutamic oxaloacetic transaminases (SGOT) activities and hypergammaglobulinemia.

In the assessment of liver damage by chloroform, the determination of enzyme levels such as AST, ALT, are largely used. Membrane damage releases the enzymes into circulation and hence it can be measured in the serum. High levels of AST and ALT indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative to cellular leakage and loss of functional integrity of cell membrane in liver. (Drotman et al., 2008). Administration of chloroform caused a significant elevation of enzymes levels such as AST, ALT, and decrease in total protein when compared to control. However, administration of (250mg/kg and 500mg/kg) of methanol extract of *G. balsamifarum* was not able to restore these enzyme levels to normal. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Silymarin and other standard hepatoprotection drugs decreased CCl4 induced elevated enzyme levels in rats, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. The increase in Lipid peroxidase level in liver induced by chloroform/ hexane suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation

of excessive free radicals. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. (Curtis and Mortiz, 1972), SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. *G. balsamifarum* did not cause any significant increase in hepatic SOD activity and thus could not reduce reactive free radical induced oxidative damage to liver. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance and Greenstein , 1992). Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Even at a higher dose (500 mg/kg), *G. balsamifarum* was not able to increases the level of CAT as produced by silymarin, the standard hepatoprotective drug. Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx) (Prakash et al., 2001). Decreased level of GSH is associated with an enhanced lipid peroxidation in chloroform/ hexane treated rats. Administration of *G. balsamifarum* had no significant increase in the level of glutathione peroxidase. Antioxidants (e.g. Vitamin C. and E.) are relevant for hepatoprotection. They prevent chain reaction intiated by free radicals via termination of the oxidation reaction. Thus they are important component of a hepatoprotective compound. However, treatment of the test group with the leave extract of *G. balsamifarum*, was not able to increase the level of serum mean of Vitamin C. and E.

CONCLUSION

Methanol extract of *G. balsamifarum* has no hepatoprotective activities. This was because of its inability to increase the level of liver enzymes (SOD, GSH, and CAT). The level of serum AST and ALT was also significantly ($P<0.05$) higher in the test group treated with this leave extract as compared to the control group. And also, there is no increase in the levels of serum Vitamin C. and E.

Again, the lipid profile of the rats as judged by the level of LDL, HDL, Total cholesterol and TAG was found to be the same in the whole groups of animal.

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