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Original Research Article

Hepatocurative Properties of *Eucalyptus camaldulensis* Stem Bark Extract

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Abstract: The research investigates the hepatocurative potentials of *Eucalyptus camaldulensis* solvents extract on CCl_4 induced liver damage using albino rats, partial characterization of the most active extract was done using GCMS techniques. Forty-two rats were placed into seven groups of six rats each. Group I and II were normal control and test control respectively. Groups III to VII were induced with liver damage using 120mg/kg of CCl_4 and administered with standard drug, n-hexane extract (E1), chloroform extract (E2), ethyl acetate extract (E) and methanol extract (E4) respectively. 24 hours after CCl_4 administration, a significant ($p<0.05$) increase in mean serum level activities of AST, ALT, ALP with decrease in conjugated bilirubin and albumin concentration was recorded in test control group compared to normal control. This was reversed upon administration chloroform and methanol extract with former been most active. Antioxidant analysis reveals a significant ($p<0.05$) decrease in level of liver tissue antioxidant enzymes (SOD, CAT and GPx) with concomitant increase in MDA of test control compared to normal control rats. After extracts administration, increase in the antioxidants enzymes (SOD, Catalase and GPx) levels was recorded in methanol and standard drug administered groups.

Keywords: Antioxidant; CCl_4; *Eucalyptus camaldulensis;* Hepatocurative and Solvents extracts.

INTRODUCTION

Eucalyptus camaldulensis usually called "red river gum" is a tree of the genus Eucalyptus. It is a plantation species in many parts of the world which is native of Austria where it is widespread especially beside inland water courses (Hill, 2019). *Eucalyptus camaldulensis* typically grows to a height of 20 meters (66 ft) but sometimes to 45 meters (148 ft) and often does not develop a lignotuber. The bark is smooth white or cream-colored with patches of yellow, pink or brown. There are often loose, rough slabs of bark near the base. The juvenile leaves are lance-shaped, 80–180 mm (3.1–7.1 in) long and 13–25 mm (0.51–0.98 in) wide. Adult leaves are lance-shaped to curved, the same dull green or greyish green color on both sides, 50–300 mm (2.0–11.8 in) long and 7–32 mm (0.28–1.26 in) wide on a petiole 8–33 mm (0.31–1.30 in) long. The flower buds are arranged in groups of seven, nine or sometimes eleven, in leaf axils on a peduncle 5–28 mm (0.20–1.10 in) long Flowering mainly occurs in summer with white flower (Brooker and Slee, 2019).

The plant has many medicinal applications especially as a cough remedy and expectorant, it is also used as febrifuge, tonic, astringent, antiseptic and homeostatic properties (Kerharo & Adam, 1974). In Senegal a leaf decoction sweetened with sugar is used to treat stomach-ache and dysmenorrhoea. In Sudan fresh leaves are applied against rheumatism, and the smoke of burnt leaves is inhaled for the treatment of respiratory problems. The gum is used medicinally to treat diarrhea and pharyngeal inflammations, and as an astringent. In Nigeria chewing sticks are obtained from the tree. The smoke of burnt leaves serves to repel insects (Ellis and Pfeiffer, 1990). An infusion of the bark is used as an eye drop for some eyes, ophthalmia and is highly effective in treating diarrhea (Boily and Vanpuyvelde, 1986).

Liver is the largest and heaviest internal organ of the body with a weight of 1 to 1.5 kg and representing 1.5% to 2.5% of the lean body mass. It is a reddish wedge shaped and covered by a network of connective tissue called Glisson capsule. It is located in the right upper quadrant of the abdominal cavity,

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resting just below the diaphragm and lies to the upper right side of the stomach and overlies the gallbladder. (Guyton and Hall, 1996). The liver regulate the flow of nutrients to the rest of the body as it controls the release of absorbed materials in to the systemic circulation and has a central role in carbohydrate, proteins and fat metabolism. The liver store substances, such as minerals, blood and vitamins, which can be released when required (Finlayson *et al*., 1995).

The liver metabolizes endogenous substances such as bilirubin and exogenous substances such as drugs, which are then excreted via kidney or the biliary system. It is the biggest reticulo-endothelial organ in the body and as such it has important immune functions in maintaining body's integrity. The basic functional units of the liver is the liver lobule. The human liver contains 50,000 to 100,000 individual lobules (Guyton and Hall, 1996). It is unique among the body's organs in that it can regenerate back cells that have been destroyed by some short term injury or disease. The liver can lose about 70 percent of its tissue to disease without ceasing to function.

Liver diseases have become one of the serious health problems and a major cause of death all over the world. Nearly 20,000 deaths and 250,000 new cases have been reported every year. Consequently, there is need to provide scientific basis for the use of plants such as *Eucalyptus Camaldulensis* in traditional medicine. It is against this background that this research was initiated in other to know the hepatocurative efficacy and bioactive compounds from *Eucalyptus Camaldulensis* stem bark.

MATERIAL AND METHODS

Equipment and Apparatus

Standard equipment and apparatus were used in the study, some of them are: Spectrophotometer, Centrifuge, Water bath, Refrigerator, weighing balance, Micropipette, measuring cylinder, Beakers, Centrifuge tubes, Pasteur pipettes, Test tubes, Test tube racks, Hand gloves, Razor Blades, masking tapes.

Chemicals and Reagents

Some of the chemicals and reagent used include: Hexane, Chloroform, Ethyl acetate, Ammonia thocyanate Sodium hydroxide, Alanine amino transferase kit, Aspartate amino transferase kit, Alkaline phosphatase kit, Total protein kit, Albumin kit, Total and direct bilirubin kit, Superoxide dismutase kit, catalase kit, glutathione peroxidase kit and Charcoal.

Study Animals

Male and female albino rats weighing between 100 g to 150g were purchased from animal house of Biochemistry Department; Yobe State University Damaturu. The animals were housed in well-ventilated aluminium cages and had access to food and clean water *ad libitum.* The rats were allowed to acclimatize for one week prior to commence of experiment. Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (NIH. 1996; Zimmermann, 1983).

Collection Identification and extraction of the plant material

Eucalyptus camaldulensis stem barks were collected from University farm of Yobe State University, Damaturu. The plant was identified and authenticated by a taxonomist at the Plant Biology Department of the same institution, shade dried and ground to powder. Five hundred grams of the powder was successfully extracted using different solvents of increasing polarity viz-a-viz hexane, chloroform, ethyl acetate and water with the aid of soxhlet extractor. The filtrate thus obtained was concentrated by complete evaporation of the solvent to yield solvents free extracts labelled **E1, E2, E3** and **E4** for Hexane, Chloroform, Ethyl acetate and aqueous extracts respectively. Extract were stored in a clean airtight plastic container at room temperature until use.

Twenty (10) gram of each extract was accurately weighed and dissolve in 100 ml of DMSO or distilled water to prepare a concentration of 100mg/ml. The volume of the extract for administration into the laboratory rats was determined based on the weight of the animals using the following relationship according to Muhammad *et al*., (2015).

$$Volume\ (ml) = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{Concentration\ of\ extract\ (mg/ml)}$$

Induction of liver damage

Liver damage was induced using Carbon tetrachloride (CCl_4) according to the method of Alhassan *et al* (2009) using 120 mg/kg of CCl_4. A stock solution of CCl_4 was prepared in 1:1 by dissolving $25cm^3$ of CCl_4 in $25cm^3$ pure olive oil. It was as administered intraperitoneally. The volume of CCl_4 administered was determined by the weight of the rat according to the following relationship:

$$Volume\ (ml) = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{Concentration\ of\ extract\ (mg/ml)}$$

Evaluation of the Effect of *Eucalyptus camaldulensis* stem bark extract on the CCl_4 induced Liver Damage

Forty-two (42) experimental rats were placed into seven groups of six rats each. Groups II to VII were induced with liver damage using 120mg/kg of CCl_4.

Group I: Normal Control.

Group II: Test Control; induced with liver damage, no extract given

Group III: Administered with standard

Group IV: Administered with 150mg/kg body weight of n-hexane extract (E1)
Group V: Administered with 150mg/kg body weight of chloroform extract (E2)
Group VI: Administered with 150mg/kg body weight of ethyl acetate extract (E)
Group VII: Administered with 150mg/kg body weight of methanol extract (E4)

The rats in groups I and II were sacrificed 24 hours after inducement with CCl_4 and blood samples were collected to confirm inducement of liver damage using 120mg/kg of CCl_4. The rats each from Group III, IV, V, VI and VII were sacrificed after two weeks oral administration of stem bark extracts of *Eucalyptus camaldulensis* and blood samples were collected for determination of liver functions indices i.e: Aspartate aminotransferase (AST) and Alanine Aminotransferases Assay (ALT) were assayed using Reitman and Frankel (1957) method, Alkaline Phosphatase (ALP) activity assayed using the method developed by Roy (1970), Bilirubin by method of Malloy and Evolyn (1937), and Total protein was determined using Biuret method by Tietz (1995). Anti-oxidants indices were assayed using sandwich-ELISA kits

Statistical Analysis

Results were expressed as mean ± standard deviation and analysed using ANOVA, with p value <0.05 considered significant, a component of GraphPad Instat3 Software version 3.05 by GraphPadInc was used for the analysis

RESULTS

Figure 1 presents the effect of administration solvents extract of *Eucalyptus camaldulensis* stem bark extract on CCL_4 induced liver damage rats. A significant increase in mean serum level activities of AST, ALT, ALP with decrease in conjugated bilirubin and albumin was observed in test control rats compared to normal control. Upon administration of the extract, significant decrease in serum concentration of AST, ALT, ALP, was recorded in methanol extract administered group compared to the test control but still higher than the normal control group and standard drug administered group.

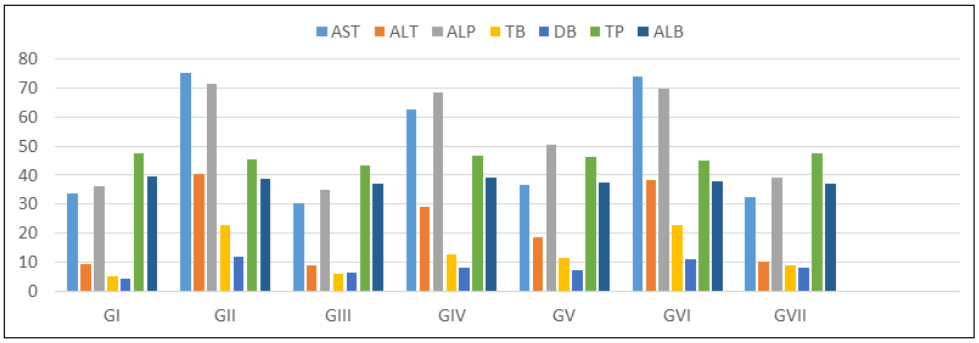

Fig 1: Serum Liver Function Indices of CCl_4 Induced Liver Damage Rats after two weeks of Oral Administration of solvents Extract of Eucalyptus camaldulensis Stem Bark

The effect on extracts administration on antioxidant indices (SOD, CAT, GPx and MDA) was presented in Table 1. A significant ($p<0.05$) decrease in level of SOD, Catalase and GPx with concomitant increase in MDA was observed in liver tissue of test control compared to normal control rats. After extracts administration, increase in the antioxidants enzymes (SOD, Catalase and GPx) levels was recorded in methanol and standard drug administered groups.

Table 1: Liver Tissue anti-oxidant levels of hepatotoxic rats administered with solvents Fractions of *Eucalyptus camaldulensis*

	SOD (µ/mgprotein)	CAT (µ/mgprotein)	GPx (µ/mgprotein)	MDA (nmol/g protein)
Group I	75.26±3.12^{a}	9.12±0.23^{a}	7.24±1.23^{a}	451.12±9.23*
Group II	25.45±1.12^{a,b,c}	2.23±0.11^{a,b,c,}	3.11±0.56^{a,b,c}	613.03±7.41^{*,f,g}
Group III	75.11±5.17^{b}	10.12±0.67^{b}	6.23±1.87^{b}	423.11±7.11^{f}
Group IV	28.87±4.27	3.42±0.82	4.87±1.45	587.62±8.21
Group V	25.23±4.11	3.11±0.34	4.71±1.23	501.03±5.48^{f}
Group VI	25.87±2.27	2.89±0.75	3.23±0.47	579.62±10.45
Group VII	65.23±4.11^{c}	8.11±0.14^{c}	6.56±1.36^{c}	470.50±18.10^{g}

Results are expressed as mean SD, n = 5. Values bearing similar superscript are significantly different at $p<0.05$ compared with each other.

Figure 1 shows the GCMS chromatogram of most active methanol fraction, analysis of the spectra shows the presence of 2,4-bis(1,1-dimethylethyl) Phenol; Phthalic acid (5-methylhex-2-yl heptadecyl ester); Stearic acid 3-(octadecyloxy)propyl ester; and α-amyrin.

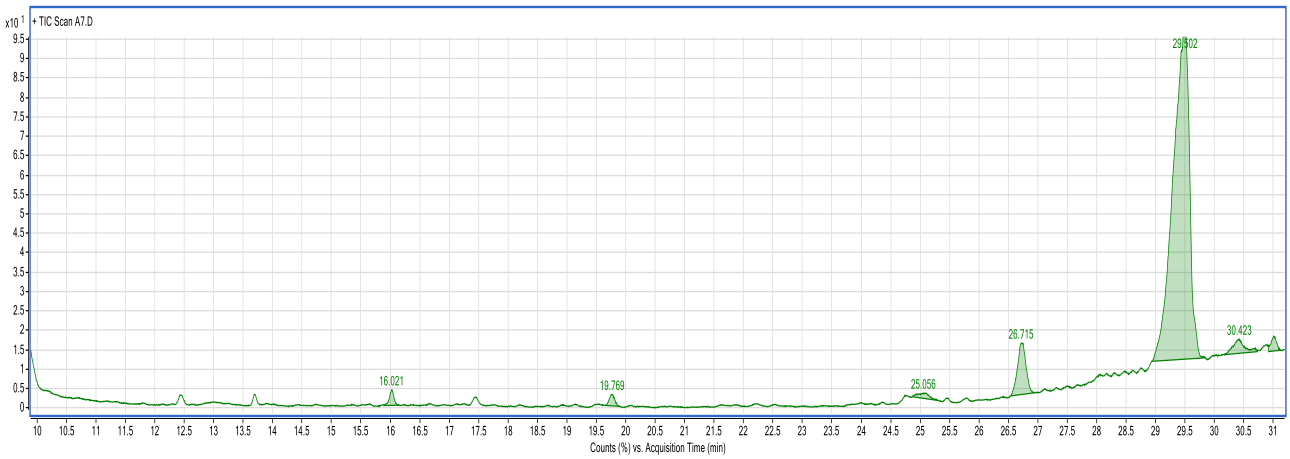

Figure 2: GC-MS chromatogram of methanol extract

DISCUSSION

Liver damage is a serious disease characterized by disturbances in the normal functions of the liver. It is clinically diagnosed by determining the serum concentration of liver enzymes (ALT, AST and ALP), bilirubin, total protein and albumin (Willett *et al.*, 2004). ALT and AST are non-plasma specific enzymes involved in transamination of aspartic acid and alanine respectively, the enzymes were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis (Sule, 2004). ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline pH. These enzymes were reported to reach higher than normal level in the blood in events of impaired liver function (Price and Stevens, 2003). Thus, they are used as serum markers of hepatic damage. Liver is the sole organ that produces albumin, despite the long life span of albumin, serum level of albumin is a good index of chronic liver disease (Alhassan *et al.*, 2009).

A significant increase in serum activities of ALT, AST and ALP ($p<0.05$) in CCl_4 induced control group compared to the normal control shows an indication of successful induction of liver damage using 120mg of CCl_4. The enzymes were reported to be higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis (Sule, 2004; Abubakar *et al*., 2009). Non-significant increase in albumin and conjugated bilirubin in hepatotoxic control rats might be due to onset the liver damage which trigger the released of newly synthesized albumin and conjugated bilirubin into the blood stream as a result of liver damage from CCl_4 administration (Muhammad *et al*., 2015).

Administration of solvent extracts from sequential partitioning led to a significant decrease ($p<0.05$) in the mean serum levels of liver enzymes, with a slight increase in total protein in group administered with ethyl acetate extract. This could be due to the continued release of newly synthesized protein/albumin by the damaged hepatocytes. This finding is in accordance with the findings of Muhammad *et al.*, (2015) who reported a lower activities of transaminases in the liver of diabetes induced rats administered aqueous extract of *K. senegalensis*. Ali *et al.*, (2011) also reported a significant decrease in the activity of liver enzymes in albino rats induced with liver damage after been treated with aqueous extract of *K. senegalensis* stem bark at doses of 250 mg/kg and 500 mg/kg body weight for 5 days.

The observed decrease in MDA and increase in level of SOD, GSHpx and CAT in hepatotoxic rats after administration with ethyl acetate extract shows that the extract possess some anti-oxidant principles and could scavenge excess free radicals generated by CCl_4. The toxicity of carbon tetrachloride is achieved through metabolic activation by cytochrome P-450 enzymes, catalyzing the addition of an electron, which then allows homolytic cleavage and the loss of a chloride ion and the formation of the trichloromethyl radical that attack lipids and protein of the endoplasmic reticulum of the liver (Dai and Cederbaum, 1995).

The exact antioxidant mechanism(s) by which the extract cures liver damage is yet to be established. However, it can be postulated that it may act through its antioxidant potentials, since amyrin was reported have antioxidant activity, it may help in restoring the damaged liver tissue (Zhou *et al*., 2016). It could act at molecular level by inducing the expression of messenger RNA for these enzymes or by acting as a natural antioxidant (Nour-Eldine *et al*., 2016)

CONCLUSION

The research demonstrated that methanol extract from of *Eucalyptus camaldulensis* stem bark possess some hepatocurative activity against carbon tetrachloride induced liver damage. The activity might be modulated through antioxidant property as amyrins were reported to possess strong antioxidant property.

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