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Full Length Research Paper

Effects of aqueous extract of *Eucalyptus globulus* on lipid peroxidation and selected enzymes of rat liver

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The effects of repeated administration of varying concentrations of the aqueous extract of *Eucalyptus globulus* leaves on some biochemical parameters of rat liver were studied. Twenty four albino rats (*Rattus norvegicus*) with a mean weight of 148.35 g were divided into four groups (A - D) of 6 rats each. Rats in group A served as control and were administered 1 ml of distilled water while those of the experimental groups (B - D) were administered varying doses of the aqueous extract (80 mg/kg, 100 mg/kg and 120 mg/kg body weight respectively) for seven days. The activities of acid phosphatase (ACP), alkaline phosphatase (ALP), superoxide dismutase (SOD) and the level of malondialdehyde (MDA) were determined in the liver and serum. ACP and ALP activities were significantly increased ($P < 0.05$) in the liver with no significant difference ($P > 0.05$) in their serum activities while the activity of SOD was significantly increased ($P < 0.05$) in the liver at concentrations of 100 and 120 mg/kg body weight (b.w) of extract. There existed a significant increase ($P < 0.05$) in the level of MDA in the liver of all the treatment groups and at 120 mg/kg b.w of extract in the serum. Over all, the results indicate that the aqueous extract of *E. globulus* leaves (despite its efficacy) may have deleterious effects on liver membrane structure and functional integrity.

Key words: Extract, *Eucalyptus globulus*, lipid peroxidation, enzymes, rat liver.

INTRODUCTION

Plants have been considered as sources of medicinal agents for the treatment of various diseases. One of such diseases is *Diabetes mellitus* which is a disorder of carbohydrate metabolism in which glucose molecules in the body are not oxidized due to a defect in either the production or function of insulin (National Diabetes Data Group, 1995). Although present therapy for *D. mellitus* relies on an arsenal of drugs developed since the introduction of insulin prior to 1922, diabetes therapy also revolved around dietary measures including the use of traditional antihyperglycaemic plants such as *Eucalyptus globulus* (Swanston-Flatt et al., 1990).

E. globulus is an evergreen tree, one of the most widely cultivated trees native to Australia and Tasmania bearing pendent leaves. The medicinal parts of the plant are the leaves from which tea is made (Chiej, 1984). *E. globulus* was reported to have antihyperglycaemic property which does not directly affect blood glucose concentration in normal rats (Bever and Zalmel, 1979). Therefore, *E. globulus* is likely to act by modulating insulin secretion and/ or insulin action (Swanston -Flatt et al., 1990). Studies us-ing clonal pancreatic cells showed that aqueous extract of *Eucalyptus* exert a dose-dependent stimulatory effect on insulin secretion. The quantity of essential oil in the leaves of *E. globulus* ranges from less than 1.5 to over 3.5%. On the average between 70 and 95% of the oil is
1.8 cineole (eucalyptol) (Chalchat et al., 1995). Other important compounds found in the leaves, buds, branches and bark of *E. globulus* include taxifolin and eriodictyol (antioxidants), rutin, tannins, gamma-terpinene and terpineol. Activities contributed by these compounds include: anaesthetic, antibronchitic, anticatarrh, antiseptic, CNS-stimulant, expectorant and sedative among others (Chalchat et al., 1995; Lee et al., 1998).

While *Eucalyptus spp*. contain high levels of phenolics and terpenoids which can be toxic, animals such as the koala which eat *Eucalyptus* have developed methods for detoxifying these compounds in the liver (Whitman and Ghazizadeh, 1994). In addition, they have bacteria that degrade tannin-protein complexes (Whitman and Ghazi-

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Table 1. Effect of daily administration of aqueous extract of *E. globulus* leaves on rat liver and serum alkaline phosphatase activity.

Groupings	Liver (µmol/min/mg protein)	Serum (µmol/min/L)
Group A (control)	288.50 ± 8.44[a]	52.66 ± 4.70[a]
Group B	378.50 ± 17.63[b]	52.66 ± 5.35[a]
Group C	406.66 ± 10.56[b]	53.167 ± 4.15[a]
Group D	650.66 ± 44.85[c]	146.00 ± 8.31[b]

Values are mean ± S.E.M for 6 rats, column values with different superscripts are significantly different ($P < 0.05$).

zadeh, 1994). Most animals however do not posses these attributes. This therefore informed the resolve to embark on this study to investigate the effects of re-peated administration of aqueous extract of *E. globulus* on lipid peroxidation (since it is reported to contain some antioxidants) and some selected enzymes of rat liver. Reports have shown that the changes in the cellular activities of such enzymes can be used as indicators of cell damage in tissues and organs (Akanji et al*.,* 1993; Akanji and Yakubu, 2000). This will aid to provide useful information on the safety/toxicity of the aqueous extract of the leaves of *E. globulus*.

MATERIALS AND METHODS

Twenty-four albino rats (*R. novergicus*) with an average weight of 148.35 g used for this study were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were fed with normal animal feed and given water *ad libitum* throughout the experimental period. Animal husbandry and experimentation were consistent with the guiding principles in the use of animals in toxicology. All the reagents used for this study were of analytical grade and were prepared in all glass-distilled water.

Preparation of plant extract

E. globulus leaves were collected from few *E. globulus* trees behind the Faculty of Science office, University of Ilorin, Ilorin, Nigeria. The leaves were air dried and milled into powder. 200 g of the powder were then percolated in 500 ml of distilled water for two weeks. The percolated mixture was filtered and evaporated on a water bath according to the method of Majekodunmi et al*.* (1996). A homogenous aqueous suspension of the extract was made before being administered to the experimental animals. *E. globulus* aqueous extract has been reported to be rich in steroids, flavonoids and tannins (Egwaikhide et al*.,* 2008).

Administration of extract

The experimental animals were randomly divided into four groups (of six rats each) which were designated A (control), B, C and D. The rats in group A were administered 1 ml distilled water orally while rats in groups B - D were administered the aqueous extract in varying doses daily (80 mg/kg, 100 mg/kg and 120 mg/kg body weights respectively) for seven days.

Sample preparation

At the end of the experimental period, venous blood was collected from the experimental animals according to the method of Narayanan et al. (1984). The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min (Ogbu and Okechuwu, 2001) and collected by pipetting. The animals were thereafter quickly dissected and the liver removed. The liver was suspended in ice-cold 0.25 M sucrose solution and homogenized. The resulting homogenates were diluted appropriately with 0.25M ice-cold sucrose solution to give a final volume of five folds the initial tissue weight. The homogenates were kept frozen overnight to ensure maximum release of the enzymes (Ngaha et al*.,* 1989).

Assay of Biochemical Parameters

Alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC.3.1.3.2) activities were determined by the method of Ahamed and King (1959). The method employed in the assay of superoxide dismutase (SOD) activity was that of Winterbourne et al. (1975) and is based on the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium by superoxide. Determination of malondialdehyde level was obtained by the method of Varshney and Kale (1990) . Protein content of the homogenate and serum were determined using the Biuret method (Gornal et al., 1949).

Statistical analysis

All data are presented as mean ± standard deviation. Statistical analyses were carried out using Duncan Multiple Range test (Montgomery, 1976). In all cases, probability level of 95% was taken as significant.

RESULTS

Effects of aqueous extract of *E. globulus* on ALP activeties of rat liver and serum are represented in Table 1. The data revealed that the repeated administration of the aqueous extract produced a significant increase ($P < 0.05$) in ALP activity in the liver in a dose-dependent manner, while the enzyme activity in the serum also increased significantly ($P < 0.05$) at a dose of 120 mg/kg body weight of the extract.

Effects of daily administration of aqueous extract of *E. globulus* on the activities of liver and serum ACP of both the control and experimental animals are presented in Table 2. Compared with the control, ACP activity was significantly increased ($P < 0.05$) in rat liver at doses of 80 mg/kg and 100 mg/kg while there was a significant de-

Table 2. Effect of daily administration of aqueous extract of *E. globulus* on the ACP activity of rat liver and serum.

Groupings	Liver (µmol/min/mg protein)	Serum (µmol/min/L)
Group A(control)	1706.33 ± 50.72^{a}	38.66 ± 2.18^{a}
Group B	2042.50 ± 28.76^{b}	40.83 ± 1.19^{a}
Group C	2094.66 ± 16.65^{b}	38.35 ± 2.15^{a}
Group D	916.16 ± 37.35^{c}	64.83 ± 1.70^{b}

Values are mean ± S.E.M for 6 rats, column values with different superscripts are significantly different (P < 0.05).

Table 3. Effect of daily administration of aqueous extract of *E. globulus* on the SOD activities of rat liver and serum.

Groupings	Liver(µmol/min/mg protein)	Serum(µmol/min/L)
Group A (control)	2057.85 ± 194.31^{a}	168.00 ± 2.86^{a}
Group B	2001.16 ± 12.96^{a}	170.66 ± .05^{a}
Group C	2246.16 ± 107.46^{b}	170.83 ± 2.70^{a}
Group D	2288.16 ± 37.54^{b}	239.83 ± 2.91^{b}

Values are mean ± S.E.M for 6 rats, column values with different superscripts are significantly different (P < 0.05).

Table 4. Effect of daily administration of aqueous extract of *E. globulus* on rat liver and serum malondialdehyde concentration.

Groupings	Liver(µmol/g)	Serum(µmol/L)
Group A(Control)	30.58±0.58^{a}	1.85±0.03^{a}
Group B	34.33±3.02^{a}	1.83±0.07^{a}
Group C	60.16±2.57^{b}	1.93±0.77^{a}
Group D	77.83±2.35^{b}	3.76±0.16^{b}

Values are mean ± S.E.M for 6 rats, column values with different superscripts are significantly different (p<0.05).

crease (P < 0.05) at a dose of 120 mg/kg body weight. In the serum however, there was significant increase (P <0.05) in ACP activity only at a concentration of 120 mg/kg body weight of extract.

Effects of daily administration of aqueous extract of *E. globulus* on rat liver and serum SOD activities are represented in Table 3. SOD activity was significantly elevated (P < 0.05) at doses of 100 and 120 mg/kg body weight when compared with the control. The serum SOD activity was also increased significantly (P < 0.05) at a dose of 120 mg/kg body weight when compared with control. The effects of daily administration of aqueous extract of *E. globulus* on the concentration of MDA in rat liver and serum is presented in Table 4. The data revealed a significant increase (P < 0.05) in the concentration of MDA (at doses of 100 mg/kg and 120 mg/kg of the extract) in the liver when compared with control. In the serum, there was a significant increase (P < 0.05) only at a dose of 120 mg/kg body weight of the extract when compared with control.

DISCUSSION

Aqueous extract of *E. globulus* has been used in the management of diabetes (Swanston-Flatt et al., 1990) with a report of high efficacy. However, there is paucity of information on the effects of this herbal regimen on the level of some enzymes and lipid peroxidation in the cellular systems of human recipients.

ALP has been reported to be the marker enzyme for plasma membrane (Wright and Plummer, 1974) and is required in certain amounts for proper functioning of organs (Brain and Kay, 1927). However, the administration of aqueous extract of *E. globulus* leaves led to a signifycant increase (P < 0.05) in ALP activity in a dose-dependent manner when compared with control. This may be due to enzyme induction by the extract (Yakubu et al., 2001) . The increase in the activity of ALP may be attributed to the effects of some divalent ions such as Mg^{2+} and Zn^{2+} reported to be present in the leaves of *Eucalyptus* (Pinto et al., 2007). These ions are known and establish-

ed activators of alkaline phosphatase (PetitClerc and Fetau, 1977) . The increase obtained may constitute a threat to the structural and functional integrity of cells which are dependent on a variety of phosphate esters for their vital processes (Butter-worth and Moss, 1966).

The significant increase in the activity of ACP of the liver of all the treatment groups when compared with control except at a high dose of 120 mg/kg body weight, may be due to induced enzyme synthesis triggered by certain component(s) of the aqueous extract possibly the metal ions (Pinto et al., 2007) while the reduction in activity at high dose may result from leakage of the enzyme due to membrane damage. This is accounted for by a corresponding significant increase in activity, at the same dose, in the serum. The reduction in the enzyme activity may also be ascribed to inhibition from flavonoid and tannin components of the extract (Emeh et al., 2000). Elevated acid phosphatase activities could result in indiscriminate hydrolysis of phosphate esters which are potential energy sources for the cell (Buttersworth and Moss, 1966). Also elevation of serum acid phosphatase may arise as a result of increased erythrocyte and platelets destruction (Ryman, 1978).

Superoxide dismutase (SOD) is an antioxidant enzyme which mops up free-radicals. It protects oxygen- metabolizing cells against harmful effects of free-radicals (Petkau et al*.,* 1975). The significant increase in SOD activity obtained at a dose of 100 mg/kg body weight may indicate the free radical generating potential of the extract which consequently triggered increased synthesis of SOD to mop the free radicals produced. Also the increased SOD activity may be due to the effects of some metal ions like zinc, copper and manganese. *Eucalyptus* extract has been reported to contain high levels of mag-nesium, copper, zinc, iron, manganese and phosphorus (Pinto et al., 2007). Zinc, copper or manganese plays sig-nificant roles in SOD activity as cofactors for SOD iso-enzymes (Kunikowska and Jenner, 2002).

Thiobarbituric acid reacting substance (TBARS) or malondialdehyde is a major product of lipid peroxidation, thus an index of measuring the degree of lipid peroxidetion. The significant increase obtained in the level of MDA in a dose dependent manner suggests a stimulation of membrane lipid peroxidation by the extract while the significant increase in MDA level of the serum obtained at a high dose of extract (120 mg/kg body weight) may imply leakage of end product of lipid peroxidation (MDA) into the serum. This may be suggestive of alteration in the cellular redox status of the animals as a result of increased lipid peroxidation. The level of antioxidant enzyme (in this case, superoxide dismutase) may also not be sufficient to cope with the level of oxidant influx caused by the extract. These elevations in SOD activity and MDA formation suggest that the extract of *E. globulus* is capable of inducing oxidative stress which may overload the endogenous detoxification mechanism of the cells. It has been reported that membrane lipid peroxidation results in the loss of polyunsaturated fatty acids, decreased membrane fluidity and severe structural changes leading to loss of enzymes and in other cases receptor activity (Van Ginkel and Sevanian, 1994) . Direct free radical damage to membrane proteins may occur as a result of lipid peroxidation leading to their activation (Dean et al., 1993). Thus, the loss of enzyme activities from tissues and sometimes the elevated activities of tissue enzymes as observed with the enzymes studied may be attributed to membrane lipid peroxidation and direct free radical damage to membrane proteins.

The result generated from this study is suggestive that the aqueous extract of *E. globulus,* despite its antidiabetic efficacy may have deleterious effect when administered repetitively.

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