Antidiabetic and Antioxidant Activity of Methanolic Leaf Extracts of *Costus Pictus* D. Don in Alloxan Induced Diabetic Rats

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Summary

The methanol extract of *Costus pictus* (*C. pictus*) D. Don (family - Zingiberaceae) leaf was investigated for its antidiabetic and antioxidant effects in Wistar Albino rats. Diabetes was induced in rats by administration of single dose of alloxan monohydrate (120 mg/kg, i.p.). The methanol extract of *C. pictus* (MECP) at doses of 120 and 180 mg/kg (p.o.) was administered as a single dose per day. Tolbutamide served as positive control at a dose of 100 mg/kg. The effect of MECP on blood glucose, lipid peroxidation (LPO), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and non enzymatic antioxidants (Vitamin A, E, C and reduced glutathione) were investigated in liver and kidney tissues. The MECP produced significant (p<0.001) reduction in blood glucose and lipid peroxidation (LPO). The extract also caused significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, vitamin A, vitamin C, vitamin E and reduced glutathione. From the above results it is concluded that MECP possesses significant antidiabetic and antioxidant effects in alloxan induced diabetic rats.

Key words: *Costus pictus*, antidiabetic, antioxidant, alloxan.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1]. The presence of DM confers increased risk of many devastating complications such as cardiovascular disease (CVD) [2], coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy, amputations and blindness [3]. Diabetes is associated with significant oxidative stress and oxidative damage to tissues may be a contributing factor in several diabetes complications [4]. Reactive oxygen species are an important part of the defense mechanisms against infection, but excessive generation of free radicals in unsaturated fatty acids has been implicated in pathogenesis of vascular diseases [5] and Gutteridge 1984). Diabetic patients have an increased incidence of vascular disease and it has been shown that free radical activity is elevated during diabetes. Normal levels of the antioxidants defense mechanism are not sufficient for the eradication of free radical induced injury. Therefore, the administration of antioxidants from a natural origin has a promising role to play. Several antioxidants of plant materials have been experimentally proven and widely used as more effective agents against oxidative stress [6].

The medicinal plant *Costus pictus* (*C. pictus*) is a very popular and fast spreading ginger belonging to the family of Zingiberaceae and it is also a newly introduced an ornamental climbing plant that has antidiabetic properties [7] due to the presence of an active constituent such as β-amyrin [8].The present study was conducted to investigate the antidiabetic and antioxidant effects of methanol leaf extract of *Costus pictus* (MECP) in alloxan induced diabetic rats.

Materials and methods

Animals

Male albino rats of Wistar strain (150g-200g) were used for this study. The animals were maintained in an air-conditioned room controlled for temperature and humidity, where they were fed a standard rat pellets, supplied by M/s Hindustan Lever Limited, Bangalore, (India) and filtered water *ad libitum*. Animals described as fasted were deprived of food for at least 16 hours but allowed free access to water. Ethical clearance for the handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and the care of laboratory animals was taken as per the guidance of committee for the purpose of control and supervision of experiments of animals (CPCSEA), Ministry of Forests and Environment, Government of India (CPCSEA No: 688/02/C – CPCSEA).

Plant material

C. pictus was collected from different areas of Kottayam District, Kerala, identified and confirmed by a taxonomist from the Botanical survey of India, Coimbatore, Tamilnadu. A voucher specimen (08903-Jun 2006) is deposited in the herbarium of Tamilnadu Agriculture University, Coimbatore, Tamilnadu, India.

Preparation of plant extract

The dried powdered form of leaves of *C. pictus* was taken and subjected to successive solvent extraction. The extraction was carried out with the following solvents in increasing order of polarity: petroleum ether, chloroform, methanol followed by water [9,10]. The yield of methanol extract was 28.5% w/v, which was done by prescribed monograph specified in I.P, 1996.

Chemicals

All chemicals and solvents used were of analytical grade. SD Fine Chem Ltd., (Mumbai, India), Ranbaxy Laboratories, (New Delhi, India) and Himedia Chemicals, (Mumbai, India).

Induction of Diabetes

Animals were allowed to fast for 24 hrs and were injected with freshly prepared alloxan monohydrate (120 mg/kg, i.p.) in sterile normal saline [11,12]. The animals were maintained in the diabetic state over a period of 21 days. Serum glucose was measured by reported method [13]. Rats showing fasting serum glucose levels (>250 mg/dl) were selected for the study.

Experimental grouping of animals

The experimental rats were divided in to five groups of six animals each group.
Group I: Animals, served as normal healthy controls, which received 0.5% w/v carboxymethyl cellulose (1 ml/kg, p.o.).
Group II: Untreated diabetic control animals treated with 0.5% w/v carboxymethylcellulose (1 ml/kg, p.o.).
Group III: Diabetic rats given tolbutamide (100 mg/kg, i.p.) single dose per day, served as positive control [14].
Group IV: Diabetic rats given MECP (120 mg/kg, p.o.) at a single dose per day in 0.5% w/v Carboxymethylcellulose sodium. The dose was selected based on the earlier reported toxicity studies on *C.pictus* [15].
Group V: Diabetic rats given MECP (180 mg/kg, p.o.) at a single dose per day in 0.5% w/v carboxymethylcellulose sodium [15].
The treatment period was 21 days.

Collection of liver, kidney and blood

Blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500rpm. The rats were sacrificed by cervical dislocation and organs were excised immediately and thoroughly washed with ice-cold physiological saline. The serum collected was used for biochemical estimations.

Estimation of biochemical parameters

Serum glucose was determined by standard procedure in an auto analyzer using Ecoline kits (E. Merk, Mumbai, India). The content of lipid peroxidation (LPO) [16], enzymatic antioxidants such as superoxide dismutase (SOD) [17], catalase (CAT) [18], glutathione peroxidase (GPx) [19], glutathione reductase (GR) [19] and non enzymatic antioxidants ascorbic acid (Vit-C) [20], β- carotene (Vit-A) [21], α-tocopherol (Vit-E) [22], glutathione [23] were estimated in liver and kidney of experimental groups. Tissue protein content was estimated by the method of Lowry et al [24].

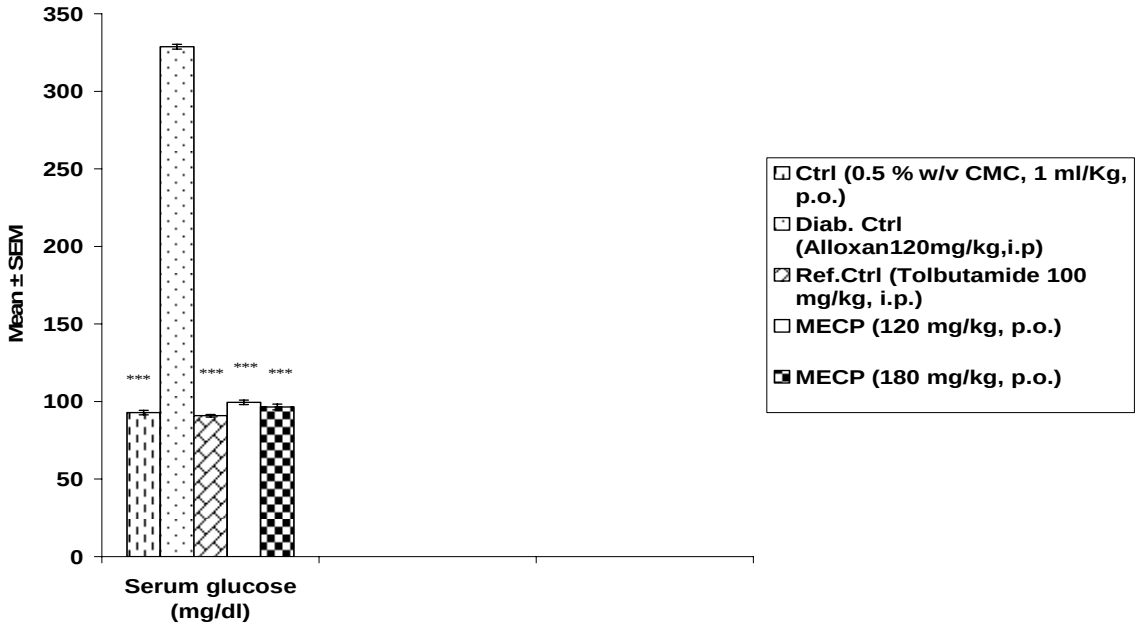
Statistical analysis

Data were expressed as mean ± SEM for 6 rats in each group. The biochemical parameters were analyzed statistically using one-way ANOVA, followed by Dunnet's Multiple Comparison test. Statistical analysis was carried out with GraphPad InStat Version 3 (GraphPad Software Inc., Camino Real, San Digeo, USA).

Results

Fig. 1 shows the level of serum glucose in normal and diabetic rats. There was a significant increase in serum glucose level (p<0.001) in diabetic rats when compared with normal rats. Administration of MECP (120 mg/kg and 180 mg/kg, p.o.) and tolbutamide at dose of 100 mg/kg significantly (p<0.001) decreased blood glucose level in diabetic rats.

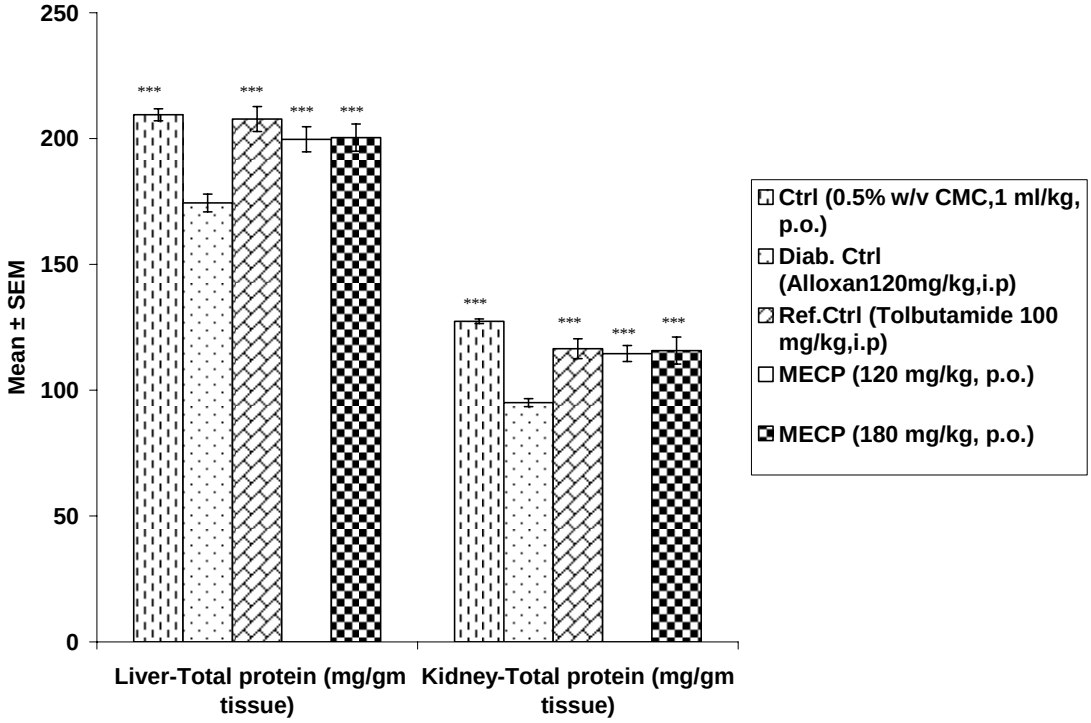
Fig. 1: Effect of MECP on serum glucose of control and experimental rats.



Values are in mean ± SEM (n=6); ***P<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.

The level of total protein (liver and kidney) decreased significantly (p<0.001) in diabetic rats and the level restored to normal significantly (p<0.001) after the treatment with MECP at doses of 120 and 180 mg/kg and tolbutamide at 100 mg/kg (Fig. 2).

Fig. 2: Effect of MECP on total protein in liver and kidney tissue of control and experimental rats.

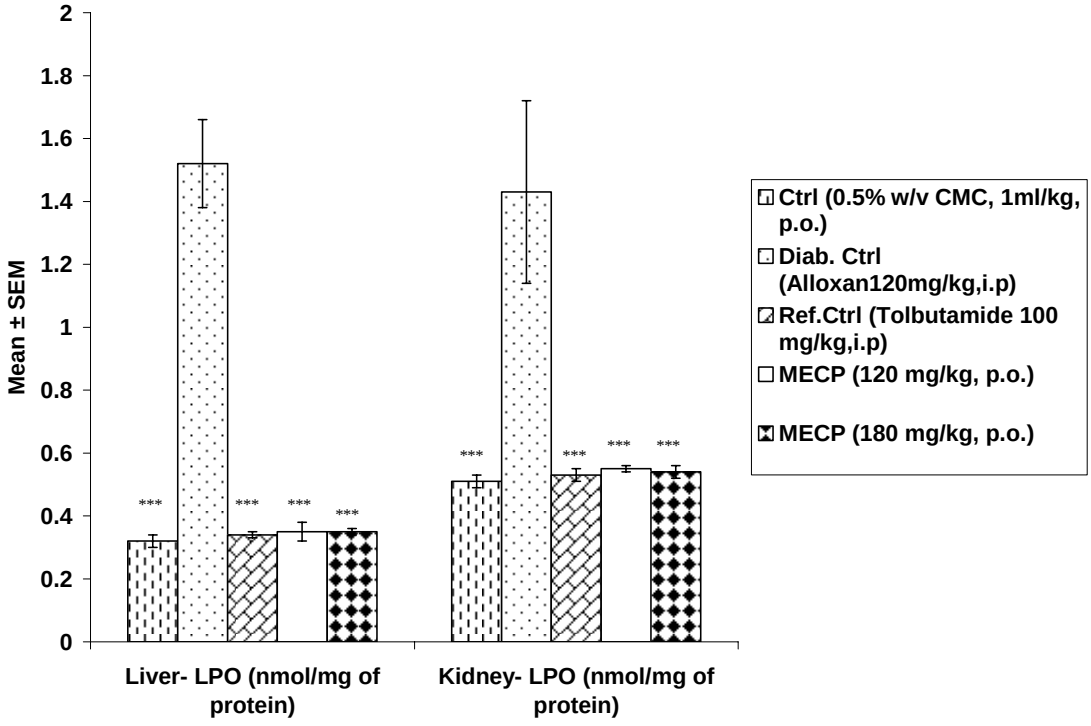


Values are in mean ± SEM (n=6);*P<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.**

Determination of antioxidant activity

The concentration of TBARS in tissues of experimental diabetic rats was shown in Fig. 3. There was a significant elevation of TBARS in alloxan induced diabetic rats when compared to normal rats. It was found that significant (p<0.001) decrease in the level of TBARS in liver and kidney of MECP at above mentioned doses. The decreased level of TBA reactive substances shows that *C.pictus* leaf extract can improve the pathological condition of lipid peroxidation in diabetes. The activity of superoxide dismutase (SOD) and catalase (CAT) in experimental animal tissue have been summarized in Fig. 4 and 5. There was a significant reduction (p<0.001) in the activity of SOD and CAT in liver and kidney during diabetes. Administration of MECP and tolbutamide at the above mentioned doses, increased (p<0.001) the activity of SOD and CAT in liver and kidney to near normal.

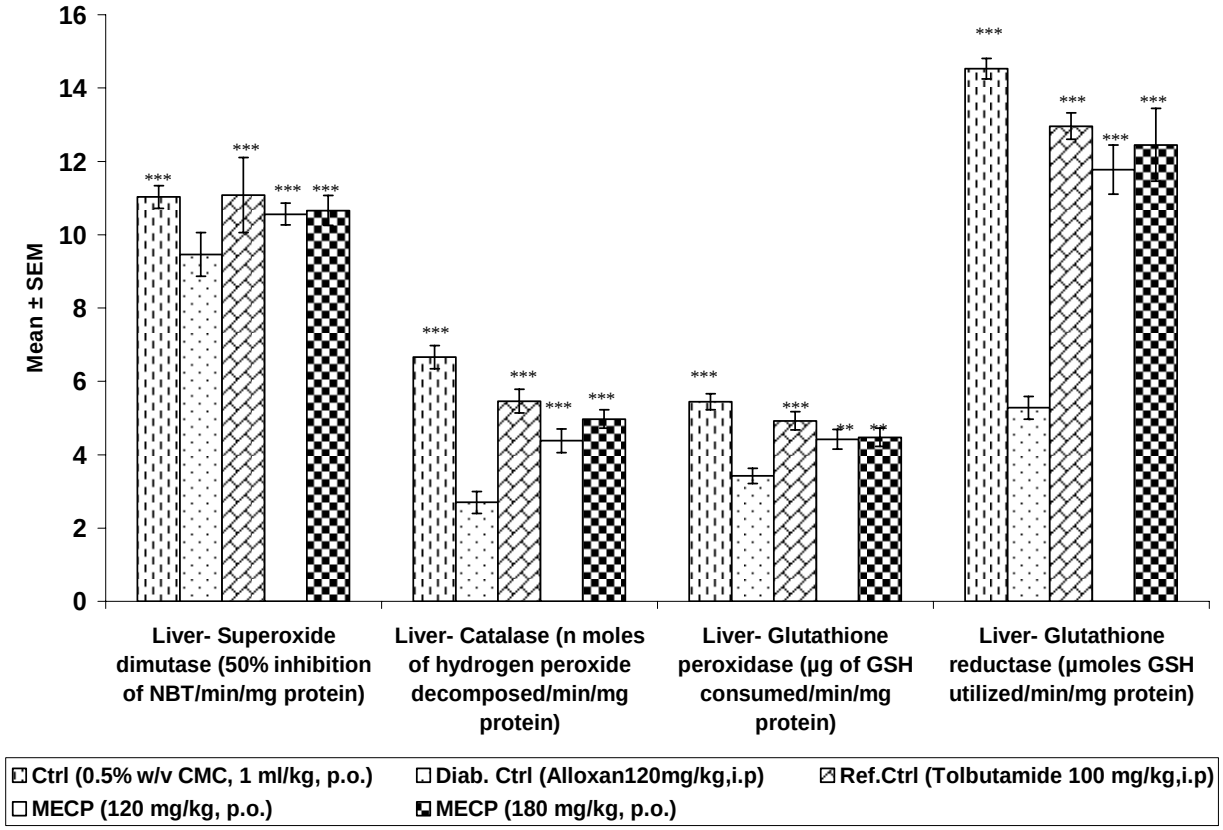
Fig. 3: Effect of MECP on lipid peroxidation in liver and kidney tissue of control and experimental rats.



Values are in mean ± SEM (n=6);*P<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.**

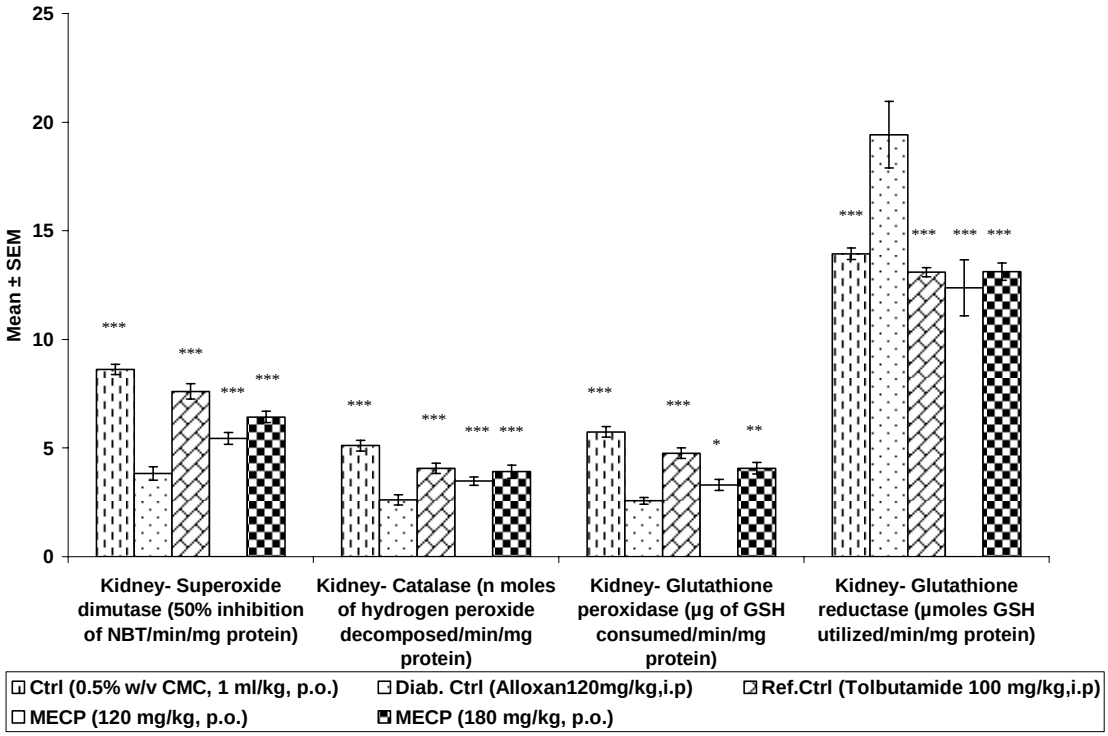
Fig. 4 and 5 showed the level of GPx and GR in liver and kidney of control and experimental rats. The level of enzymatic antioxidants such as glutathione peroxidase (GPx) and glutathione reductase (GR) in liver and kidney of diabetic rats was significantly (p<0.001) reduced when compared with control rats. Administration of MECP and tolbutamide at previously mentioned doses to diabetic rats for 21 days significantly (p<0.01 and p<0.001) respectively reversed the volume of glutathione peroxidase in the tissue to normal. Administration of MECP and tolbutamide at above mentioned doses to diabetic rats significantly (p<0.001) reversed the value of glutathione reductase in the tissue to normal.

Fig. 4: Effect of MECP on superoxide dimutase, catalase, glutathione peroxidase, glutathione reductase in liver tissue of control and experimental rats.



Values are in mean ± SEM (n=6); ****P*<0.05 Vs Diabetic control rats; *****P*<0.01 Vs Diabetic control rats;****P*<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.**

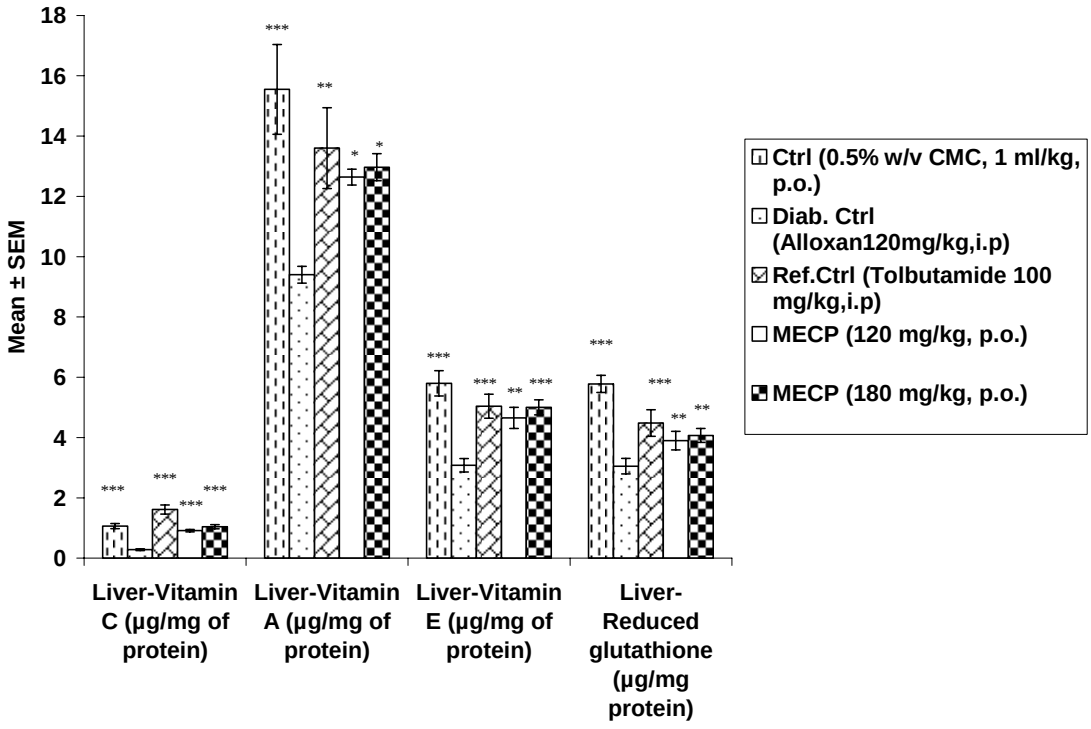
Fig. 5: Effect of MECP on superoxide dimutase, catalase, glutathione peroxidase, glutathione reductase in kidney tissue of control and experimental rats.



Values are in mean ± SEM (n=6); ****P*<0.05 Vs Diabetic control rats; ******P*<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.

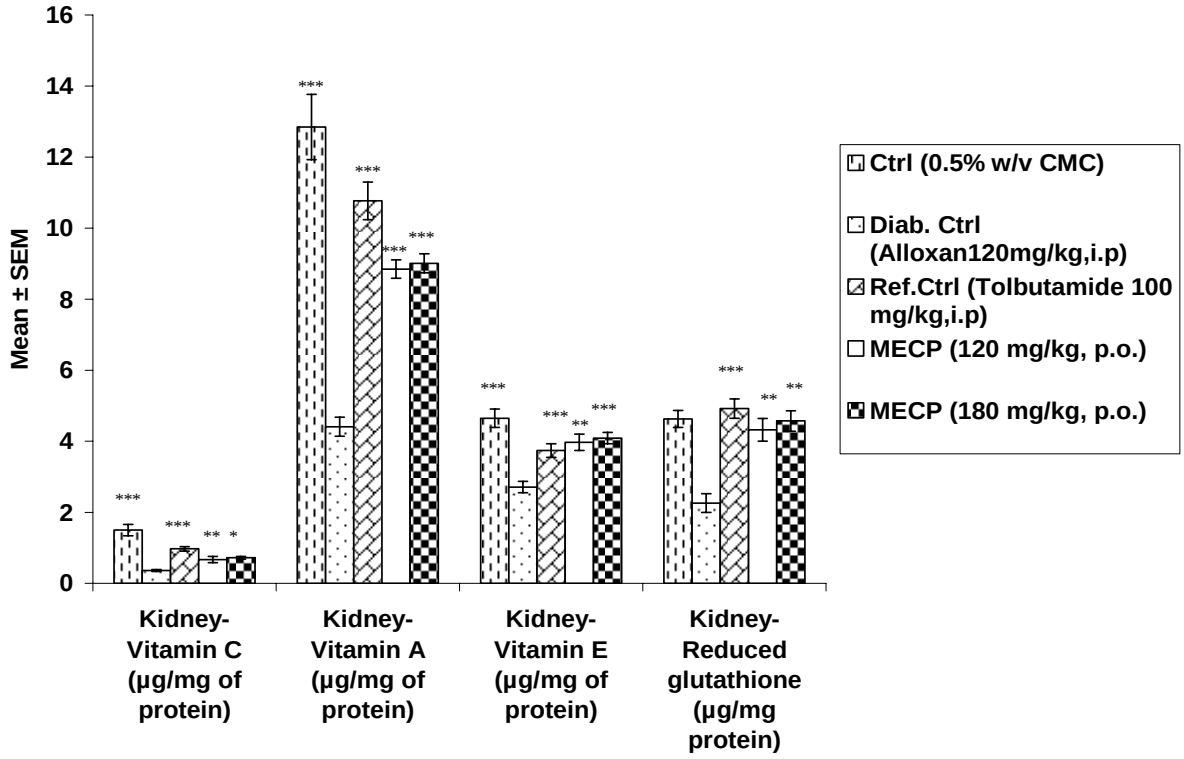
The level of non-enzymatic antioxidants such as vitamin C and vitamin A in tissues of control and experimental rats are shown in Fig. 6 and 7. The level of non-enzymatic antioxidants in tissues were significantly *(p*<0.001) reduced in diabetic rats. Administration of MECP at a dose of 120 mg/kg body weight followed by 180 mg/kg body weight and tolbutamide at a dose of 100 mg/kg body weight significantly (*p*<0.001) reversed the value of vitamin C in the liver to normal. Administration of MECP and tolbutamide at the above mentioned doses significantly (*p*<0.05), (*p*<0.01) reversed the value of vitamin A in the liver tissue where as the doses of 120 mg/kg, 180 mg/kg and tolbutamide at a dose of 100 mg/kg significantly (*p*<0.05, *p*<0.01 and *p*<0.001) reversed the value of vitamin C in the kidney tissue to normal with respect to 120 mg/kg, 180 mg/kg body weight of test doses and 100 mg/kg of reference dose. The level of vitamin E and reduced glutathione in the tissue of experimental and diabetic rats are shown in Fig. 7.

Fig. 6: Effect of MECP on vitamin C, A, E and reduced glutathione in liver tissue of control and experimental rats.



Values are in mean ± SEM (n=6); ****P*<0.05 Vs Diabetic control rats; *****P*<0.01 Vs Diabetic control rats;****P*<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.**

Fig. 7: Effect of MECP on vitamin C, A, E and reduced glutathione in kidney tissue of control and experimental rats.



Values are in mean ± SEM (n=6); ****P*<0.05 Vs Diabetic control rats; *****P*<0.01 Vs Diabetic control rats;****P*<0.001 Vs Diabetic control rats; One-way ANOVA followed by Dunnet's multiple comparison tests.**

The level of non-enzymatic antioxidants significantly decreased in diabetic rats. After the administration of MECP and tolbutamide at above mentioned doses significantly (p<0.01, p<0.001 and p<0.001) reversed the value of vitamin E in the tissue to normal. The level of glutathione in the liver tissue reversed significantly (p<0.01 and p<0.001) to the scheduled doses of test and positive control. MECP (120,180 mg/kg) and tolbutamide (100 mg/kg) significantly (p<0.01 and p<0.001) restored the value of reduced glutathione in the kidney tissue to normal.

Discussion

DM is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defect in insulin secretion or action [25]. Free radical mediated biomembrane lipid peroxidation has been implicated in the pathogenesis of many pathological conditions including diabetes mellitus and its complications. In the present study MECP at a dose of 120 and 180 mg/kg body weight possess marked anti-hyperglycemic activity by lowering the blood glucose level in alloxan induced diabetic rats. The anti-hyperglycemic activity was due to protection of *β*-cell by the leaf extract which produces insulin that enhances glycogen synthase [26,27]. The MECP at various doses (120 and 180 mg/kg) were compared with the positive control of tolbutamide (100 mg/kg). The anti-hyperglycemic activity of different doses was almost similar to that of tolbutamide.

Administration of MECP at different doses restored the protein levels to near normal. Insulin deficiency leads to various metabolic aberrations in animals such as decreased protein content. Insulin deficiency causes excessive catabolism of proteins and amino acids released are used for glucogenesis and lipolysis in adipose tissue which gives rise to hyperlipidemia respectively [28]. LPO is a process whereby oxygen radicals interact with polyunsaturated fatty acids. In the present study, there was an observed increase in LPO in liver and kidney of alloxan induced diabetic rats. The reduction of two electrons from alloxan gives dialuric acid, which undergoes oxidation and leads to the generation of $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$. Dialuric acid has been observed to stimulate LPO [29]. Oral administration of MECP decreased the level of LPO. This extract might have scavenged or inhibited free radical formation and effectively prevent liver and kidney damage.

SOD and CAT are the two scavenging enzymes that remove toxic free radicals [30] are considered primary enzymes, since they are involved in direct elimination of reactive oxygen species [31]. SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals [32] and CAT is a hemoprotein which catalyzes the reduction of H_2O_2 and protects tissue from highly reactive $OH^{\bullet}$ radicals [33]. The reduced activity of SOD and CAT in the liver and the kidney observed during diabetes may be due to deleterious effect of the accumulation of superoxide anion radicals and hydrogen peroxide. The result clearly shows that methanolic leaf extract of *C.pictus* contain a free radical scavenging activity which could exert a beneficial action against pathological alteration caused by the presence of $O_2^{\bullet-}$ and $OH^{\bullet}$. Glutathione peroxidase is a selenium containing enzyme present in significant concentrations, detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione [34]. Depression of glutathione peroxidase activity observed in the diabetic liver and kidney has been shown to be an important adaptive response to increased peroxidative stress. The decrease in the activity of these (GPx and GR) enzymes results in the involvement of deleterious oxidative changes and also insufficient availability of GSH.

Vitamin C contributes about 20% of the total peroxy radical trapping capacity so that the decrease may be due to increasing utilization in the trapping of the free radicals [35].

Vitamin C is an excellent hydrophilic antioxidant in plasma and disappears faster than other antioxidants on exposure to reactive oxygen species [36]. The decreased level of vitamin C in diabetic rats may be due to either increased utilization as an antioxidant defense against increased reactive oxygen species or due to decreased glutathione level, since glutathione is required for recycling of ascorbic acid [37]. Vitamin A acts as a powerful, free radical scavenger (singlet oxygen) and chain breaking antioxidant [38]. Vitamin A functions as a radical scavenging antioxidant, it can protect the cell from oxidative damage [39]**,** and probably assists vitamin E in the inhibition of lipid peroxidation by recycling the vitamin E. Vitamin E reduces lipid hydroperoxides generated during the process of peroxidation and protects cell structures against damage [40]. The decreased level of vitamin E found in the liver and kidney of diabetic rats as compared with control rats could be due to increased oxidative stress, which accompanies the decrease in the level of antioxidant and may be related to the cause of diabetes mellitus [41]. GSH is a major endogenous antioxidant which counters balance free radical mediated damage. It is well known that GSH is involved in protection of the normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions. The decrease in liver GSH levels represents increased utilization due to oxidative stress [42]**.** Thus in conclusion, the MECP plant had anti-hyperglycaemic effect and gives the impression to be effective in reducing oxidative stress and free radical mediated diseases.

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