MODULATORY ROLE OF *MOLLUGO CERVIANA* ON ERYTHROCYTE MEMBRANE ANTIOXIDANT IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The aim of the present study was designed to investigate the protective effects of *Mollugo cerviana* extract on erythrocyte membrane in Alloxan induced diabetic rats. Diabetic rats were treated with *Mollugo cerviana* extract and diabetic rats were also treated with glibenclamide as drug control, for 30 days. In this study, erythrocytes antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxide and non enzymatic antioxidants mainly vitaminC, vitaminE, reduced glutathione (GSH) levels were altered. Moreover, *Mollugo cerviana* extract reversed the lipid peroxidation markers, osmotic and antioxidant status in Alloxan induced diabetic rats. These results suggest that *Mollugo cerviana* extract could provide a protective effect on diabetes by decreasing oxidative stress associated diabetic complications.

KEYWORDS: *Mollugo cerviana* extract, Antioxidant, Erythrocyte membrane

INTRODUCTION

Diabetes is a major health problem; globally it affects about 5% of the population. Based on epidemiological studies and clinical trials reports hyperglycemia is the principal cause of complications. Undiagnosed or poorly controlled disease may be linked with late complications of diabetes such as enhanced atherosclerosis, blindness, renal insufficiency,

stroke, and amputation of extremities. Diabetes is also accompanying with a diminution in life expectancy (Martin_Gallan et al. 2013). Reactive oxygen species (ROS) induced oxidative stress is currently suggested to be a mechanism underlying diabetes and diabetic complications. ROS are generated in biological systems through metabolic processes and through exogenous sources In normal physiological conditions, several antioxidant defense systems protect against the adverse effects of free radical production *in vivo.* Hyperglycemia may disturb cellular antioxidant defense systems and damage cells. (Martin_Gallan et al. 2013).

Erythrocytes are frequently undergone membrane protein oxidation or carbonylation in diabetes (Dalle_Donne et al. 2003). Hence, protein carbonyls are indicators of oxidative damage to proteins in cells (Dalle_Donne et al. 2003). Diabetes mellitus also depreciates the components of antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), vitamin_C, vitamin_E and reduced glutathione (GSH) (Pari and Saravanan 2007). These decreased antioxidant components accelerates the oxidative attack on erythrocyte membrane lipids, proteins, and cytoskeletal proteins. Besides, the structure and function of erythrocyte membrane lipid bilayer may change, which further damages the erythrocyte membrane as indicated by increased osmotic fragility and modifications in erythrocyte morphology (Mohamed et al. 2013).

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-220g were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2°C and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatization to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee (Ethical No: MC/1416/a/11/CPCSEA) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Plant material

The whole plant of *Mollugo cerviana* was collected in January 2015 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The whole plant was identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Joseph's College, Thiruchirappalli, Tamil nadu, India.

Preparation of plant extract

The whole plant of *Mollugo cerviana* was first washed well and dust was removed from the plant. Whole plant was washed several times with distilled water to remove the traces of impurities from the plant. The whole plant was dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Mollugo cerviana* extract (MCE) was stored in refrigerator until used.

Experimental design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows.

Group 1: Control rats (untreated) were fed with standard diet and water *adlibitum.*

Group 2: Diabetic rats.

Group 3: Diabetic rats treated with *Mollugo cerviana* by oral gavage daily at a dose of 500 mg/kg body weight for a period of 30 days.

Group 4: Diabetic treated with glibenclamide (0.5mg/kg body weight) for a period of 30 days.

Preparation of erythrocyte membrane and hemolysate

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Blood was collected in heparinized tubes and centrifuged at 3000 rpm for 15 min. The buffy coat was removed and the packed cells were washed three times with physiological saline. The washed cells were lysed by suspending in hypotonic buffer and centrifuged at 15,000g for 30 min. The resulting pellet is the erythrocyte membrane fraction, and the supernatant represents the hemolysate.

Biochemical estimation

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al* (1979). Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar *et al.* (1984) in plasma. The activity of catalase was assayed by the method of Beers and Sizer (1952). The activity of mitochondrial glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). The level of ascorbic acid was estimated by the method of Omaye *et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980).

RESULTS

Effect of *Mollugo cerviana* on erythrocyte membrane lipid peroxidation and antioxidant defence in control and experimental rats

Erythrocytes, the unique carriers of oxygen are highly susceptible to oxidative stress conditions. Therefore, present study was concentrated on the role of MCE in augmenting the functions of antioxidants and determines the level of lipid peroxidation in erythrocyte membrane of alloxan treated rats. Supplementation of MCE to diabetic rats may probably related to a counteraction of free radicals by its antioxidant nature of MCE. MCE strengthening of endogenous antioxidant defense by its ability to restored the levels of SOD, CAT, GPx, vitamin C, vitamin E and increased GSH content and also its ability to decreased the levels of lipid peroxidation (Table. 1 and Fig. 1).

Table 1: Effect of *Mollugo cerviana* extract on RBC membrane MDA content, activities of SOD, CAT, GPx and contented of GSH, Vitamin C and E in experimental animals.

PARAMETERS	GROUP - I	GROUP - II	GROUP - III	GROUP –IV
MDA	5.26 ± 0.45^a	18.51 ± 0.38^b	7.6 ± 0.43^a	6.99 ± 0.36^a
SOD	0.92 ± 0.11^a	0.44 ± 0.09^b	0.92 ± 0.13^a	0.91 ± 0.11^a
CAT	6.47 ± 0.07^a	1.57 ± 0.09^b	6.35 ± 0.05^a	5.98 ± 0.09^a
GPx	5.86 ± 0.17^a	2.11 ± 0.15^b	5.66 ± 0.21^a	5.95 ± 0.13^a
VITAMIN-C	3.23 ± 0.16^a	1.90 ± 0.10^b	3.03 ± 0.13^a	2.90 ± 0.19^a
VITAMIN-E	1.61 ± 0.02^a	0.30 ± 0.03^b	1.86 ± 0.02^a	1.98 ± 0.01^a
GSH	7.35 ± 0.15^a	5.55 ± 0.16^b	7.64 ± 0.12^a	7.33 ± 0.13^a

Values are expressed as Mean $\pm$ SD for six rats. Mean values within a row followed by different letters are significantly different from each other at P <0.05 level comparison by Duncan's multiple range test (DMRT).

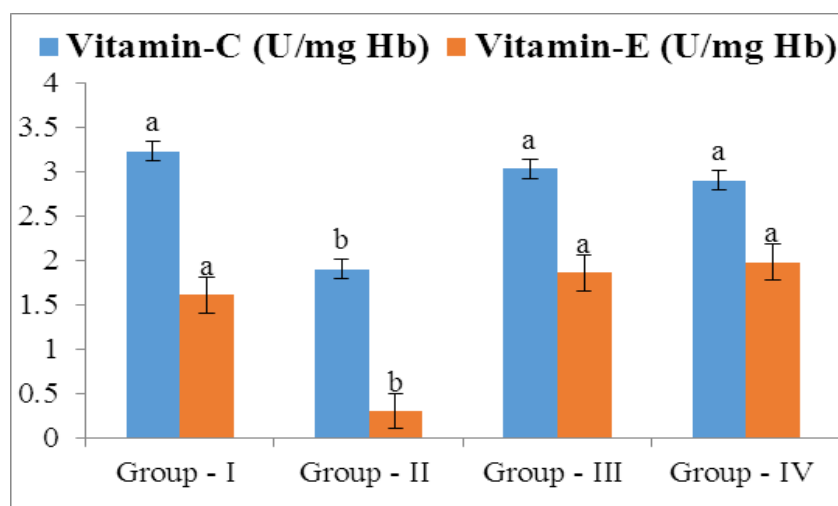MDA= is formed nmole/ mg Hb; SOD, GPx, Catalase = U/mg Hb. GSH =µg/mg Hb.
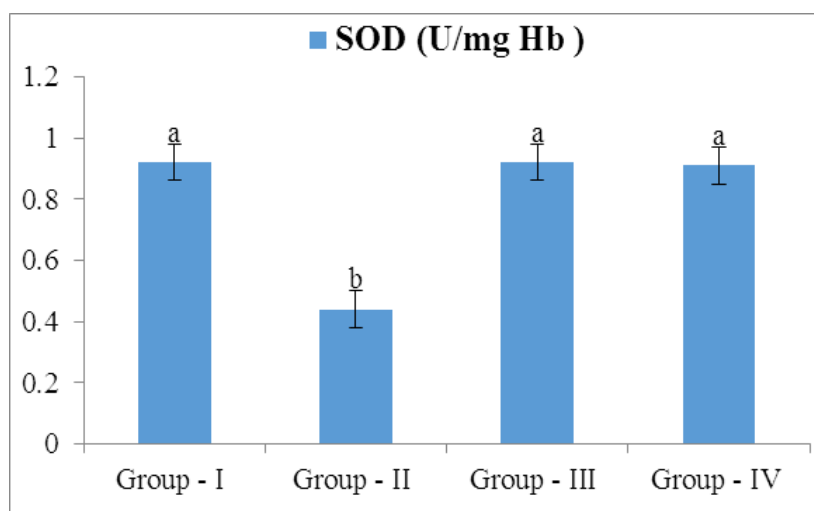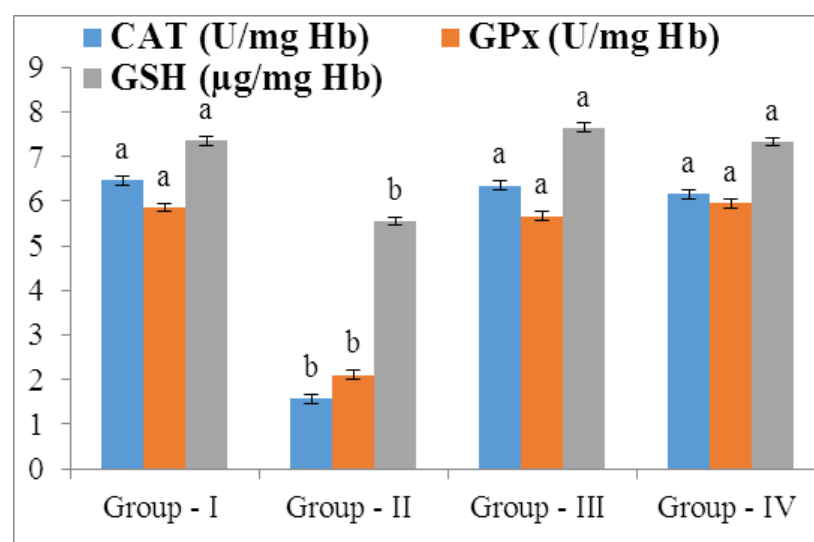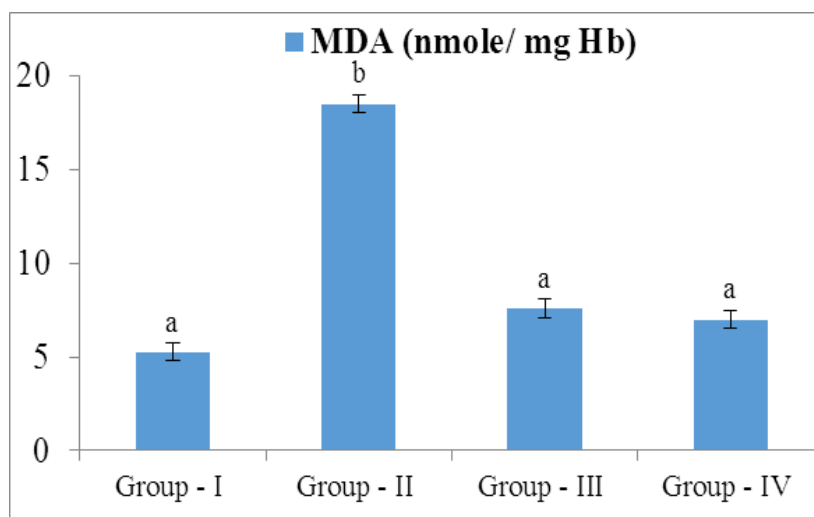
Fig.1: Effect of *Mollugo cerviana* extract on RBC membrane MDA content, activities of SOD, CAT, GPx and contented of GSH, Vitamin C and E in experimental animal.

DISCUSSION

Effect of *Mollugo cerviana* on erythrocyte membrane lipid peroxidation and antioxidant defence in control and experimental rats

In general, the erythrocytes in normal physiological conditions are resistant to oxidative damage because of their efficient protective mechanisms. The interior of the cell is rich in antioxidant enzymes, such as CAT, SOD, GPx and glutathione reductase (Picot et al., 1992; Sies, 1997). Erythrocyte membrane is also rich in α-tocopherol concentration (Burton et al., 1983. However, under oxidative stress condition the erythrocytes may be susceptible to oxidative damage due to the presence of haeme-iron, PUFA and oxygen, which may initiate the reactions that induce oxidative changes in red blood cells. Ney et al. (1990) had reported that erythrocytes and their membranes are very sensitive to oxidative damage due to their content of unsaturated fatty acids, which are continuously exposed to high concentration of oxygen.

Persistent hyperglycemia and elevated oxidative stress are major players in the development of secondary diabetic complications. During oxidative stress, hydrogen peroxide (H_2O_2) and hydroperoxide stimulates iron release from hemoglobin and glycosylated hemoglobin, which enhances the iron-mediated free radical reactions. This could be lead to structural, conformational modification and functional alterations in erythrocyte.

Hyperglycemia-induced lipid peroxidation can depletes antioxidant status in the erythrocytes. The antioxidants like vitamin-C and vitamin-E have been shown to decrease oxidative stress

in experimental diabetes (Madhu and Devi 2000). In this study, erythrocyte vitamin-C was reduced and vitamin-E was elevated in diabetic rats as reported earlier (Ramesh and Pugalendi 2005). This might be due to enhanced utilization and elevated erythrocyte membrane damage. Since circulating erythrocytes act as a sink for free radicals, both $O_2^{\bullet-}$ and H_2O_2 have the ability to penetrate the membrane of the cells (Arai et al. 1989). *Mollugo cerviana* and glibenclamide treated diabetic rats showed a significant restoration in vitamin-C and vitamin-E levels. This might be due to vitamin-C and other antioxidant compounds which are present in *Mollugo cerviana*, may decrease peroxidation and ameliorate vitamin-C and vitamin-E of erythrocyte membrane (Andarwulan et al. 2012; Loganayaki et al. 2012; China et al. 2012; Dethe et al. 2014).

GSH plays a vital role in defending against oxidative haemolysis. In the present study, the diminished level of GSH may be the result of increased oxidation of GSH to GSSG, increased degradation or reduced synthesis. Besides, decrease in erythrocyte GSH concentrations have been reported that free radical induced oxidative stress and enhanced release of lipid peroxidation products (Ramesh and Pugalendi 2005; Pari and Saravanan 2004; Saravanan and Ponmurugan 2013; Ozkol et al. 2013; Matough et al. 2014). GSSG act as a physiological indicator of the intracellular defense system against free radicals. An increase in GSSG levels in erythrocytes of diabetic rats was supported that enhanced oxidative challenge, such as by lipid peroxides, would be expected to result in depletion of cellular GSH pool and a corresponding elevation in GSSG. *Mollugo cerviana* administration was increased the GSH level by providing NADPH for the reduction of GSSG to GSH, catalyzed by GR and G6PDH. Measurement of GSH/GSSG ratio has been used to estimate the redox environment of a cell (Schafer and Buettner 2001). We observed a significant decrease in the redox index in diabetic rats, which may be related to the diminished GSH and increased GSSG concentrations. The redox index has reverted to near normal by *Mollugo cerviana* treatment in diabetic rats.

SOD and CAT are the two important scavenging enzymes that eradicate toxic free radicals from the cells. Earlier studies have reported that the activities of SOD and CAT are low in the erythrocytes of diabetic rats (Ramesh and Pugalendi 2005; Pari and Saravanan 2004; Saravanan and Ponmurugan 2013; Ramkumar et al. 2014). A reduction in the activity of these antioxidant enzymes can lead to an excess availability of $O_2^{\bullet-}$ and H_2O_2 in biological systems, which in turn produce hydroxy radicals, resulting in initiation and propagation of

lipid peroxidation. Treatment with *Mollugo cerviana* and glibenclamide significantly increased the SOD and CAT activities in diabetic rats. The result of increased activities of these enzymes suggest that *Mollugo cerviana* contains a free radical scavenging activity, which could exert a beneficial effect against pathological changes caused by the presence of $O_2\bullet-$ and $OH\bullet$. The increased activity of SOD enhances dismutaion of $O_2\bullet-$ to H_2O_2, which is eliminated by CAT.

GSH-related enzymes such as GPx, GR and GST function either directly or indirectly as antioxidant. GSH is the primary non-enzymatic antioxidant which acts as a free radical scavenger in the repair of free radical-induced oxidative damage in presence of GPx. GST and GPx plays a major role to reduce organic hydroperoxides within cell membranes in presence of GSH (Hayes and Pulford 1995). Therefore, diminished activities of GPx with a concomitant reduction in the activity of GSH-regenerating enzyme GR suggest the utilization of GSH while protecting against the alloxan-induced oxidative stress, as they help to sustain cellular redox status. In this study, a significant decrease in GPx activity was observed in the erythrocytes of diabetic rats in agreement with previous reports (Ramesh and Pugalendi 2005; 574 Pari and Saravanan 2004; Murugan and Pari 2007; Ozkol et al. 2013). Interestingly, *Mollugo cerviana* could markedly restored the diminution of these antioxidant enzymes activity in the erythrocytes of diabetic rats, this might be attributed to its antioxidant and free radical scavenging properties which could be due to the presence of bioactive compounds present in *Mollugo cerviana* like vitamin-C, vitamin-A, Kaempferol, Quercetin, Rutin, Ferulic acid, Saponins, etc., (Andarwulan et al. 2012; Loganayaki et al. 2012; China et al. 2012; Dethe et al. 2014).

The present investigation showed that *Mollugo cerviana* possesses an antioxidant activity, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular enzymatic and non-enzymatic antioxidant defense contributing to the protection against oxidative damage in alloxan induced diabetes.

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