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## Efficacy of *Achyranthes aspera* (Linn.) on Lipid Peroxidation and Antioxidants Status in Alloxan-Induced Diabetic Rats



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### ABSTRACT

The present study was aimed to investigate the antioxidant and antihyperglycemic efficacy of alcoholic fruits extract of *Achyranthes aspera* in alloxan induced diabetic rats. The male albino Wistar rats were randomly divided into five groups with six animals in each group. Diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg BW). After being confirmed the diabetic rats were treated with *A.aspera* fruits extract (100 mg/kg b.w.) for 15 days. The biochemical estimation like hepatic marker enzymes, lipid peroxidation, and antioxidants in the plasma was performed. The diabetic rats showed elevated levels of blood glucose and alanine transaminases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and  $\gamma$ -glutamyl transferase (GGT) and lipid peroxidation by products such as thiobarbituric acid-reactive substances (TBARS), Lipid hydroperoxides (LOOH), and conjugated dienes (CD). It also showed decreased in the activities/levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), vitamin C and vitamin E. Oral administration of *A. aspera* fruits extract (100 mg/kg BW) to diabetic rats for a period of 30 days significantly decreased the blood glucose, hepatic marker enzymes, lipid peroxidation by products and increases activities/ levels of the enzymic and nonenzymic antioxidants. This effect may be due to the presence of phytochemicals such as alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols in the extract. The antioxidant and antihyperglycemic effects mediated by *A.aspera* may also be anticipated to have biological significance and provide a scientific rationale for the use of *A.aspera* as an anti-diabetic plant.



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## 1. INTRODUCTION

Diabetes mellitus is a steady rise in the rate of prevalence in worldwide. It is estimated that one in five may be diabetic by the year 2025 (Junk *et al.*, 2006 and Malviya *et al.*, 2010). Diabetes mellitus is one of the most common disease affecting millions of people. Worldwide 190 million people suffer from diabetes mellitus (WHO, 2005). Diabetes in India is slowly becoming a killer disease next to coronary heart disease. Currently 36 million Indians are affected by diabetes and this current figure will be expected to be 60 million by the year 2025 (Sridhar, 2000). Study of such a drastic disease is an important concern at a number of traditions comes to dominate the practice of herbal medicine in the western world at the end of the 20<sup>th</sup> century.

A number of chemical agents have been used to induce the experimental diabetes in rats. However, alloxan is the most commonly used agents. It selectively destroys  $\beta$ -cells of Langerhans, and is cytotoxic agent and possibly involves oxygen free radicals. Alloxan is rapidly reduced in the body to form dialuric acid and this undergoes auto-oxidant to yield detectable amount of  $H_2O_2$ , superoxide ion and hydroxyl free radicals. These radicals breakup DNA strands and activates poly ADP synthetase which utilizes NAD as a substrate causing depletion of pyrimidine nucleotide and it also results in cellular dysfunction and cell-death (Prince and Menon, 2000; Jelodar *et al.*, 2003; Grover *et al.*, 2000).

Since antique, plants have been an ideal source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties (Vadivelan *et al.*, 2011). The commonly practiced treatment of diabetes includes oral antidiabetic drugs, insulin injection and management through diet. Drawbacks of insulin therapy are like local pain, inconvenience of multiple injections, insulin edema, lipohypertropy, insulin allergy, resistance and above all of this are weight gain. Moreover, oral hypoglycemic agents have their own limitations due to selective mechanism of action.

*A.aspera L.* belonging to family Amarathaceae, is commonly found in India. It is known as Apamarg in Sanskrit, Chirchitta in Hindi and Prickly chaff flower in English, Naayuruvi in Tamil. The plant is used in indigenous structure of traditional medicine systems as an antibacterial (Bisen *et al.*, 2009), antiviral (Eychmüller and Rogach, 2000), anticancer (Franzblau

*et al.*, 1998), antioxidant (Girach, 1992), anti-inflammatory and antiarthritic (Harborne, 1973) anti-fertility, and antiplasmodic (Kapoor and Harkishan, 1967). It is also used in antitumor activities (Manandhar,2002; Guzmán, 2009). It is commonly used in treatment of cold, cough, headache, asthma, bleeding, dog bites, dysentery, ear complications, leucoderma, pneumonia, renal complication and the plant possesses anti inflammatory effect, anticancer activity and immunomodulatory effect (Anand *et al.*, 2014). Owing to its wide pharmacological activity and increased medical uses the current aims to evaluate its antioxidant and antihyperglycemic effect.

## **2. MATERIALS AND METHODS**

### **2.1. Preparation of plant extracts**

The fruit body of *Achyranthes aspera* was collected in and around Sivakasi, Virudhunagar (District), Tamilnadu, India using sterile polythene bag and knife. The plant materials were shadow dried for one week and then it was powdered with help of mixer grinder and used for preparation of plant extraction. Ten gram of powdered plant material was taken in clean sterile Soxhlet apparatus and extraction done with 100 ml of ethanol different. After extraction the extracts were dried in room temperature.

### **2.2. Chemical and solvents**

Alloxan monohydrate was obtained from S.D. Fine, Mumbai and all the other chemicals used were of analytical grade and were acquired from commercial sources.

### **2.3. Alloxan induction**

Diabetes mellitus was induced in by intraperitoneally administration (150 mg/kg BW) of alloxan monohydrate dissolved in physiological saline after 16 hrs starved. This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of sugar levels by the analysis of blood and urine samples.

### **2.4. Experimental animals**

Wister albino rats used for the present investigation were obtained from Tetrox biosuppliers, Madurai and reared in laboratory under standard conditions of light and darkness (12-12 hrs) and temperature ( $22 \pm 2^{\circ}\text{C}$ ). The rats were fed standard laboratory rat pellet feed (Lipton Ltd.,

Mumbai) (Consisting protein 15-17%, fat 4-5%, carbohydrate 45-55%, fiber 15%, vitamin A 7000 IU/kg, vitamin E 40 mg/kg, vitamin K 2 mg/kg, vitamin B 1 g/kg and Hawk-Oser salt 11 g/kg) and water *ad libitum* to all the animals. The access to animal room was limited and kept to minimum.

## 2.5. Experimental design

A total number of 30 rats were randomized into 5 groups and were treated as follows. From our preliminary study, the ethanolic extract of fruits of *A.aspera* (100 mg/k.g BW) was effective than other extracts. Hence the effective dose is used in this study.

- Group I: Served as untreated control (normal) and did not receive any other treatment.
- Group II: Animals were treated with single intraperitoneal (i.p) injection of alloxan monohydrate (150 mg / kg BW) after overnight fast for 12 hours.
- Group III: Animals were received ethanol extract of *A.aspera* (100 mg /kg BW) for 45 days after the diabetic state was assessed in alloxan induced diabetic rats.
- Group IV: Animals received ethanol extract of *A.aspera* (100 mg / kg BW) for 45 days.
- Group V: Animals received tolbutamide (standard antidiabetic drug) (100 mg/ kg BW) after inducing diabetes.

After the experimental period of 45 days, the animals were fasted over night, anesthetized with ketamine hydrochloride (30 mg/kg, i.p) and then sacrificed by cervical decapitation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 min. The blood, collected in a heparinised centrifuge tube was centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration. Liver was excised, washed in ice-cold saline, weighed and the tissue homogenate was prepared in appropriate buffer.

## 2.6. Biochemical Estimations

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using the method of Reitman and Frankel (1957), Alkaline phosphatase(ALP) and  $\gamma$ -glutamyl transferase (GGT) were estimated using the method of Kind and King (1954), and Rosalki (1972). The levels of TBARS, LOOH and CD were estimated by the methods of Niehaus and

Samuelson (1998), Jiang et al. (1992), Rao and Recknagel (1968), respectively. The levels of vitamin C, vitamin E and GSH were estimated by the methods of Roe and Kuether (1943), Baker et al. (1980), Ellman et al. (1959), respectively. SOD and CAT, were assayed with the methods of Kakkar et al. (1984) and Sinha (1972) respectively.

## 2.7. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using Statistical Package for the Social Science (SPSS, Chicago, IL) software version 11.5. Values were given as means  $\pm$  S.D. for six samples. The limit of statistical significance was set at  $p \leq 0.05$ .

## 2.8. Histopathological investigation

Liver tissue was excised, washed and placed in 10% formalin. They were later sectioned with a microtome, dehydrated in ethanol, embedded in paraffin wax. Five micrometer thick sections were stained with hematoxylin and eosin (H&E) and studied by a routine light microscope.

## 3. RESULTS

Table 1 reveals the levels of blood glucose in control and experimental rats. Alloxan induced rats showed elevated levels blood glucose when compared to the normal control rats. Whereas alloxan induced rats treated with *A.aspera* (100 mg /kg BW) and tolbutamide (100 mg/ kg BW) significantly reduced the blood glucose when compared to the untreated alloxan induced rats.

Effect of *A.aspera* and tolbutamide on the activities of plasma hepatic marker enzymes such as AST, ALT, ALP and GGT in the control and experimental rats were given in Table 2. The hepatic marker enzymes were significantly ( $p < 0.05$ ) elevated in alloxan induced diabetic rats when compared to control rats. Oral administration of *A.aspera* and tolbutamide to alloxan induced diabetic rats significantly ( $p < 0.05$ ) decreased the activities of these hepatic marker enzymes. Control rats treated with *A.aspera* did not show any significance change when compared to the normal control rats.

The levels/activities of lipid peroxidation byproducts, enzymic and nonenzymic antioxidants were given in Table 3. Alloxan induced diabetic rats showed elevated levels of TBARS, LOOH,

CD and decreased the activities/levels of SOD, CAT, GPx, GSH, vitamin C and vitamin E in the plasma of control and experimental rats. Treatment of *A.aspera* and tolbutamide to diabetic rats significantly decreased the levels of lipid peroxidation byproducts (TBARS, LOOH and CD) and increased the activities/levels of enzymatic antioxidants (SOD, CAT, GPx and GST) and nonenzymic antioxidants (vitamin C, E and GSH).

Histopathological evaluation of liver of control and control rats treated with *A.aspera* exhibited no pathological abnormalities (Fig.1a&1b). Liver of alloxanised rats showed more hepatocytes with cytoplasmic vacuolar degeneration and pyknotic nuclei (Fig.1c). In case of alloxanised rats treated with ethanol extract of *A.aspera* no inflammation and showing mild fatty infiltration were observed (Fig.1d). Further, regeneration of the histology of liver was evident due to the influence of ethanol extract of *Achyranthes aspera*.

#### 4. DISCUSSION

In light of the results, our study indicates that ethanolic extracts of *A.aspera* has good antidiabetic activity in alloxan-induced hyperglycemic rats; they can also improve the condition of *Diabetes mellitus* as indicated by parameters like hepatic markers enzymes, lipids peroxidation byproducts, and endogenous antioxidants. The pancreas is an organ located in the abdomen. It plays an essential role in converting the food we eat into fuel for the body's cells. The pancreas has two main functions: an exocrine function that helps in digestion and an endocrine function that regulates blood sugar. The endocrine component of the pancreas consists of islet cells that create and release important hormones directly into the bloodstream. Two of the main pancreatic hormones are insulin, which acts to lower blood sugar, and glucagon, which acts to raise blood sugar (Edem, 2009). Maintaining proper blood sugar levels is crucial to the functioning of key organs including the brain, liver, and kidneys. Alloxan is a toxic glucose analogue, when administered to rodents and many other animal species causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycaemia. This causes an insulin-dependent diabetes mellitus in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008). Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, cholesterol, increased

levels of alkaline phosphate and transaminases (Begum and Shanmugasudnaram, 1978; Shanmugasundaram *et al.*, 1983).

Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. The TBARS value was measured as biomarkers of lipid peroxidation. Lipid peroxidation and protein oxidation were significantly higher in the alloxan induced rats compared with control group. The marked increase in the production of TBARS could be due to the superoxide radical overload, indicating the presence of oxidative stress and a subsequent increase in the production of hydrogen peroxide. This was consistent with previous reports that alloxan induces critical oxidative damage (Sharma *et al.*, 2010). In the present investigation ethanolic fruit extract of *A.aspera* demonstrated that the extract can reduce the levels of serum lipid peroxidation byproducts and reduces the vital organ liver, the mechanism could be in pancreas, thereby reducing the causation of diabetes in the experimental animals. Free radicals scavenging activities are mainly due to the presence of the phenolic compounds especially alkaloids (Kaveti, 2013). The phytochemical analysis of *A.aspera* fractions contain rich source of polyphenol (Patel and Patel, 2010). The presences of flavonoids, which are efficient antioxidants, play a crucial role in cytoprotection and scavenging of free radicals; thereby protect the  $\beta$ -cells from oxidative damage. Evidence of the C-glycosyl flavones in plants with antidiabetic properties and their efficacy in the treatment and prevention of diabetes has been extensively documented (Joyce, 1987). Recent reports stated that the extraction obtained from the seed of *A.aspera* showed more protection against Superoxide and Nitric oxide. In order to maintain redox homeostasis, cells have antioxidants and help to scavenge superoxide and hydroxyl radicals, thus maintain homeostasis and cell viability in response to oxidants' damage. SOD catalyses the conversion of superoxide anion to hydrogen peroxide ( $H_2O_2$ ). CAT and GPx scavenge excess  $H_2O_2$  as well as other free radicals in response to oxidative stress. The equilibrium between these antioxidants is important for the effective removal of oxidative stress in intracellular organelles. GSH is a major defense mechanism against oxidative stress. The lowered activities of SOD, CAT and GPx were observed in alloxan induced diabetic rats. These antioxidants might be utilized by the cells to neutralize the produced free radicals. Treatment with *A.aspera* could enhance the antioxidants in alloxan induced rats which may be due to antioxidant properties polyphenol and saponin present in the extract.



Apart from the enzymatic antioxidants, non-enzymatic antioxidants such as vitamin C, vitamin E, reduced glutathione (GSH) play an excellent role in preventing the cells from oxidative threats. Vitamin E is the most ancient antioxidant in the lipid phase (Ingold et al., 1987). In our study, vitamin C, vitamin E and GSH were decreased in alloxan induced diabetic rats, which could be due to increased membrane damage by reactive oxygen species. Treatment with *A.aspera* brought vitamin E, vitamin C and GSH to near normal levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation. Histological examination of alloxin induced rats show micro and macrovesicular infiltration. *A.aspera* treated to the alloxon fed rats suppressed the diabetic effects on hepatocytes with only few hepatocytes exhibiting microvesicular type of fatty changes. Control rats liver treated with *A.aspera* appeared normal but exhibited Kupffer cell hyperplasia.

## 5. CONCLUSION

On the basis of above results, it could be concluded that the extract of *A.aspera* exert a significant antihyperglycemic, antioxidant and antilipidperoxidative effect. This could be due to the presence of different types of active principles, each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present armamentarium of antidiabetic drug.

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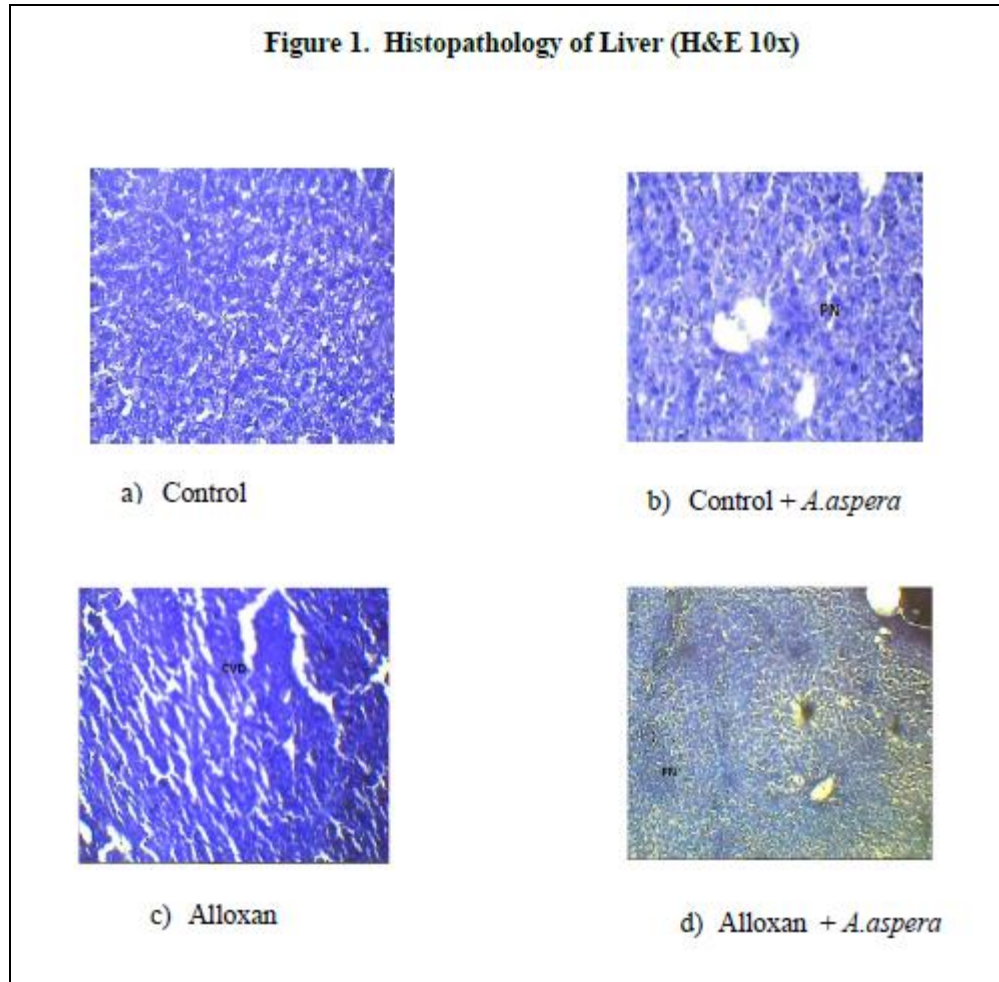
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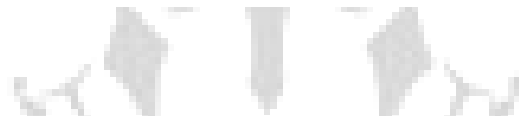


**Table 1. Effect of *A. aspera* on blood glucose levels in control and experimental rats.**

List of parameters/ Groups	Blood sugar (mg / 100ml)	
	15 Days	30 Days
Control	86.0 ± 6.59 <sup>a</sup>	85.05 ± 4.88 <sup>a</sup>
Control + <i>A. aspera</i> extract	83.0 ± 5.29 <sup>a</sup>	81.0 ± 3.25 <sup>a</sup>
Alloxan	335 ± 20.86 <sup>b</sup>	386.60 ± 21.95 <sup>b</sup>
Alloxan + Tolbutamide	194.0 ± 4.83 <sup>c</sup>	172.74 ± 13.55 <sup>c</sup>
Alloxan + <i>A. aspera</i> extract	210 ± 16.15 <sup>c</sup>	180.25 ± 15.25 <sup>c</sup>

Values are means ± SD of 6 rats in each group. ANOVA followed by DMRT.

Values not sharing a common alphabet as superscripts are significantly different from each other at the level of  $p < 0.05$



**Table 2: Effect of *A. aspera* on hepatic function marker enzymes in serum of the control and experimental rats**

Groups/parameters	Control	Control + <i>A. aspera</i>	Alloxan	Alloxan + Tolbutamide	Alloxan + <i>A. aspera</i>
ALT (IU/L)	28.71 ± 2.17 <sup>a</sup>	25.18 ± 2.10 <sup>a</sup>	61.06 ± 4.11 <sup>b</sup>	33.27 ± 2.81 <sup>c</sup>	36.11 ± 2.20 <sup>c</sup>
AST (IU/L)	63.76 ± 3.84 <sup>a</sup>	62.31 ± 5.17 <sup>a</sup>	128.25 ± 8.95 <sup>b</sup>	79.49 ± 7.20 <sup>c</sup>	83.80 ± 6.73 <sup>c</sup>
ALP (IU/L)	74.33 ± 4.99 <sup>a</sup>	72.17 ± 4.19 <sup>a</sup>	141.27 ± 10.19 <sup>b</sup>	90.34 ± 7.25 <sup>c</sup>	94.45 ± 8.04 <sup>c</sup>
GGT (IU/L)	16.54 ± 1.94 <sup>a</sup>	16.23 ± 1.41 <sup>a</sup>	39.67 ± 3.38 <sup>b</sup>	24.49 ± 2.31 <sup>c</sup>	27.19 ± 2.15 <sup>c</sup>

Values are means ± SD of 6 rats in each group. ANOVA followed by DMRT. Values not sharing a common alphabet as superscripts are significantly different from each other at the level of  $p < 0.05$

**Table 3: Effect of *A. aspera* on circulatory lipid peroxidation byproducts and antioxidants status in the control and experimental rats**

Groups /Parameters	Control	Control + <i>A.aspera</i>	Alloxan	Alloxan + Tolbutamide	Alloxan + <i>A.aspera</i>
<b>Lipid peroxidation markers</b>					
TBARS (nmoles/dL)	0.16 ± 0.03 <sup>a</sup>	0.14 ± 0.02 <sup>a</sup>	0.59 ± 0.04 <sup>b</sup>	0.24 ± 0.03 <sup>c</sup>	0.30 ± 0.03 <sup>c</sup>
LOOH (nmoles/dL)	1.99 ± 0.11 <sup>a</sup>	1.95 ± 0.15 <sup>a</sup>	3.77 ± 0.26 <sup>b</sup>	2.30 ± 0.21 <sup>c</sup>	2.38 ± 0.17 <sup>c</sup>
CD (nmoles/dL)	0.70 ± 0.06 <sup>a</sup>	0.69 ± 0.04 <sup>a</sup>	1.12 ± 0.07 <sup>b</sup>	0.83 ± 0.06 <sup>c</sup>	0.87 ± 0.08 <sup>c</sup>
<b>Enzymic antioxidants</b>					
SOD <sup>*</sup>	2.83 ± 0.24 <sup>a</sup>	2.80 ± 0.24 <sup>a</sup>	0.99 ± 0.08 <sup>b</sup>	2.41 ± 0.16 <sup>c</sup>	2.14 ± 0.10 <sup>c</sup>
CAT <sup>#</sup>	3.12 ± 0.29 <sup>a</sup>	3.10 ± 0.27 <sup>a</sup>	1.95 ± 0.18 <sup>b</sup>	2.91 ± 0.20 <sup>c</sup>	2.59 ± 0.20 <sup>c</sup>
GPx <sup>@</sup>	13.87 ± 0.69 <sup>a</sup>	13.79 ± 1.21 <sup>a</sup>	5.91 ± 0.48 <sup>b</sup>	10.12 ± 0.73 <sup>c</sup>	9.79 ± 0.64 <sup>c</sup>
<b>Non enzymic antioxidants</b>					
Vitamin-C (mg/dL)	1.68 ± 0.14 <sup>a</sup>	1.74 ± 0.16 <sup>a</sup>	0.87 ± 0.07 <sup>b</sup>	1.38 ± 0.12 <sup>c</sup>	1.29 ± 0.11 <sup>c</sup>
Vitamin-E (mg/dL)	1.71 ± 0.16 <sup>a</sup>	1.79 ± 0.12 <sup>a</sup>	0.92 ± 0.06 <sup>b</sup>	1.46 ± 0.13 <sup>c</sup>	1.33 ± 0.11 <sup>c</sup>
GSH (mg/dL)	22.27 ± 1.17 <sup>a</sup>	23.08 ± 1.98 <sup>a</sup>	10.38 ± 1.07 <sup>b</sup>	16.21 ± 0.94 <sup>c</sup>	15.05 ± 1.05 <sup>c</sup>

Values are means ± SD of 6 rats in each group. ANOVA followed by DMRT. Values not sharing a common alphabet as superscripts are significantly different from each other at the level of  $p < 0.05$ . SOD: Superoxide dismutase; <sup>\*</sup>50% NBT reduction/min/mg Hb, CAT: Catalase; <sup>#</sup>µmoles of H<sub>2</sub>O<sub>2</sub> utilized/min/mg Hb; GPx: Glutathione peroxidase; <sup>@</sup>µmoles of GSH utilized/min/mg Hb; GSH: Reduced glutathione.

