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Changes by Herbs on Oxidative Stress

Raval Jeetendra K¹, Prasad Maheshchandra², Parmar Hitesh C³, Patel Jignesh M⁴, Vihol Priti D⁵, Patel Jatin H⁶, Kalyani I. H⁷

¹Assistant Professor, ²Ex.JD (Research) IVRI, Vanbandhu College of Veterinary Science and A.H Navsari Agricultural University. Navsari- Gujarat India

³Scientist, Krishi Vigyan Kendra, Surat. Navsari Agricultural University. - Gujarat India

^{4, 5, 6}Assistant Professor, Vanbandhu College of Veterinary Science and A.H. Navsari Agricultural University. Navsari- Gujarat India

⁷Department of Veterinary Pathology, Vanbandhu College of Veterinary Science and A.H, Navsari Agricultural University – Navsari-396450, Gujarat, India

Abstract: The treatment with *Eugenia jambolana* aqueous seeds extract caused significantly lower level of Lipid Peroxidation (LPO) and higher level of Superoxide dismutase (SOD) at all the stages in comparison to diabetic control group which indicated amelioration of diabetes (STZ induced) induced oxidative stress. The value of LPO were found to be significantly lower whereas the value of SOD found to be significantly higher in comparison to Glimepiride on all the stages. These observations could suggest higher efficiency of *Eugenia jambolana* aqueous seeds extract in ameliorating diabetes induced oxidative stress as compared to glimepiride.

Keywords: Type-2 diabetes, Rats, *Eugenia jambolana*, Glimepiride, SOD, LPO.

I. INTRODUCTION

Diabetic patients are reported to have reduced antioxidant capacity making them more prone to risk of oxidative stress related ailments like vascular damage and digestive tract ulcerations. In hyperglycaemia, glucose undergoes auto-oxidation and produces superoxide and free radicals that in turn leads to lipid peroxidation in lipoproteins (Sabu and Kuttan, 2004). To control reactive oxygen species, aerobic cells have developed their own defense systems; the antioxidant system based on enzymatic control which includes Superoxide Dismutase, Catalase and Glutathione peroxidase (Gul *et al.*, 2000).

The seed kernel of *Eugenia jambolana* has been reported to increase the hepatocellular reduced glutathione content and also to increase the activities of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase in the liver of experimental diabetic animals (Ravi *et al.*, 2004a).

II. MATERIALS AND METHOD

A. Chemicals

Diabetes inducing agents i.e Streptozotocin and Nicotinamide along with other chemicals were purchased from Himedia Labs (P) Ltd, Mumbai.

B. Animals

Fifty five male and fifty five female wistar rats were procured from Zydus Research Centre, Ahmedabad, Gujarat and Laboratory Animal Resources (LAR), Sun Pharma Advanced Research Company Limited, Vadodara, Gujarat, India. Ethical clearances for performing the experiments on the rats were obtained from Institutional Animal Ethical Committee (IAEC) (No. 030-VCN-VPP-2015.)

C. Induction of Type-II Diabetes in Rats

Type-II diabetes mellitus was induced in rats by administering Nicotinamide (NAD) and Streptozotocin (STZ). The animals were first subjected to intraperitoneal injection of NAD @ 230 mg/kg (dissolved in normal saline) followed by the administration of STZ @ 65 mg/kg (dissolved in 0.1 Molar citrated buffer) intraperitoneally after 15 minutes (Masiello *et al.*, 1998; Bisht, 2013). Two days post STZ-NAD administration blood glucose level was estimated by glucometer to confirm diabetes. Thirty male and Thirty female rats with glucose level $\geq 180 \pm 8$ mg/dl were considered as diabetic and were selected for the study. Control group rats were injected normal saline followed by 0.1 Molar citrated buffer.

D. Grouping and Treatment Provided

The control group (Gr.1) consisted of 10 normal male and 10 normal female rats. The diabetic rats (30 males and 30 females) were divided into three groups (i.e Gr.2, Gr.3 and Gr.4) each consisting of ten males and ten females. Among these; Gr.2/ Diabetic control rats were treated with distilled water, Gr.3/ Diabetic rats were treated with glimepiride alone (180 mg/kg bwt. per os) and Gr.4/Diabetic rats were treated with aqueous extract of *Eugenia jambolana* seeds alone (150 mg/kg bwt. per os) for the period of 90 days. The blood sample was collected at the time points of Day 0, Day 30, Day 60 and Day 90.

The packed red blood cells were separated by centrifugation and used for the assessment of oxidative stress related parameters namely Lipid Peroxidation and Superoxide Dismutase with the help of colorimetric assay kit (Make: Biovision, USA).

E. Lipid Peroxidation (LPO) Analysis Procedure

Membrane peroxidative damage in erythrocyte was measured in terms of malondialdehyde (MDA) production by a readymade kit which was based on the method of Shafiq-U-Rehman (1984). The underlying principle is the reaction between thiobarbituric acid with MDA, a secondary product of lipid peroxidation (LPO) formation at pH 4. Colorimetrically, optical density values were recorded at 532 nm which indicate the extent of peroxidation. The activity of LPO was expressed as $\mu\text{mol} / \text{mg protein}$.

F. Superoxide Dismutase (SOD) (U/g Hb) Analysis Procedure

SOD level was estimated by a readymade kit which was based on the method of Madesh and Balasubramanian (1998). It involves the generation of superoxide by pyragallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-yl) 2,5 diphenyl tetrazolium bromide] to its formazan. The reaction was terminated by the addition of dimethyl sulfoxide, which helps to solubilize the formazan formed. Colorimetrically, OD value was taken at 570 nm and is expressed as SOD Units (1 unit of SOD is the amount (μg) of haemoglobin required to inhibit the MTT reduction by 50 %).

G. Statistical Analysis

The oxidative stress related quantitative data were subjected to statistical analysis using statistical software (Statistical Package for Social Science (SPSS) version 16.0) (Anonymous, 2007). One-way analysis of variance (ANOVA) followed by Dunnett's test was performed to determine intergroup differences. The criterion for statistical significance was $P < 0.05$. The mean values are presented with the standard error (SE) and the number of observations (N).

III. RESULT AND DISCUSSION

The lipid peroxidation (in terms of Malondialdehyde production) and superoxide dismutase activities recorded in different groups (Gr.1 to Gr.5) have been shown in Table as below.

Table 1. Average Oxidative Stress Parameters Values

Parameters studied	Groups	Values	Day 30	Day 60	Day 90
LPO (nmol/ mg protein)	Gr.1	Mean \pm SE	2.38 ^a \pm 0.09	2.10 ^a \pm 0.12	2.45 ^a \pm 0.09
		n	20	20	20
	Gr.2	Mean \pm SE	6.30 ^c \pm 0.09	7.10 ^c \pm 0.07	8.20 ^c \pm 0.23
		n	19	18	18
	Gr.3	Mean \pm SE	5.09 ^d \pm 0.04	6.00 ^d \pm 0.08	6.50 ^d \pm 0.18
		n	20	19	19
	Gr.4	Mean \pm SE	4.50 ^c \pm 0.11	5.10 ^c \pm 0.09	5.40 ^c \pm 0.16
		n	20	19	19
	Gr.1	Mean \pm SE	11.00 ^d \pm 0.16	10.60 ^c \pm 0.17	10.80 ^c \pm 0.19
		n	20	20	20
SOD Unit	Gr.2	Mean \pm SE	7.20 ^a \pm 0.11	5.60 ^a \pm 0.15	4.24 ^a \pm 0.10
		n	19	18	18
	Gr.3	Mean \pm SE	9.20 ^b \pm 0.14	8.00 ^b \pm 0.16	5.20 ^b \pm 0.11
		n	20	19	19
	Gr.4	Mean \pm SE	10.10 ^c \pm 0.17	9.00 ^c \pm 0.21	7.90 ^c \pm 0.22
		n	20	19	19

Note: Means bearing different superscript in a column differ significantly ($P < 0.05$)

- 1) Group 1: Vehicle /non diabetic control
- 2) Group 2: Streptozotocin (STZ)- Nicotinamide (NAD) Induced diabetic rats
- 3) Group 3: Diabetic rats treated with Glimepiride @ 180 mg/kg per os
- 4) Group 4: Diabetic rats treated with *Eugenia jambolana* aqueous seeds extract @ 150 mg/kg per os

A. Effects of Diabetes

- 1) Group 2 (Streptozotocin (STZ)- Nicotinamide (NAD) Induced diabetic rats): The values of LPO remained significantly higher while SOD activity maintained significantly lower in STZ-NAD induced diabetic rats (Gr.2) when compared to normal control group at all the stages of oxidative stress parameter assessment (i.e day 30,60 and 90). These changes were attributed to development of diabetes in this group. Both human and experimental animal models of diabetes exhibited higher oxidative stress due to persistent and chronic hyperglycemia which depletes the activity of free radical scavenging enzymes and thus promotes free radicals generation (Bonnefont-Rousselot *et al.*, 2000; Sabu and Kuttan, 2004). Oxidative stress has been reported to be responsible for the β -cell dysfunction caused by glucose toxicity (Evans *et al.*, 2003). This is a major factor of defective insulin secretion and enhanced apoptotic potentiality of pancreatic cells. Moreover, reactive oxygen species produced by β -cell in response to metabolic stress affect mitochondrial structure and function leading to β -cell failure (Du *et al.*, 2012). As opined by Kakadiya and Shah (2010), the level of malondialdehyde formation/ lipid peroxidation (LPO) in heart tissue was significantly increased while reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were found to be significantly decreased in STZ-NAD induced diabetic rats. There are reports of increased (Matkovics *et al.*, 1982; Omotayo *et al.*, 2010), decreased (Mahdi *et al.*, 2003; Kaleem *et al.*, 2005) as well as unchanged (Kesavulu *et al.*, 2001) SOD activity in diabetic animals. Slight elevation in the concentration of lipid peroxide in streptozotocin induced diabetic rats was also reported by Renuga *et al.* (2013). In addition, it is worth full to mention that the alloxan injection was able to significantly ($P < 0.05$) decrease the activity of SOD in liver and pancreas after 30 days (Nagy and Mohamed, 2014).

B. Assessment of Different Antidiabetic Treatments

- 1) Group 3 (Diabetic rats treated with Glimepiride @ 180 mg/kg per os): LPO level remained significantly lower while SOD activity on at all the stages remained significantly higher in this group rats when compared to diabetic control group. These observations were suggestive of antioxidative action of gliempiride on diabetes altered oxidative stress parameters. At no timepoints, further increase in LPO and decrease in SOD was noticed in comparison to diabetic control group which proved nontoxic nature of glimepiride. In comparison to Gr.4, LPO level remained significantly higher whereas SOD activities remained significantly lower in Gr.3 rats at all the stages. These observations indicated lower competence of glimepiride alone in correcting oxidative stress level as compared to *Eugenia jambolana* aqueous seeds extract (Gr.4). In comparison to Gr.5, the LPO level remained significantly higher while SOD activity remained significantly lower at all the stages of analysis. These observation could be attributed to lower competence of glimepiride alone in alleviating diabetes induced oxidative stress as compared to combination of glimepiride and *Eugenia jambolana* aqueous seeds extract (Gr.5). Our findings in serum oxidative stress parameters supported the findings of Jaeschke *et al.* (2012) who registered significant increase ($p < 0.05$) in testicular lipid peroxidation (LPO) in the glibenclamide treated diabetic rats. Treatment with glimepiride in STZ-NAD induced diabetic rats had significantly restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation in compared to diabetic control group as reported by Kakadiya and Shah (2010). Glimepiride is well reported to possess antioxidant properties (Krauss *et al.*, 2003). It was reported that pretreatment with glimepiride prevented renal ischemia/reperfusion-induced lipid peroxidation and protected the kidneys (Kakadiya *et al.*, 2010). Our findings also strengthened the report of Kakadiya *et al.*, (2009) who described improvement in oxidative stress markers in hepatic and renal tissues of glibenclamide treated rats. On the contrary administration of glibenclamide to diabetic rats did not produce any significant difference on thiobarbituric acid reactive substance as an indirect measure of lipid peroxidation levels when compared to diabetic control rats (Omotayo *et al.*, 2010).
- 2) Group 4 (Diabetic rats treated with *Eugenia jambolana* aqueous seeds extract @ 150 mg/kg per os): This group revealed significantly lower level of LPO and higher level of SOD at all the stages in comparison to diabetic control group which indicated amelioration of diabetes induced oxidative stress. The value of LPO were found to be significantly lower and SOD remained significantly higher in comparison to Gr.3 on all the stages. These observation could suggest higher efficiency of *Eugenia jambolana* aqueous seeds extract alone in ameliorating diabetes induced oxidative stress as compared to

glimepiride alone (Gr.3) Our findings supported the earlier findings of Migliato (2005) who observed hepatocyte regeneration in paracetamol damaged liver of rats when treated with *Eugenia jambolana* pulp extract. Slight elevation in the concentration of lipid peroxide in streptozotocin induced diabetic rats was restored almost to normal level when treated with *Eugenia jambolana* seeds protein as reported by Renuga et al., (2013). Further, the use of *Eugenia jambolana* leaves have been shown to reduce radiation-induced DNA damage in cultured lymphocytes in human being (Migliato, 2005). Prince and Menon, (1998) when administered jamun alcoholic seeds extract at different dose rates in alloxan induced diabetic rats produced a significant reduction in plasma lipid peroxide and elevation in plasma reduced glutathione in comparison to glibenclamide (an allopathic antidiabetic drug). Prince et al. (2003) also observed that oral administration of jamun seeds aqueous extract (5 g/kg) produced a significant decrease in lipids and thiobarbituric acid content in the brain of alloxan induced diabetic rats. They further remarked that the antioxidative effects of jamun seeds extracts were better when compared to glibenclamide (600µg/kg). The jamun seeds was reported to decrease the lipid content, thiobarbituric acid reactive substances while found to increase in catalase and superoxide dismutase in diabetic rats (Prince et al., 1998, 2003).

IV. CONCLUSION

The experimentally induced Type-2 diabetic rats showed significant increase in the value of LPO and decrease in the value of SOD. The treatment with *Eugenia jambolana* aqueous seeds extract proved to have better antioxidative property in terms reducing the diabetic LPO level and increasing SOD level on day 30, 60 and 90 in comparison to Glimepiride. These observation could suggest higher efficiency of *Eugenia jambolana* aqueous seeds extract in ameliorating diabetes induced oxidative stress as compared to glimepiride alone (Gr.3).

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VI. COMPETING INTERESTS

The authors declare that they have no competing/ conflicting interests.

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