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Research Article

AMELIORATIVE EFFECTS OF *EUGENIA JAMBOLANA* SEEDS AQUEOUS EXTRACT ON DIABETES INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

The present study was carried out to study the ameliorative effects of *Eugenia jambolana* aqueous seeds extract (150 mg/kg body wt.) in comparison to Glimepiride (allopathic drug) in STZ-NAD (Streptozotocin-Nicotinamide) induced Type 2 diabetic rat model. The treatment with *E. 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 induced oxidative stress. The values of LPO were found to be significantly lower whereas the value of SOD found to be significantly higher in comparison to Glimepiride at all the stages. These observations could suggest higher efficiency of *E. jambolana* aqueous seeds extract in ameliorating diabetes induced oxidative stress as compared to glimepiride.

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

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. 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.

The seed kernel of *E. 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.*, 2004).

MATERIALS AND METHODS

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

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.).

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 M citrated buffer) intraperitoneally after 15 minutes (Masiello *et al.*, 1998; Bisht and Bhattacharya, 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 ≥180±8 mg/dl were considered as diabetic and were selected for the study. Control group rats were injected normal saline followed by 0.1M citrated buffer.

Grouping and Treatment Protocols: 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 *E. jambolana* seeds alone (150 mg/kg bwt. per os) for the period of 90 days. The blood sample was collected at Day 30, 60 and 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 (Bio-vision, USA).

Lipid Peroxidation (LPO) Analysis: Membrane peroxidative damage in erythrocyte was measured in terms of malondialdehyde (MDA) production involving use of readymade kit. The underlying principle is the reaction between thiobarbituric acid with MDA, a secondary product of lipid peroxidation (LPO) formation at pH 4.

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Calorimetrically, optical density values were recorded at 532 nm which indicate the extent of peroxidation. The activity of LPO was expressed as nmol/mg protein.

Superoxide Dismutase (SOD) (U/g Hb) Analysis: SOD level was estimated by a kit which is based on the principle which involves generation of superoxide by pyragallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 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. Calorimetrically, OD value was taken at 570 nm and is expressed as SOD Units (1 unit of SOD is the amount (ìg) of haemoglobin required to inhibit the MTT reduction by 50%.

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). 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).

RESULT AND DISCUSSION

The lipid peroxidation (in terms of Malondialdehyde production) and superoxide dismutase activities recorded in different groups (Gr.1 to Gr.4) have been shown in Table 1.

Effects of Diabetes

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.*, 2006). Oxidative stress has been reported to be responsible for the α-cell dysfunction caused by glucose toxicity. This is a major factor of defective insulin secretion and enhanced apoptotic potentiality of pancreatic cells. Moreover, reactive oxygen species produced by α-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/LPO in heart tissue was significantly increased while reduced glutathione (GSH), catalase (CAT) and SOD were found to be significantly

Table 1
Oxidative stress parameters in different groups

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

Means bearing different superscript for individual parameter in a column differ significantly ($P < 0.05$)

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

decreased in STZ-NAD induced diabetic rats. There are reports of increased (Omotayo *et al.*, 2010), decreased as well as unchanged 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).

Assessment of different antidiabetic treatments:

Group 3 (Diabetic rats treated with Glimepiride @ 180 mg/kg per os): LPO level remained significantly lower while SOD activity at different time intervals remained significantly higher in this group rats when compared to diabetic control group. These observations were suggestive of antioxidative action of glimepiride 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).

Our findings in serum oxidative stress parameters supported the findings of Jaeschke *et al.* (2012) who registered significantly 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. 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).

Group 4 (Diabetic rats treated with *E. 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 was found to be significantly lower and SOD remained significantly higher in comparison to Gr.3 at all the stages. These observations could suggest higher efficiency of *E. 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 *E. 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 *E. jambolana* seeds protein as reported by Renuga *et al.* (2013). Further, the use of *E. 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 *E. jambolana* 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 *E. jambolana* 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 *E. jambolana* seeds extracts were better when compared to glibenclamide (600µg /kg). The *E. jambolana* seeds were 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.*, 2003).

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 *E. 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 observations could suggest higher efficiency of *E. jambolana* aqueous seeds extract in ameliorating diabetes induced oxidative stress as compared to glimepiride alone (Gr. 3).

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