ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antioxidant Activity of *Erythrina variegata* and *Breynia vitis-idaea* in Alloxan Induced Diabetic Rats

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Manuscript Info

Manuscript History:

Received: 14 November 2015
Final Accepted: 26 December 2015
Published Online: January 2016

Key words:
Erythrina variegata, Breynia vitis-idaea, Alloxan, Antioxidant.

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Abstract

The present work was carried out to study the effect of roots of *Erythrina variegata* and leaves of *Breynia vitis-idaea* on antioxidant enzymes levels in Alloxan induced diabetic rats. Alloxan (120 mg/kg, i.p) induced diabetic rats were treated with alcohol and aqueous extract at a dose levels of 300 and 600 mg/kg for 21 days. Antioxidant enzymes levels viz. Lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) were measured in liver homogenate. After 21 days of experimental period the level of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) significantly increase, while elevated level of lipid peroxidation (LPO) significantly decrease. It suggests that because of its antioxidant effects its administration may be useful in controlling the diabetic complications in experimental diabetic rats. So, it can be concluded that both the extracts of *Erythrina variegata* and *Breynia vitis-idaea* are very promising candidate for the design of new drugs based on its pharmacological effects of antioxidant adequacy.

Introduction:-

Diabetes mellitus has been considered an important health hazard because of the morbidity and mortality associated with it. Diabetes mellitus may be caused and or exacerbated by certain chemicals or compounds which elicit oxidative stress in the exposed individual. On the other hand, antioxidants have been involved in the amelioration of oxidative stress mediated pathologies. Hence oxidative stress and antioxidants have been weighed side by side in diseases states including diabetes mellitus (Ogugua and Aroh, 2006). There are many clinical and experimental evidences indicating involvement of oxidative stress in pathogenesis of diabetes mellitus and its complications namely diabetic neuropathy, retinopathy and nephropathy. Increased oxidative stress is due to excessive reactive oxygen species and inadequate antioxidant defences (Murugan, 2006). In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant material for their potential medicinal value (Singh et al., 2010).

The main objective of the study was to assess the antioxidant activity of Indian coral tree *Erythrina variegata* (Leguminosae) and Coral berry tree *Breynia vitis-idaea* (Euphorbiaceae). *Erythrina variegata* Linn. is a medium sized deciduous ornamental small tree with prickly stem and branches. Leaves are alternate, compound, trifoliate, entire margin, lanceolate stipules and leaflets are commonly triangular medium to light green, heart shaped. Flowers are large; emerge in dense, orange red colour. Flowering is normally followed by lavish production of seeds. The pods are thick and black in colour. Each pod contains 5-10 reniform seeds. These are glossy brown in colour (Kumar et al., 2010; Baskar et al., 2010 Yoganarasimhan, 2000).

Breynia vitis-idaea (Burm.f.) is an evergreen 1.5-5m tall glabrous tree or large erect shrub with horizontal branches found in the Gangetic plain, Western Peninsula, China, Malay Peninsula and Sri Lanka. These plants are planted as ornamental hedge in garden. Leaves are 1-3cm long, elliptic to elliptic-ovate, alternate dark brown or black when

dry. Bark is yellowish grey; flowers are small, greenish yellow or pink. The fruits are fleshy, pink to red which turns black when ripe and measures 2-3 mm in diameter. The seeds are black and have a very hard seed coat (Pullaiah, 2002; Chandrashekar et al., 2011; Yoganarasimhan, 2000).

Materials and methods:-

Plant Material:-

The roots of *Erythrina variegata* and leaves of *Breynia vitis-idaea* were collected from the vicinity of Tirunnelveli (Tamil Nadu, India). Taxonomic identification was carried out by V. Chelladurai, Research Officer- Botany (Retired scientist-CCRAS). A voucher specimen of each plant (JCNagar1 & JCNagar2) was deposited in the herbarium of the department of Pharmacognosy in the college for future reference.

The crude drugs were washed thoroughly in tap water to remove any unwanted matter and then dried under shade for two to three weeks. After complete drying, crude drug were pulverized into coarse powder. The powder stored in airtight container in cool & dark place to prevent deterioration by elevated temperature, light and moisture.

Preparation of Crude Extracts:-

Coarse powder of both drugs was charged in soxhlet apparatus and successive hot extraction was carried out using ethanol (70% v/v) for 24 h. The liquid extract was concentrated in rotary flash evaporator at a temperature not exceeding 50ºC. The alcohol extract was formulated as a suspension in distilled water using 2% v/v Tween-80 as suspending agent for animal studies. The aqueous extract was prepared by maceration method. The coarse powders were kept with chloroform water for 24h. The macerate was filtered and filtrate concentrated in rotary flash evaporator. Aqueous extract was prepared by dissolving in distilled water for animal studies. The extracts were preserved in desiccators for further experiments.

Animals Used:-

Swiss albino mice weighing 20-30g and albino rats (Wistar strain) weighing 170±10g of either sex were used for the study. The animals were procured and housed in the animal house at least 2 weeks prior to the study, for acclimatization. Animal house was well maintained under standard hygienic conditions, at a temperature (15-20±5°C), room humidity (60%±10%) with 12 h day and night cycle with food and water *ad libitum*. All the pharmacological experiments were as per CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) norms after obtaining approval of the Institutional Animal Ethics Committee (Reg.No.870/ac/08/CPCSEA).

Acute Toxicity Studies:-

These studies were carried out to study the acute toxic effects and determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing between 20-30 g fasted overnight, were used for the study. Each extract was orally administered at doses of 30, 100, 300, 1000 and 3000 mg/kg body weight to separate groups of mice. Subsequent to administration of drug extracts, the animals were observed closely for the first three hours, for any toxic manifestations, like increased motor activity, salivation, clonic convulsions, coma and death. Subsequently observations were made at regular intervals for 24 hours. The animals were under further investigation up to a period of 1 week (Ghosh, 2005).

Induction of Diabetes Mellitus :-

In the present work alloxan monohydrate was used to induce hyperglycemia in animals at the dose of 120 mg/kg body weight by intraperitonial injection. The fasting blood glucose levels were determined after 72 hours of Alloxan administration. Rats having blood glucose level above 200 mg/dl were selected for the study. Normal and diabetic rats were divided in eleven groups; each group comprised of six rats (Ragavan and Krishnakumari, 2006; Madhavan et al., 2007; Ilango and Chitra, 2009).

Group 1 - Normal control.
Group 2 - Positive control-Untreated Alloxan diabetic rats.
Group 3 - Standard- Alloxan diabetic rats treated with glibenclamide (500µg/kg, *p.o.*)
Group 4 - Diabetic rats treated with alcohol extract of *Erythrina variegata* (300mg/kg, *p.o.*)
Group 5 - Diabetic rats treated with alcohol extract of *Erythrina variegata* (600mg/kg, *p.o.*)
Group 6 - Diabetic rats treated with aqueous extract of *Erythrina variegata* (300mg/kg, *p.o.*)
Group 7 - Diabetic rats treated with aqueous extract of *Erythrina variegata* (600mg/kg, *p.o.*)
Group 8 - Diabetic rats treated with alcohol extract of *Breynia vitis-idaea* (300mg/kg, *p.o.*)
Group 9 - Diabetic rats treated with alcohol extract of *Breynia vitis-idaea* (600mg/kg, *p.o.*)

Group 10 - Diabetic rats treated with aqueous extract of *Breynia vitis-idaea* (300mg/kg, *p.o.*)
Group 11 - Diabetic rats treated with aqueous extract of *Breynia vitis-idaea* (600mg/kg, *p.o.*)

Determination of Antioxidant Activity:-
Doses of aqueous extract, alcohol extract, standard drug and normal saline were calculated according to the body weight of each animal. Suspension of extracts, standard drug and normal saline were administered orally to each animal using stainless steel feeding needle fitted on a plastic syringe. The treatment schedule was once daily for 21 days and animals were fed on laboratory diet of pellet chow and water *ad libitum*.

At the end of study animals liver were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.2 were prepare in 0.15 M Tris-HCl buffer) and processed the estimation of lipid peroxidation (Ohkawa et al., 1979). A part of homogenate after precipitating proteins was used for estimation of glutathione (Sedlak and Lindsay, 1968). The rest of the homogenate was centrifuge at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD (Kakkar et al., 1984). CAT activity was measured by the method of (Sinha,1972).

Statistical Analysis:-
The data obtained were statistically analyzed by one way analysis of variance (ANOVA) and expressed as mean ± S.E.M. followed by Tukey Kramer Multiple Comparison Test using instat software.

Results and discussion:-

Acute toxicity study revealed the nontoxic nature for both the extracts. There was no mortality and no toxic reactions found at any of the doses tested until the end of the study period. As per OECD guidelines, therapeutic range was considered between 1/10 to 1/5 times of LD_{50}. Accordingly, 300 mg/kg and 600 mg/kg BW doses for both the extracts were selected for determination of pharmacological studies. The present study focused the scientific explanation about the antioxidant activity for alcohol and aqueous extracts of *Erythrina variegata* and *Breynia vitis-idaea*. Experimental animals were made diabetic using alloxan. Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin dependent diabetes mellitus in these animals, with characteristics similar to

type-1 diabetes in humans (Szkudelski, 2001; Lenzen, 2008).
The results showed increased lipid peroxidation in liver of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver of diabetic rats (Ananthan et al., 2004). This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and there levels were significantly reduced after the supplementation of the alcohol and aqueous extract of *Erythrina variegata* and *Breynia vitis-idaea* (Table 1).

The levels of superoxide dismutase, catalase and reduced glutathione were significantly reduced in liver of alloxan induced diabetic rats. These adverse changes were reversed to near normal values in alcohol and aqueous extract treated (Table 1). It is well known that CAT, SOD play an important role as protective enzymes against free radical formation of tissues (Oberly and Buettner, 1974). SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen (Mc Crod et al., 1976), hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes (Sozmen et al., 2001).

The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide (Sathishsekar and Subramanian, 2005). Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase. Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Searle and Wilson, 1980). The decrease in CAT activity could result from inactivation by glycation of enzyme (Yan and Harding, 1997). Reduced activities of SOD and CAT in the liver have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides (Wohaieb and Godin, 1987). GSH is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences.

Table-1: Effect of *Erythrina variegata* and *Breynia vitis-idaea* on liver LPO, SOD, CAT and GSH in the normal, diabetic and drug treated rats.

Group	Treatment	Antioxidant enzyme			
		LPO **(nanomol/mg protein)**	SOD **u/mg protein**	CAT **u/mg protein**	GSH **(u/mg protein)**
I	Normal Control	1.89±0.04	12.4±0.07	65.6±1.48	25.85±0.41
II	Positive control	4.59±0.04	8.91±0.16	47.93±2.24	16.01±0.48
III	Standard	2.36±0.05***	11.75±0.12***	62.18±0.87***	23.26±0.85***
IV	EV Alc Ex (300 mg/kg)	3.55±0.15***	9.84±0.11**	56.30±1.39**	21.05±0.63**
V	EV Alc Ex (600 mg/kg)	2.87±0.05***	10.70±0.19***	57.73±1.51***	22.41±0.82***
VI	BV Aq Ex (300 mg/kg)	3.30±0.11***	9.74±0.14**	56.73±1.36**	20.63±0.80**
VII	BV Aq Ex (600 mg/kg)	2.85±0.10***	10.77±0.17***	57.33±1.02**	20.63±1.01**
VIII	EV Alc Ex (300 mg/kg)	3.14±0.08***	9.82±0.09**	56.90±1.67**	20.56±0.76**
IX	EV Alc Ex (600 mg/kg)	2.87±0.08***	10.71±0.14***	60.08±1.15***	22.15±0.80***
X	BV Aq Ex (300 mg/kg)	3.16±0.06***	9.76±0.16**	56.85±1.80**	20.85±0.82**
XI	BV Aq Ex (600 mg/kg)	2.76±0.08***	10.92±0.10***	60.06±1.52***	22.68±0.91***

EV- *Erythrina variegata*, BV- *Breynia vitis-idaea*, Alc- Alcohol, Aq- Aqueous, Ex- Extract
Values expressed as Mean ± SEM. One way ANOVA (*** $p < 0.001$, ** $p < 0.01$)
Tukey Kramer Multiple Comparison Test in comparison with diabetic control group.

Conclusion:-

Screening of Ayurvedic drugs/plants for biological activity assumes prime importance to establish physiological action of the drug. To obtain required evidence that will demonstrate drug's safety and effectiveness for its proposed use, a carefully designed and progressive sequence of preclinical (animal) and clinical (human) studies are undertaken.

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