IJPSR (2013), Vol. 4, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 July, 2013; received in revised form, 11 October, 2013; accepted, 17 November, 2013; published 01 December, 2013

PROTECTIVE ACTIVITY OF S*HOREA ROBUSTA* LEAF AGAINST OXIDATIVE STRESS IN RATS

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Keywords:

Shorea robusta, Free radical scavenging activity, Lipid peroxidation, Oxidation damage, Cytoprotective

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ABSTRACT: Currently there has been an increased interest globally to identify the plant origin with free radical scavenging properties and have great importance as therapeutic agents in several diseases due to oxidative stress. Lipid peroxidation of cellular membranes has also been implicated in various disease processes, including cardiac ischemia-reperfusion injury. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. *Shorea robusta* has wide range of pharmacological activity but the cytoprotective activity against oxidative damage has not yet been documented. The present study was investigated the cytoprotective activity of *Shorea robusta* extract (SRE). The results of the study indicate that the cytoprotective role of SRE may be related to counteraction of free radicals. The mechanism of cytoprotective is to be worked out in future studies.

INTRODUCTION: Oxidative damage to cellular plays an important role in the pathobiology of both chronic and acute tissue injury. Unsaturated fatty acids present in the membrane (phospholipids, sterols, glycolipids, and glycerides) and in transmembrane proteins containing oxidizable amino acids, are particularly susceptible to free radical damage.

Increased membrane permeability caused by lipid peroxidation and oxidation of structurally important proteins can disrupt transmembrane ion gradients and cellular metabolic processes. Lipid peroxidation of cellular membranes has also been implicated in various disease processes, including cardiac-ischemia diperfusion injury [12].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(12).4754-57

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(12).4754-57

Bioactive compounds commonly found in fruits, vegetables, herbs, and other plants have been possible health benefits with antioxidative, anti-carcinogenic, atherosclerosis, antimutagenic, and angiogenesis inhibitory activities [21].

Interestingly, many herbs are known to contain large amounts of phenolic anti-oxidants other than well-known vitamin C, vitamin E, and carotenoids. Phenolic antioxidants in herbs are mainly composed of phenolic acids [3], flavonoids [11] and catechins [19].

Shorea robusta Gaertn. F. (Sal) is one of the dominant tree species in tropical deciduous forests (moist as well as dry types) in India. Its fruits are also used in diarrhea.

The aqueous extract of leaves of *Shorea robusta* found to possess Tannins, flavonoids, cardiac glycosides and steroids, which may involve in showing Antibacterial activity [5] and the leaves used for the treatment of painful inflammatory conditions [7].

Shorea robusta resin was used for anti- aging activities especially for better skin health [4]. Bark extract of *Shorea robusta* on modulated immune response in rats [9].

MATERIALS AND METHODS:

Animals: Animals albino Wistar strains rats of male were obtained from the Bangalore they were kept in clean cages placed in well ventilated housed condition with food and water ad libitum and maintained on a natural 12; 12 h l; D cycle. The procedure used is the study was formed in accordance with the National Institute of Health Guide for the care and use of laboratory animals.

Plant Materials: *Shorea robusta* leaves were purchased from kolli hills at January 2012. Dried and powdered leaves of *Shorea robusta* (10 g) were extracted with 100 ml of 70% ethanol. The filtrate obtained from extraction was poured and concentration using vacuum evaporator to yield a semisolid residue. The yield of the extract EESR was % (W/W).

Experimental Design: Body weight of animals was recorded and they were divided in to three groups of six animals each as follows.

Group I: Normal animals received oral administration of leaf of *Shorea robusta* (500 mg/kg body weight) daily for three consecutive days.

Group II: Animals received in peritoneal injection of diethyl dithio carbonate (800 mg/ kg/ h/ wt) for the three consecutive days.

Group III: Treatment group received diethyl dithio carbonate (800kg/ h/ wt/ I,p) as group III. After 24 hours of last administration of diethyl dithio carbonate treatment was started at a dose of 500mg/ kg body weight of leaf of *Shorea robusta* for seven days (Xi et al., 1993).

Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters. Parameters such as, malondialdehyde (Beuge and Aust, 1978), superoxide dismutase (Kakkar et al, 1984), catalase (Beers and sizer, 1952), glutathione peroxidase (Rotruck et al, 1973), reduced glutathione(Moran et al, 1979), ascorbic acid (Omaye et al 1979), alpha- Tocopherol (Baker et al,1980).

RESULT AND DISCUSSION: *Shorea robusta* has wide range of pharmacological activity but the cytoprotective activity against oxidative damage has yet been documented. The present study was to investigate the effect of a *Shorea robusta* extract (SRE) against oxidative cell damage induced by DDC intoxicated rats supplementation of SRE to DDC intoxicated rats exert the following effects: Reduced MDA content in serum and liver, improved the enzymatic antioxidants such as catalase, GPx, GSH, Vitamin C and Vitamin E level (**Table 1**). DDC treated rats showed significant increase in lipid peroxidation as evidenced by the increase in MDA levels. The levels of lipid peroxidation products observed might be attributed to oxidative stress. Table 1 showed the level MDA, GSH, Catalase, GPx, Vitamin-E and vitamin-C in the levels of normal and experimental rats.

TABLE 1: EFFECT OF *SHOREA ROBUSTA* EXTRACT ON BIOCHEMICAL PARAMETERS IN THE CONTROL AND EXPERIMENTAL RATS:

Groups	MDA (nmole/mg)	GSH (µg/dl)	Catalase (mg/dl)	Gpx (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Group I	2.21±0.28	8.16±0.34	6.54±0.95	2.33±0.34	10.10±2.51	5.23± 1.23
Group II	4.27±0.19	6.4±0.24	3.24±0.721	1.15±0.26	6.32±1.46	3.54 ±0.62
Group III	2.03±0.19*	8.53±0.42*	5.58±0.87*	2.28±0.39*	9.18±1.85*	5.12±0.86*

Values were expressed as mean ± Standard deviation for six rats in each group. Significantly different from Group II.

Ethanolic Extract of *S. robusta* might be capable of effectively countering cytoprotective activity. *Shorea robusta* administration reduces lipid peroxidation in serum and liver of DDC treated rats.

The reduction in lipid peroxide levels may be due to the electron and H+ donating capacity of flavonoids present in *Shorea robusta*, which seems to contribute to the termination of lipid per-oxidation chain reaction based on their reducing power.

Several studies have shown that flavonoids interact with cell membranes, improving their fluidity, thereby protecting them from lipid peroxidation [17]. DDC induced oxidative stress rats showed a significant decreased in the level of GSH and GPx liver. DDC induced oxidative stress and these Rats treated with *Shorea robusta* extract significantly increased in the activity of GPx as compared to Group II. DDC induced oxidative stress rats showed a significant decreased in the level of Vitamin C and E and these rats treated with *Shorea robusta* extract significantly increased in the level of Vitamin C and E when compared to Group II.

Crucial components of the antioxidant defense system in the body are cellular antioxidant enzymes. (SOD and glutathione), which are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organism as well as in the detoxification of certain compounds of exogenous origin, thus, playing a primary role in the maintenance of a balanced redox status [13]. Hence, they can serve as a potential marker of susceptibility, early and reversible tissue damage, and of decrease in antioxidant defense [10].

The ethanolic extract of *Shorea robusta* also restored the levels of antioxidant enzymes such as SOD and CAT almost back to the normal levels. SOD plays an important role in the elimination of ROS and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in liver and kidney tissue [6]. CAT considered as most important H2O2 removing enzyme and also a key component of anti-oxidative defense system. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage [18].

Thus, in our investigation the enzymatic oxidants such as glutathione, SOD, catalase, and peroxidase were improved in drug treated group as compared to control. This might be due to some phenolic compounds, present in ethanolic extract of *Shorea robusta* and be involved in the removal of the free oxy radicals. It is further observed that administration of alcoholic extract of *Shorea robusta*, prevented the ethanol induced changes of oxidative stress parameters, in the experimental animals.

Since, the *Shorea robusta* could reverse the influences of the free radicals induced membrane damages.

SUMMARY AND CONCLUSION: In conclusion, enzymatic and non-enzymatic antioxidants are improved on treatment with SRE and it suggested that there is prevention of the free radical mediated membrane damage by SRE. The results of the present study indicated that the cytoprotective role of SRE and it may be related to counteration of free radicals. Thus, SRE stimulates the repair of membranes and improve membrane function. These findings concluded that the cytoprotective activity of SRE may indeed play a pivotal role in attenuating free radical damage and stabilize cellular structural integrity. The mechanism of action of Shorea robusta has to be worked out in future studies.

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How to cite this article:
Karthika C, Chitra M and Radhika K: Cytoprotective by *Shorea robusta* leaf against DDC mediated oxidative stress in rat. *Int J Pharm Sci Res* 2013; 4(12): 4754-57. doi: 10.13040/IJPSR. 0975-8232.4(12).4754-57

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