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Protective effect of *Adhatoda vasica* on D-galactosamine induced liver damage in rats with reference to lipid peroxidation and antioxidant status

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Abstract

The present investigation was carried out to evaluate the lipid peroxidation and antioxidant nature of aqueous extract of *Adhatoda vasica* (AEAV) on D-galactosamine (D-GalN) induced hepatic damage in wistar albino rats. Hepatic damage was induced by intraperitonial injection of DGalN (500 mg/kg, bw) for 2 days. AEAV extract (350 mg/kg, bw) was given orally for 21 days before the induction of hepatic damage. The levels of lipid peroxides (LPO) under basal and also in the presence of inducers (H_2O_2, ascorbate and FeSO4) were estimated in liver of control and experimental animals. enzymic such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non-enzymic antioxidants like glutathione (GSH), vitamin C (vit C) and vitamin E (vit E) and total thiols (TSH) levels were determined in all the groups of animals. A significant increase in LPO levels were observed while the levels of enzymic and non-enzymic antioxidant were decreased, in D-GalN induced rats. These altered enzyme levels were ameliorated significantly in AEAV pretreated animals. This protective effect of AEAV was associated with inhibition of LPO induced by D-GalN and to maintain the antioxidant enzyme levels. From this study it is concluded that the pre-treatment of AEAV has an antioxidant activity against D-GalN induced hepatic damage.

Key words: D-galactosamine, *Adhatoda vasica*, lipid peroxidation, antioxidants enzymes, liver damage.

1. Introduction

It is well known fact that lipid peroxidation has long been known to be responsible for numerous effects observed in biological systems, especially after initiation it concurrently proceeds by a free radical reaction mechanism [1].

Therefore, lipid peroxidation (LPO) is a very attractive hypothesis for explaining many diseases induced by drugs, chemicals and other xenobiotics [2]. The process arising from the reaction of radicals with lipids is considered as

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an important feature of the cellular injury leading to the deterioration of cellular constituents including lipids, proteins and nucleic acids [3]. The peroxidative attack cause extensive damage of cells, therefore, the large molecules like enzymes are able to leak out from the cells [4]. In particular, the peroxidation of endogenous lipid has been shown to be a major factor in the cytotoxic action of D-GalN induced in hepatic damage animals [5]. For instance, D-GalN induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH-), the stimulator of lipid peroxidation and the source of destruction and damage to cell membrane as suggested by [6]. Previous reports by [7] (Sakaguchi and Yakota 1995) showed that the injection of D-GalN to animals result in lipid peroxides formation and membrane damage in experimental animals, causing decreased level of scavengers or quenchers of free radicals. A major defense mechanism is the antioxidant enzymes which convert active oxygen molecules into non-toxic compounds [8]. The activities of antiperoxidative enzymes, viz superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), have also been previously reported to decrease in galactosamine induced hepatitis in rats [9]. *Adhatoda vasica* Linn. (Acanthaceae) is an Ayurvedic medicinal plant which is a home remedy for several diseases and human requirements. It is mentioned in Vedas as a herbal remedy for treating cold, whooping cough, asthma, hepatic disorders and cancer [10,11]. Roots of this plant have number of secondary bioactive metabolites such as alkaloids (vasicine and vesicinone and vesinol) steroids (vasakin) and essential oil (betane). These compounds are essential to elucidate pharmacological activity of this plant [12]. Previously studies have led to the isolation of a number of alkaloids some of which have range of biological activities including hepatic disorders, antifungal and

bronchial inflammation [13]. [14] Bhattacharya *et al* (2005) there are reports that an extract of *A.vasica* leaves had inhibitory activity against D-GalN induced hepatic damage in rats. However, there is a paucity of information regarding the antioxidant property of this plant. Therefore, in this study the hepatoprotective and antioxidant role of the aqueous extract of *A. vasica* roots on Dgalactosamine induced liver damage in rats is evaluated.

2.Materials and methods

2.1 Collection of plant material

The *Adhatoda vasica* Linn. (Acanthaceae) roots were collected in the month of September-November 2002 in Chennai, Tamil Nadu, India. The plant was identified and authenticated by the chief botanist of Arignar Anna Hospital of Indian Medicine, Arumbakkam, Chennai, Tamil Nadu, India. A voucher specimen (No.265) has been deposited in the herbarium of the same department.

2.2 Preparation of plant extracts

The roots were dried in the sunshade. A dried and pulverized root of *Adhatoda vasica* (500 g) was extracted with double distilled water at 100/°C for 5 h. The (15%) aqueous extract was then filtered, freeze-dried and kept at 4/°C. Quantitative phytochemical screening showed presence of alkaloids, essential oil and steroids.

2.3 Animals

Healthy male Wistar albino rats weighing 120 ± 30 g were obtained from Tamil Nadu University of Veterinary and Animal Sciences (TANUVAS), Chennai, Tamil Nadu, India. The animals were randomised and housed in polypropylene cages (four per cage) with rice husk for bedding and maintained in an air-conditioned room at 25 ± 2/°C, a relative humidity of 36 ± 6 % with 12 h dark cycle; they were fed with normal rat chow, marketed

by M/s Hindustan Lever Limited, Mumbai, India and water *ad libitum*. Experimental animals were handled according to the University and Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.4 Experimental design

The experimental animals were divided into four groups, each comprising of 6 rats. Group I served as the controls. Group II animals were administered with intraperitonial injection of D-GalN 500 mg/kg body wt/day, for 2 days. Group III animals were pre-treated with the aqueous extract of AEAV (350 mg/kg body wt/day), orally for 21 days and then treated with D-GalN as group II. Group IV animals were treated with AEAV alone for 21 days. At the end of the experimental period, all rats weighted were fasted overnight, anesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p) and then sacrificed by cervical decapitation. The liver was excised immediately, weighted and washed in ice-cold saline. The tissues were homogenized in ice-cold 0.1M Tris-HCl buffer in a Teflon homogenizer and centrifuged at 5000 g for 10 min. The aliquots of the homogenate were suitably processed for the assessment of following biochemical parameters.

2.5 Lipid peroxidation

Lipid peroxidation (LPO) was determined by the procedure of [15] Hogberg *et al.*,(1974) and *in vitro* induction of peroxidation with inducers (H_2O_2, ascorbic acid, FeSo4) [16]. Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a coloured product that can be measured optically at 532 nm.

2.6 Assessment of enzymatic antioxidants

Superoxide dismutase (SOD) was assayed according to the method of referd [17] Marklund and Marklund (1974). The unit of enzyme activity was defined as the enzyme required for 50% inhibition of pyrogallol auto-oxidation. The activity of catalase (CAT) was assayed by the method of [18] Sinha (1972). In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced was measured colorimetrically at 610 nm. Glutathione peroxidase (GPx) was assayed by the method of [19] Rotruck *et al.* (1973), which is based on the reaction between glutathione remaining after the action of GPx and 5, 5-dithiobis-(2-nitrobenzoic acid) to give a compound that absorbs light at 412 nm.

2.7 Estimation of non-enzymic antioxidant

Total reduced glutathione (GSH) was estimated by the method of [20] Moron *et al.* (1979), where the colour developed was read at 412 nm. Ascorbic acid was assayed by the method of [21] Omaye *et al.* (1979). Ascorbic acid (Vit C) was oxidized by copper to form dehydroascorbic acid and diketoglutaric acid, which were treated with DNPH to form the derivative of bis-2, 4-dinitrophenyl hydrazine. This compound in strong sulphuric acid undergoes a rearrangement to form a product, which was measured at 520 nm. A mildly reducing medium with thiourea was used to prevent non-ascorbic chromogen interference. Tocopherol (Vit E) was estimated by the method of [22] Desai (1984). Total thiol and non-protein sulphydryl groups was estimated by the method of [23] Sedlak and Lindsay (1968) where the colour developed was read at 412 nm. Protein content was determined by the method of [24] Lowry *et al.* (1951).

2.8 Statistical analysis

The values were expressed as mean ± SD. The data were analyzed using one-way ANOVA followed by using SPSS computer software version 7.5. Statistical significance at Pvalues < 0.001, <0.01, <0.05 were considerd.

3. Results

The effect of AEAV on liver of control and experimental animals are presented in Table 1. The levels of LPO were found to be significantly increased in groub II II D-GalN induced hepatic damage animals compared with control animals (Table 1) under basal conditions and also inducers (H_2O_2, FeSo4, and Ascorbate) (*p< 0.001). Conversely, reduced levels of LPO observed in AEAV treated (group III) animals when compared with group II animals (p<0.001).

Table 2 presented the alterd activites of SOD, CAT, GPx, vit E and vit C in the liver of control and experimental animals are presented in

The levels of LPO in liver tissue were found to be significantly increased in group. Liver of group II D-GalN induced animals shows (table 2) a significant decrease in enzymic and non-enzymic antioxidant levels (p<0.001). However, the levels of SOD, CAT, GPx, vit E and vit C were increased significantly in AEAV group III treated animals (p<0.001) when compared with group II animals.

Table 1. Levels of lipid peroxidation in liver of control and AEAV treted animals. experimental animals.

Parameters	Group I	Group II	Group III	Group IV
Basal	0.74 ± 0.03	2.45 ± 0.22 [a*]	2.02 ± 0.25 [b #]	0.77 ± 0.7 [a]
H_2O_2 induced	1.00 ± 0.16	2.85 ± 0.14 [a*]	2.15 ± 0.13 [b@]	1.04 ± 0.12 [a]
Ascorbate induced	1.96 ± 0.10	2.49 ± 0.18 [a*]	2.01 ± 0.13 [b #]	2.02 ± 0.14 [a]
FeSo4 induced	1.02 ± 0.15	2.12 ± 0.21 [a*]	1.88 ± 0.17 [b@]	1.08 ± 0.08 [a]

Values are expressed as mean ± S.D. for six rats in each group. Enzyme activities are expressed as TBARS formed/min/ mg protein. Comparisons are made between: 'a'compared with group-I; 'b' compared with group II. *p< 0.001, @p< 0.01, #p<0.05.

Table 2. The activities of enzymic and non-enzymic antioxidants in liver of control and AEAV treted animals. experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD	8.02 ± 1.03	4.20 ± 0.71 [a*]	5.96 ± 1.85 [b*]	7.46 ± 1.03 [a]
CAT	81.76 ± 6.54	49.67 ± 5.80 [a*]	70.94 ± 6.82 [b*]	79.18 ± 6.10 [a]
GPx	75.08 ± 6.8	31.21 ± 2.93 [a*]	62.5 ± 6.90 [b*]	73.05 ± 5.9 [a]
Vit C	1.63 ± 0.15	0.51 ± 0.04 [a*]	1.29 ± 0.13 [b*]	1.58 ± 0.10 [a]
Vit E	5.28 ± 0.26	3.01 ± 0.26 [a*]	4.25 ± 0.32 [b*]	4.62 ± 0.38 [a]

Values are expressed as mean ± S.D. for six rats in each group. Enzyme activities are expressed as follows: SOD, superoxide dismutase: units/mg protein (1U = amount of enzyme that inhibits the auto-oxidation of pyrogallol by 50%); CAT, catalase: μmolof H_2O_2 consumed/min/mg protein; GPx, glutathione peroxidase: μ g of GSH utilized/min/ mg protein ; Vit C and Vit E : mg/protein.

Table 3. The level of non-enzymic antioxidants enzymes in the liver of control and AEAV treted animals. experimental animals.

Parameters	Group I	Group II	Group III	GroupIV
Glutathione (µg of GSH/mg protein)	6.42 ± 1.04	$3.07 \pm 0.25^{a*}$	4.49 ± 0.35 [b*]	5.44 ± 0.41 [c]
Total thiol (µg of GSH/mg protein)	4.03 ± 0.26	1.53 ± 0.36 [a*]	2.42 ± 0.34 [b*]	3.07 ± 0.84 [c]
Protein sylphydryl (µg of GSH/mg protein)	3.22 ± 0.17	0.42 ± 0.18 [a*]	2.08 ± 0.13 [b*]	2.60 ± 0.27 [c]

Each value represents mean $\pm$ SDM *$p<0.001$, @$p<0.01$, # $p<0.05$, a-compared with group I; b-compared with group II; c-compared with Group I, Values are expressed as mean $\pm$ S.D. for six rats in each group

Table 3 shows the activities of GSH, total thiol and sulphydryl conten of liver of control and experimental animals.

the activities of GSH ($p<0.01$), total thiol and sulphydryl content ($p<0.05$) were significantly decreased (Table 3) in Group II D-GalN induced animals. However, a significant increase of these enzymes was observed in AEAV treated group III animals. However, group IV AEAV control animals did not show noticeable changes in these parameters when compared with group I control animals indicating no appreciable adverse side effects due to the administration of AEAV in group IV animals.

4. Discussion

Oxidative damage is usually refers to the impairment of the function of cellular components such as enzyme, nucleic acid, membrane and proteins by reactive oxygen species such as superoxide radicals (O^{-2}), hydroxyl free radical (OH^-) and hydrogen peroxides (H_2O_2). These agents initiate or extend cell injury by extracting hydrogen atom from poly unsaturated fatty acid and cause a degeneration process known as lipid peroxidation [25]. LPO is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation. The increase in LPO reported here, may be the result of increased production of free radicals

and/or a decrease in antioxidant status. Since LPO associated membrane damage is a key feature of D-GalN induced liver injury, the lipid peroxides were estimated and used as an index of oxidative stress. It is well known D-GalN cause fatty changes in the liver, decrease the activities of antiperoxidative enzymes and increase the content of lipid peroxidation products [26], which results in liver damage.

The increased level of lipid peroxides under basal and also in presence of inducers (H_2O_2, Feso4 and ascarbate) in the group II animals may be due to free radicals produced by DGalN. LPO reported to exert deleterious effects such as increased membrane rigidity, osmotic fragility, cellular deformation, erythrocytes and membrane fluidity [27]. Inhibition of antioxidant enzymes due to D-GalN treated animals result in over production of reactive oxygen species. This might also lead to the accumulation of lipid peroxide products. In the present investigation, increase in the levels of LPO and failure of the antioxidant defense mechanism were observed. In this context, [28] Livingstone (1990) is of the opinion that D-GalN induced oxidative stress leads to lipid peroxidation and results in the alteration of both the enzymic and non-enzymic antioxidants. In the present investigation it is interestingly observed that administration of AEAV showed inhibition of LPO. Generally, the presence of alkaloids may be contributed to beneficial effects, because they are potent

inhibitors of enhanced spontaneous production of malondialdehyde (MDA), end product of LPO and are able to inhibit lipid peroxidation [29]. Hence, the generation of MDA and related substances from lipid that react with thiobarbituric acid was found to be inhibited by the extracts. SOD is one of the most important enzymes in the enzymic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide, hence diminishing the toxic effect caused by this radical [30]. Activities of SOD and CAT were lower in D-GalN treated rats, which is in accordance with the findings of [31] Korda (1996). Increase in the activity of SOD is a sensitive index in hepatocellular damage [32]. CAT is a peroxisomal haemoprotein that catalyses the removal of H_2O_2 formed during the reaction catalyzed by SOD. Thus it is understood that increased levels of ROS activities leads to decreased levels of SOD, and it further leads to the fall in the level of CAT as a SOD, CAT, GPx, vit E and vit C chain reaction. AEAV treatment also restored the depleted SOD and CAT levels near to normal levels. It reflects that correlation between the enhanced SOD and CAT levels and the reduced lipid peroxidation levels and vice-versa [33]. GPx is also considered to be an important H_2O_2 removing enzyme in mammalian cells and is more important than CAT for removing H_2O_2 [34]. GPx is involved in the defence mechanism against oxidative damage, it reduces the H_2O_2 and hydroperoxides. The present study reveals that the activity of GPx in liver was significantly decreased D-GalN treated animals. The decreased activity of GPx in D-GalN condition may be due to excessive production of lipid hydroperoxides. Enzymic antioxidants are inactivated by hydroxyl radicals, and hence the presence of non-enzymic antioxidant is presumably essential for the removal of these radicals. Glutathione plays a critical role in

important cellular functions, the destruction of H_2O_2, lipid peroxides and translocation of amino acids across cell membrane [35]. In oxidative stress condition, GSH is converted to GSSG and depleted leading to LPO. Therefore, the role of GSH as a responsible marker for evaluation of oxidative stress is important as it act as an antioxidant both extra cellular and intracellular [36]. The decreased level of GSH in D-GalN induced rats may be due to its utilization by excessive amount of free radicals. In the present investigation, the GSH levels were maintained to normal in AEAV pretreated rats compared with experimental control rats. Vit C is a water soluble antioxidant that removes free radicals from cytosol by reacting directly with them [37]. Thus, the decreased level of vit-C found in D-GalN treated animals may be due to the utilization of antioxidant to scavenge the free radicals. The availability of vit C is a determined factor in controlling and potentiating many aspects of host resistance against hepatic damage. It can protect cell membrane and lipoproteins from oxidative damage by regenerating the antioxidant from vit E [38] . Thus vitamin E and C act synergistically for scavenging wide variety of reactive oxygen species. Vit E is a chain breaking antioxidant by donating its labile hydrogen atom from phenolic hydroxyl groups to propagating lipid peroxyl and alkoxyl radical intermediates of LPO [39]. Decreased levels of vit E content in D-GalN treated animals might be due to the excessive utilization of this antioxidant for quenching enormous free radicals produced in these conditions.

TSH is water soluble antioxidants associated with membrane protein and important for the antioxidant system. Thiols, which are the main components of the intracellular nonprotein sulphydryl groups, participate in many cellular functions including drug metabolism and

detoxification of free radicals [40] (Lai *et al.*,1991). Hence, the present observations infer that, vit E and TSH levels were found to be decreased in liver of D-GalN treated animals. These alterations were significantly reversed towards normal level in AEAV treated animals when compared with control.

Thus the results of the present study showed that AEAV decreased the lipid peroxides levels and increased the enzymic and non-enzymic antioxidant in drug treated animals compared with D-GalN treated animals.

5. Conclusion

Therefore, the recoupment of these enzymes in D-GalN treated animals may be due to free radical scavenging activity of AEAV of the plant with favorable biological activity. The ability of the AEAV to enhance the antioxidant enzyme production and metabolism of DGalN in vivo is novel finding that they may have important pharmacological and toxicological implications. Hence, AEAV can be considered for further development as therapeutic agent in hepatic damage via its antioxidant potency.

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