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ORIGINAL ARTICLE

RENAL ANTIOXIDANT AND LIPID PEROXIDATIVE EFFICACY OF *BOERHAAVIA DIFFUSA* (LINN.) AGAINST D- GALACTOSAMINE INDUCED TOXICITY IN KIDNEY OF MALE ALBINO WISTAR RATS

I. Arivu and *M. Muthulingam

Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar – 608 002.

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ABSTRACT

Herbal medicines have been used by human for thousands years to treat medical illness or to improve physical performance. Plants have been always a major source of nutrition and health care for both humans and animals. The methanolic extract of *Boerhaavia diffusa* leaves was investigated for its antioxidant and lipid peroxidative efficacy on D- Galactosamine (400 mg/kg) induced liver damage in male albino wistar rats. Antioxidant and lipid peroxidative activity was measured by using biochemical parameters such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione reductase, glutathione-S- transferase and TBARS in kidney. Oral administration of the methanolic leaf extract of *Boerhaavia diffusa* at the doses of (50, 100 and 200 mg/kg) to D- Galactosamine treated rats produced significantly reduced the level of TBARS and elevated levels of antioxidant activity in kidney. The effects aqueous leaf extract of *Boerhaavia diffusa* were comparable to standard drug silymarin. These results suggest that methanolic leaf extract of *Boerhaavia diffusa* have potential therapeutic value in the treatment of kidney diseases, probably by its antioxidative nature.

Keywords: Antioxidant enzymes, *Boerhaavia diffusa*, Lipid peroxidation, D- Galactosamine.

1.INDRODUCTION

Hepatotoxic effect is associated with the depletion of UTP nucleotides followed by the formation of UDP hexosamines (Kmiec et al., 2000; Sinha et al., 2007) and leads to the inhibition of transcription and consequently the translation processes (Keppler et al., 1974). The development of acute liver failure due to the administration of galactosamine is well documented (Keppler et al., 1968; Makin et al., 1997; EI-Mofty et al., 1975). Javle et al reported that galactosamine induced liver injury is associated with the development of renal failure (Javle et al., 1998). Recently, the term hepatorenal syndrome (HRS) has been introduced to define the development of renal failure in the absence of clinical, anatomical or pathological causes of that failure. Classically, HRS is associated with the end-stage liver cirrhosis and it has been observed that renal failure occurs with this liver disease in about 50% of patients (Epstein, 1990 ; Sinha et al., 2007).
Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin (Porter and Bennett, 1981). A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, NSAID's, chemotherapeutic agents have been added to the therapeutic arsenal in recent years (Hoitsma et al., 1991). Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy (Paller, 1990). Nephroprotective agents are the substances which possess protective activity against Nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances (Mohana lakshmi et al., 2012).

Boerhaavia diffusa, belonging to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India and its traditional name is Punarnava. *Boerhaavia diffusa* is up to 1 m long or more, having spreading branches. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerably ovate - oblong, round, or subcordate at the base and smooth above (Kuldeep and Mishra, 2011). However there are no reports regarding the antioxidant and lipid peroxidative role of methanolic extract of *Boerhaavia diffusa*.

*Corresponding author: ***Dr. M. Muthulingam,*** *Department of Zoology, Annamalai University, Annamalainagar-608 002, India,*

The present study was aimed to evaluate the renal antioxidant and lipid peroxidative role of extract of *Boerhaavia diffusa* against D-Galactosamine induced toxicity in rats.

2.MATERIALS AND METHODS

PROCUREMENT AND REARING OF XPERIMENTAL ANIMALS

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (28 ± 2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 160-200 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water *ad libitum* and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. The study was approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College (160/1999/CPCSEA, Proposal No. 1025), Annamalai University, Annamalainagar, Chidambaram.

Preparation of Methanolic extract

The collected *Boerhaavia diffusa* leaves were air dried and powdered. The powdered *Boerhaavia diffusa were* kept in airtight containers in a deep freeze until the time of use. A sample containing 1 kg of *Boerhaavia diffusa* was mixed with 4000 mL of methanol and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extract was approximately 38.55 g.

The suitable optimum dosage schedule were identified by administering the methanolic extract of *Boerhaavia diffusa* extracts at different dosages (50, 100, 200, 400 and 800 mg/kg body weight) in a day daily for twenty eight days. The optimum doses were selected as 50, 100 and 200 mg/kg body weight of the animals for twenty eight days respectively.

EXPERIMENTAL DESIGN

The animals were divided into 7 groups of 6 rats each.

Group 1 : Control rats given physiological saline solution 10 mL/kg body wt..

Group 2 : Rats given D-Galactosamine (400 mg/kg body wt./ip) for one day only.

Group 3 : Rats given D-Galactosamine + *Boerhaavia diffusa* (50 mg/kg body wt.) administered orally using an intragastric tube.

Group 4 : Rats given D-Galactosamine + *Boerhaavia diffusa* (100 mg/kg body wt.) administered orally using an intragastric tube.

Group 5 : Rats given D-Galactosamine + *Boerhaavia diffusa* (200 mg/kg body wt.) administered orally using an intragastric tube.

Group 6 : Rats given D-Galactosamine + silymarin (25 mg/kg body wt.) administered orally using an intragastric tube.

Group 7 : Rats given *Boerhaavia diffusa* (200 mg/kg body wt.) alone administered orally using an intragastric tube.

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. The kidney tissues were excised immediately and washed with chilled physiological saline.

Biochemical analysis

Kidney tissues were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Biochemical parameter such as lipid peroxidation (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione-S-transferase (GST) activities were estimated according to standard methods (Niehaus and Samuelson, 1968; Ellman, 1959; Kakkar et al., 1984; Sinha, 1972; Horn and Burn, 1978; Habig et al., 1974) respectively.

STATISTICAL ANALYSIS

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at $p \leq 0.05$(Duncan, 1957).

3.RESULTS

Renal lipid peroxidation

The level of lipid peroxidation in kidney was estimated in normal and experimental rats. There was a significant elevation in lipid peroxidation in rats treated with D-Galactosamine when compared with the corresponding control rats. Administration of *Boerhaavia diffusa* 50, 100, 200 mg/kg and silymarin to D-Galactosamine treated rats caused a significant reduction in lipid peroxidation when compared with D-Galactosamine alone treated rats. No effects were observed on lipid peroxidation activities when extract alone was administered (Table 1).

Renal enzymatic and non-enzymatic antioxidant

The levels of kidney non-enzymatic antioxidant (GSH) and enzymatic antioxidant such as SOD, CAT, GST and GR were analysed in normal and experimental rats. There was a significant decrease in kidney reduced glutathione (GSH),

Table 1. Activities of SOD, CAT, GR, GST, GSH and TBARS in kidney of control and experimental groups

Groups	SOD (Units^{A})	CAT (Units^{B})	GR	GST	GSH	TBARS
Control	6.22 ± 0.47^{e}	55.75 ± 4.24^{e}	5.85 ± 0.45^{d}	5.98 ± 0.46^{e}	6.18 ± 0.47^{e}	1.04 ± 0.08^{a}
D-Galactosamine (400mg/kg)	3.35 ± 0.26^{a}	28.13 ± 2.14^{a}	2.67 ± 0.20^{a}	2.52 ± 0.19^{a}	3.30 ± 0.25^{a}	3.25 ± 0.25^{d}
D-Galactosamine + *Boerhaavia diffusa* (50 mg/kg)	3.88 ± 0.30^{b}	32.46 ± 2.47^{b}	2.94 ± 0.22^{a}	2.98 ± 0.23^{b}	3.87 ± 0.30^{b}	3.09 ± 0.24^{d}
D-Galactosamine + *Boerhaavia diffusa* (100 mg/kg)	4.65 ± 0.35^{c}	39.49 ± 3.01^{c}	3.68 ± 0.28^{b}	3.65 ± 0.28^{c}	4.62 ± 0.35^{c}	2.36 ± 0.18^{c}
D-Galactosamine + *Boerhaavia diffusa* (200 mg/kg)	5.84 ± 0.45^{de}	51.83 ± 3.95^{de}	5.47 ± 0.42^{cd}	5.58 ± 0.43^{de}	5.88 ± 0.45^{e}	1.58 ± 0.12^{b}
D-Galactosamine + Silymarin (25 mg/kg)	5.39 ± 0.41^{d}	47.76 ± 3.64^{d}	5.08 ± 0.39^{c}	5.12 ± 0.39^{d}	5.24 ± 0.40^{d}	1.74 ± 0.13^{b}
Boerhaavia diffusa (200 mg/kg) alone	6.26 ± 0.47^{e}	55.89 ± 4.26^{e}	5.89 ± 0.45^{d}	5.58 ± 0.38^{e}	6.20 ± 0.47^{e}	1.01 ± 0.08^{a}

All the values are mean ± SD of six observations; Values which are not sharing common superscript differ significantly at 5% level ($P < 0.05$) Duncan Multiple Range Test (DMRT); Units^{A} = one unit is as 50% inhibition of NBT/min/mg protein ; Units^{B} = □ moles of H_2O_2 utilised/min/mg protein. GST = □ moles of GSH- CDNB conjugate formed/min/mg protein; GR = □ moles of NADPH oxidized/min/mg protein.; TBARS= n moles of TBARS/mg protein, GSH= µgm of GSH consumed (min) mg protein

superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione-S-transferase (GST) in rats treated with D-Galactosamine when compared with the corresponding control rats. Oral administration of methanolic extract of *Boerhaavia diffusa* 50, 100, 200 mg/kg and silymarin to D-Galactosamine induced hepatic damage rats caused a marked increase in the activities of GSH, SOD, CAT, GR and GST as compared with D-Galactosamine alone treated rats. The extract alone treated rats did not show any significant alterations when compared with control group (Table 1).

4.DISCUSSION

Study of herbal drugs is gaining more attention due to their ameliorating effect on acute and chronic disease conditions. The plant extracts have been used in traditional medicines for centuries, since they act as a source of antioxidants and efficient pharmacophores (Rajamurugan et al., 2012). Molecular oxygen is an indispensable element for the life of aerobic organisms because it enables the formation of reactive oxygen species (ROS) which in small quantities are essential for many physiological processes (Tarkang et al., 2013).

In all living organisms, reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause deleterious cytotoxic effects to mammalian cells. These free radicals include various forms of activated oxygen and nitrogen such as superoxide anion ($O2^{-}$), hydroxyl (OH^{-}), nitric oxide radicals (NO^{-}) and non –free radical species such as hydrogen peroxide, nitrous acid (HNO2) which lead to generation of free radicals (Stojkovic et al., 2011). These free radicals are continuously formed inside the human body as a result of exposure to exogenous chemicals in the environment or due to various endogenous metabolic reactions involving bioenergetic electron transfer and redox enzymes (Thirumalai et al., 2011). Oxidative stress in mammalian life can be defined as the imbalance between generation of reactive oxygen species (ROS) and the rate of their suppression by antioxidants (Chamy et al., 2006). Antioxidants have been reported to scavenge free radicals by interfering with the oxidation process and chelating metal ions. Thus oxidative stress is prevented by the action of antioxidants (Taju et al., 2011).

Lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical induced cellular damages (Halliwell and Gutteridge, 1992). Peroxidation reactions in biological systems are the underlying causes for a variety of pathological condition (Estuo and Hiroyuki, 1990). Lipid peroxidation is a measurement of function of cellular membranes. The levels of TBARS are an indirect measurement of the lipid peroxidation (Halliwell et al., 1995). The reactive free radicals initiate cell damage through two major mechanisms of covalent binding to cellular macromolecules and lipid peroxidation (Slater, 1984; Brattin et al., 1985). The free radicals initiate lipid peroxidation and could produce a range of enzymatically damaging consequences and could result in membrane disorganization by peroxidizing mainly the highly unsaturated and polyunsaturated fatty acids by attacking the methylene bridge hydrogen (Slater, 1972).

Antioxidants had been proven to play an important role in the regulation of a vast array of physiological and pathological processes. They principally contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Plants play an important role in maintenance of human health primarily via nutrition and also contribute greatly to the management of various ailments. The antioxidant enzymes SOD, catalase and peroxidases constitute a mutually supportive team of defense against reactive oxygen species (ROS) (Bandhopadhay et al., 1999; Tabatabaie and Floyd, 1994). GST binds to lipophilic compounds and acts as an enzyme for GSH conjugation reactions (Anandan et al., 1999). The decrease in the activity of GST during D- Galactosamine induced hepatotoxicity may be due to the decreased availability of GSH and suggests a total inhibition of drug metabolism during D- Galactosamine induced hepatotoxicity. Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption (Anandan et al., 1999). Oral administration of methanolic extract of *Boerhaavia diffusa* 50, 100, 200 mg/kg and silymarin to D-Galactosamine treated rats restored the altrations in the activity of the enzymatic,

non-antioxidant enzymes and lipid peroxidation. Simillarly oral administration of extracts of *Astracantha longifolia* on carbon tetrachloride treated rats shows minimize the lipid peroxidation levels and enhance the antioxidant activities in kidney (Muthulingam, 2002). Sinha et al., (2007) addressed that administration of *Cajanus indicus* isolated protein to galactosamine treated kidney shows that an elevated levels of antioxidant enzyme activities. Rajamurugan et al., (2012) noticed that administration of *Brassica nigra* to galactosamine treated rats shows enrichment of antioxidant activity in kidney. Administration of D- Galactosamine to herbal preparation treated rats shows that enhance the activities of antioxidant enzymes and suppress the level of lipid peroxidation (Padmanabhan and Jangle, 2014). Medicinal plants-derived antioxidants enhance endogenous antioxidants ability to protect renal damage through reduction of lipid peroxidation (Baradaran et al., 2015).

5.CONCLUSION

It is concluded that treatment with methanolic extract of *Boerhaavia diffusa* decreases the D-Galactosamine induced toxicity in biochemical parameters. These findings suggest that the methanolic extract of *Boerhaavia diffusa* was effective in bringing about functional improvement of kidney. Oxidative stress is an important factor contributing to kidney damage by increasing production of oxidants, particularly insufficiency of endogenous antioxidant defense system. Medicinal plants antioxidants have been shown to ameliorate oxidative induced kidney damage by reduced the level of lipid peroxidation and potentially increased the scavenging ability of antioxidant enzymes. Supplementation of medicinal plants shows enhance the antioxidants might be considered as an important remedies to reduce the oxidative stress in kidney.

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