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Research Article

BENEFICIAL EFFECT OF *BACOPA MONNIERI* EXTRACT ON GENTAMICIN INDUCED NEPHROTOXICITY AND OXIDATIVE STRESS IN ALBINO RATS

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ABSTRACT

Gentamicin (GM) is an antibiotic widely used in treating severe gram-negative infections. However, a major complication of therapeutic doses of GS is nephrotoxicity. In the present study, we investigated the protective effect of *Bacopa monnieri,* an indigenous ayurvedic medicinal plant on gentamicin- induced nephrotoxicity in albino rats. The significant renal damage was observed in GM-treated rats by increased levels of urea, creatinine and decreased levels of albumin and uric acid. Moreover, the oxidative stress markers such as SOD, GR, GST, CAT, TBARS, CD, GPx and GSH were also determined in renal tissues and were found to be altered. Supplementation of two different doses of *Bacopa monnieri* (viz.100 and 200 mg/kg) to gentamicin intoxicated rats restored the altered above mentioned markers in dose dependent manner. Thus, our results suggested the nephroprotective effect of *Bacopa monnieri*, which could be by enhancing antioxidant activity with natural antioxidants and scavenging the free radicals.

Keywords: Antioxidant stress, *Bacopa monnieri,* Gentamicin, Nephrotoxicity, Renal damage, Biochemical parameters.

INTRODUCTION

Gentamicin (GM), an aminoglycoside antibiotic, is widely used against serious and life-threatening infections, but its usefulness is limited by the development of nephrotoxicity and ototoxicity[1]. It can be highly nephrotoxic, particularly if multiple doses accumulate over a course of treatment. Hence, it is usually dosed by body weight. The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum). The exact mechanism of GM-induced nephrotoxicity is unknown. However, GM has been shown to enhance the generation of ROS[2] causing deficiency in intrinsic antioxidant enzymes. ROS have been suggested as a cause of death for many cells in different pathological states including various models of renal and cardiac diseases. A number of therapeutic agents are experimentally evaluated against GM-induced nephrotoxicity, but none of them exhibited effective protection against it.

Bacopa monniera, (a member of the *Scrophulariaceae* family) also referred to as *Bacopa monnieri*, *Herpestis monniera*, water hyssop, and "Brahmi," has been used in the Ayurvedic system of medicine for centuries. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration[3] and to provide relief to patients with anxiety or epileptic disorders[4]. The compounds responsible for the pharmacological effects of Bacopa include alkaloids (Brahmine and herpestine), saponins (d-mannitol and hersaponin, acid A, and monnierin) and other active constituents such as betulic acid, stigmastarol, beta-sitosterol, as well as numerous bacosides and bacopasaponins. The constituents responsible for Bacopa's cognitive effects are bacosides A and B[5]. Its aerial parts contains phytochemicals like betulinic acid, wogonin and oroxindin[6] and the whole plant contains three new triterpene glycosides bacopasides VI-VIII , together with three known analogues, bacopaside I, bacopaside II, and bacopasaponsin C[7]. Two common flavonoids, luteolin and apigenin, were present in all samples of *B. monnieri*[8]. It is used in traditional Indian medicine, the Ayurveda, for the treatment of anxiety, and in improving intellect and memory for several centuries[9]. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory[10] and neuropharmacological[11] disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative[12,] free radical scavenging and anti-lipid peroxidative[13], reversible spermatogenesis and fertility[14].

The present study was undertaken to investigate nephroprotective and antioxidant properties of *B. monnieri*. The effect of plant extract in GM-induced nephrotoxicity was evaluated by determining the levels of blood urea, uric acid, creatinine and albumin in all the experimental groups. The activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) were determined from kidney homogenates. Further, the levels of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) were also estimated from renal samples.

MATERIALS AND METHODS

Plant Material and Drug Preparation

Bacopa monnieri (Linn) (Scrophulariaceae) was collected from Trichy district, Tamil Nadu, India in the month of January 2009. The whole aerial parts was dried under shade and then powdered with a mechanical grinder to obtain coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C[15]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccator.

Chemicals

Reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, bovine serum albumin (BSA), 1,2-dithio-bisnitrobenzoicacid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), gentamycin, 2,6dichlorophenolindophenol, thiobarbituricacid (TBA), picricacid, sodium tungstate, sodium hydroxide, trichloroaceticacid (TCA) and perchloric acid (PCA) were purchased from Sigma Chemicals Co., St. Louis, USA.

Study Animals

Studies were carried out using healthy albino rats (120 to 180g) obtained from Indian Institute of science, Bangalore. The animals were grouped and housed in polypropylene cages and with not more than six animals per cage and maintained in under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum.* All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All

procedures described were reviewed and approved by the Instituitional Animal Ethical Committee.

Gentamicin induced nephrotoxicity in rats

Animals were randomized and divided into four groups (1 to 4) of six animals (n=6) in each groups. Group 1 served as untreated control and was fed orally with normal saline (5 ml/kg body weight) daily for 15 days. Group 2 rats were treated with GM (80 mg/kg body weight), in 0.9% saline by intraperitoneal injection for 15 days. Group 3 and 4 rats were treated similarly as group 2 along with ethanolic extract of *Bacopa monnieri* at a dose of 100 mg/kg and 200 mg/kg body weight for 15 days, respectively.

Biochemical markers estimation

Following termination of the experiment on the day 15, the rats were fasted overnight. Blood samples were collected by cardiac puncture with 21G needle mounted on 5 ml syringe (under diethyl ether anesthesia) and centrifuged for 10 min at 5000 rpm. The obtained clear sera were stored at -20°C for subsequent measurement of blood urea, creatinine, uric acid and albumin. The urea and creatinine levels in all the sample sera were estimated by modified methods based on diacetylmonoxime reaction[16] and Jaffe's reaction[17] respectively and albumin level was determined by Lowry[18]. The serum uric acid was estimated by the method Caraway (1963)[19].

Preparation of renal homogenate and determination of antioxidant markers

The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at -8°C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of superoxide dismutase (SOD)[20], glutathione reductase (GR)[21], Glutathione S-transferase (GST)[22], catalase (CAT)[23], lipid peroxidation (TBARS)[24], conjugated dienes (CD)[25], glutathione peroxidase (GPx) and reduced glutathione (GSH)[26] using colorimetric assay.

Statistical analysis

Data were expressed as mean ± S.D. Statistical comparisons were performed by Student's t-test using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

In this present study, serum urea and creatinine concentrations were significantly increased ($p < 0.001$) in the GM treated animals (Group 2) compared to the normal animals (Group 1). These elevations were significantly ($p<0.001$) attenuated in rats treated with the ethanol extract of *B. monnieri* in dose related fashion (figure 1). But, the levels of uric acid and albumin significantly decreased ($p<0.001$) in gentamicin treated groups (Group 2), when compared to the control group. Treatment with ethanol extract of *B. monnieri* significantly (Group 3 & 4 respectively) increased the uric acid and albumin levels, compared to the GM treated group.

Free oxygen radicals can induce lipid peroxidation in cells; TBARS is formed during oxidative degeneration and accepted as an indicator of lipid peroxidation. In this study, the TBARS and CD concentrations were significantly increased ($p < 0.001$) in GM treated group of animals (Group 2) compared to normal animals (Group 1). Treatment with ethanol extract of *B. monnieri* showed significant ($p< 0.001$) decrease in concentrations of TBARS and CD compared to the GM treated group in a dose dependent manner (figure 2). However, maximum nephroprotection was offered by the 200 mg/kg of extract.

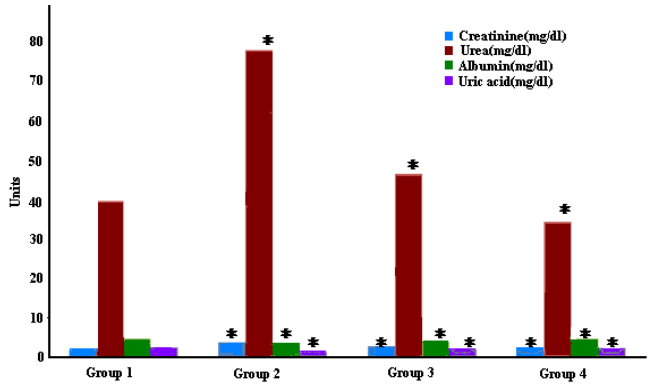

Fig. 1: Effect of treatment with ethanol extract of *Bacopa Monnieri* on the serum creatinine, urea, albumin and uric acid levels in rats with gentamicin induced nephrotoxicity. All values are mean ± S.D., (n = 6). *p < 0.001 with respect to control

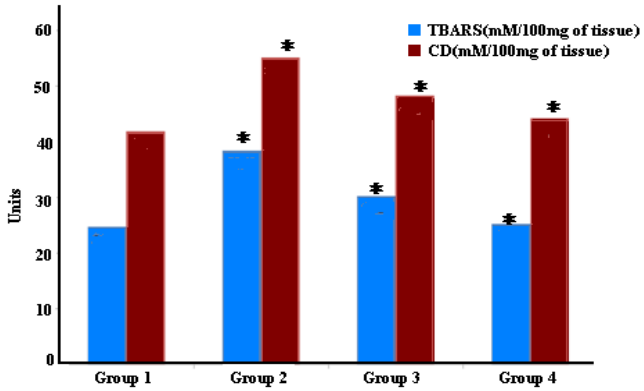

Fig. 2: Effect of treatment with ethanol extract of *Bacopa monnieri* on lipid peroxidation and conjugated diene levels in rats with gentamicin induced nephrotoxicity. All values are mean ± S.D., (n = 6). *p < 0.001 with respect to control

Table 3 depicts the enzymatic and non-enzymatic antioxidants activities in the kidney of control and experimental rats.GM produced a decrease in GSH levels (nmol/mg protein) from 14.03 ± 0.32 to 9.56 ±0.57. Treatment with ethanol extract of *B. monnieri* (group 3 and 4) prevented the GM-induced decline in GSH level and group IV caused 80% increase in GSH content ($p < 0.001$) compared to GM treated group and restored near to its normal level. Likewise, the decreased GPx activity as a result of the treatment with GM (Group 2) was also restored by the *B. monnieri* extract for Group 3 and 4 as compared to the normal group. The activities of GR and GST were significantly decreased in the kidney ($p<0.001$) of rats exposed to gentamicin (group 2) as compared with control rats. The decreased activities of GR and GST were significantly enhanced ($p<0.001$) by administration of 200 mg/kg (group 4) of ethanol extract of *B. monnieri* while both the activity was only slightly changed with 100 mg/kg (group 3) treatment in the kidney when compared with control rats.SOD (U/mg protein) and CAT activities (U/mg protein) were decreased in renal tissues of GM treated rats from 15.28±0.92 to 9.67±0.36 (for SOD) and from 15.85±1.41 to 9.56±0.48 (for CAT). However, the reduced SOD and CAT activities were increased by 82% and 84% respectively after 200mg/kg of *B. monnieri* administration. But treatment with the extract (100 mg/kg) (Group 3) show less significance ($p < 0.001$) in the SOD and CAT levels as compared to the Group 4 animals.

DISCUSSION

Various environmental toxicants and clinically used aminoglycoside like kanamycin, gentamicin can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxides and oxygen reactive species[27]. The nephrotoxicity of GM has been widely investigated with various experimental models. The administration of 80 mg/kg body weight/day of gentamicin sulphate (i.m.) consistently produced nephrotoxicity as reflected by the progressive increase in serum urea and creatinine and these consistent effects have been recognized[28]. As all other aminoglycosides, GM-loaded endocytic vacuoles fuse with lysosomes where the drug accumulates[29]. An earlier study, reported that the renal accumulation of GM across both apical and peritubular membranes of proximal tubular epithelium[30] in rats and there was a close relationship between the accumulation of aminoglycosides in the kidney and the extent of nephrotoxicity[31]. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance[32]. Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity[33]. Similarly, a number of studies demonstrated that proteins are inducible in tubular epithelial cells following renal ischemia/reperfusion[34] and toxic injury[35]. Thus, elevations of serum concentrations of these markers are indicative of renal injury[36]. In our study, we have observed that serum urea and creatinine concentrations were significantly increased ($p <0.001$) in the Group 2 animals than Group 1 which indicating the induction of severe nephrotoxicity. Supplementations of the ethanol extract of *B. monnieri* (100 and 200mg/kg) to Group 3 and 4 rats prevent the elevation of these markers in both groups (figure 1). In contrast, albumin and uric acid levels were decreased with GM treated animals than normal animals in this study and then treatment with ethanol extract of *B. monnieri* in both doses enhanced albumin and uric acid levels in Group 3 and 4 animals.

Evidence from earlier studies, suggested that various enzymatic and non-enzymatic systems have been developed by the cell to cope with the oxidative stress that is associated with reactive oxygen species (ROS) and other free radicals generated[37] in ischemic and renal failure[38]. Reactive oxygen metabolites have been shown to affect several biological processes potentially important in glomerular diseases[39]. Obtained results from the earlier studies revealed that aminoglycosides have the ability to catalyze the formation of free radicals *in vitro* and in intact cells[40] and ROS and reactive nitrogen species (RNS) have been implicated in several renal diseases[41] including the nephrotoxicity induced by the antibiotic GM[42]. Another study reported that GM induces superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO) production from renal mitochondria[43]. In addition, H_2O_2 generation[44], lipoperoxidation[45], and the content of nitrotyrosine[46] are increased and that of reduced glutathione is diminished[47] in renal cortex from GM treated rats. Moreover, the administration of several compounds with antioxidant properties, ROS scavengers, and/ or antioxidant enzymes are able to ameliorate the severity of GM-induced nephrotoxicity[48]. In addition, the kidneys from GM-treated rats are more vulnerable to ROS because of they are deficient in the antioxidant enzymes Mn-superoxide dismutase (Mn-SOD) [49], glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT).

In this present study, we have found that the levels of lipid peroxidation stress markers significantly increased ($p<0.001$) in the GM treated groups (Group 2), when compared to the control group and the groups treated with ethanol extract of *B. monnieri* (figure 2).The activities of GPx, GR, GST, SOD and catalase were significantly ($p < 0.001$) increased with the ethanol extract of *B. monnieri* (Group III and IV) when compared to the GM treated animals (Group II) (Table 3). Equally, GSH activity significantly ($p<0.001$) reduced in the GM treated animals (Group II) than in the normal (Group I) and GM with *B. monnieri* treated animals (Group III).

Table 3: Effect of treatment with ethanol extract of *Bacopa monnieri* on antioxidant stress markers in gentamicin induced nephrotoxicity.

Parameters	Group I	Group II	Group III	Group IV
GSH (nmol/mg protein)	14.03 ± 0.32	9.56 ±0.57*	10.83 ± 0.41*	11.23 ± 0.52*
GPX(Units/mg Protein)	29.06±0.95	22.27±0.21*	25.58±0.45*	27.72±0.48*
GR(Units/mg Protein)	7.49±0.32	4.38±0.15*	5.03±0.48*	5.86±0.38*
GST (IU/L)	46.38±1.18	39.82±2.14*	42.39±2.10*	44.25±2.01*
SOD(Units/mg Protein)	15.28±0.92	9.67±0.36*	11.05±1.24*	12.39±1.36*
CAT(Units/mg Protein)	15.85±1.41	9.56±0.48*	11.84±0.38*	13.23±0.83*

Values were expressed as mean ± S.D, (n=6). *$p < 0.001$ with respect to control

It is reported that the medicinal plants mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids and extract of *B. monnieri* modulates antioxidant activity, and enhances the defense against ROS generated damage in diabetic rats and in various diabetic complications including neuropathy, nephropathy and cardiopathy[50]. A protective effect for Bacopa on the hepatic or renal antioxidant status in morphine-treated rats was reported[51]. The ethanolic extract of *B. monnieri* is reported to be rich in saponins[52]. We have also confirmed the presence of saponins in the ethanolic extract through our preliminary phytochemical screening. Many researchers suggested that antioxidant activity of *B. monnieri* might be due to the presence of saponins. Thus, the saponins in the extracts may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in GM-induced nephrotoxicity.

CONCLUSION

The results of present study indicates that *Bacopa monnieri* extract attenuates renal injury in rats following gentamicin treatment, possibly by inhibiting lipid peroxidation and enhancing or maintaining the antioxidant potentials. These findings suggest the

probable efficacy of extract as a novel nephroprotective agent. Further investigations on the mechanism of action of *Bacopa monnieri* are required and may have a considerable impact on future clinical treatments of patients with renal failure.

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