International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 8 (5): 364-372, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.364.372

Benfotiamine Ameliorate Gentamicin-induced Nephrotoxicity: Effect on Renal Oxidative Stress Markers and Plasma Platelets Activating Factor Acylhydrolase Activity

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Abstract: Benfotiamine (BFT) is lipophilic thiamine precursor elicit many biochemical roles. The aim of this work was to investigate the possible protective effect of BFT against gentamicin (GM) nephrotoxicity. In this study, rats were divided into four groups. Group-1 (control) received normal saline. Group-2 received BFT (100 mg/kg/day). Group-3 received gentamicin (80 mg/kg/day). Group-4 was supplemented with BFT one week after this; they received GM simultaneously for 8 days (BGM). Daily urinary total protein level was estimated to assess kidney dysfunction. On the end of experiment, the rats were dissected sacrificed; kidneys were homogenized then used for biochemical investigations. Blood Urea Nitrogen (BUN), creatinine (CRE), phospholipids, nitrites levels as well as platelets activating factor acylhydrolase (PAF-AH) activity were measured in the blood. Moreover, glutathione (GSH), malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-s-transferase (GST), glucuronidase, N-acetylglucosaminidase (NAG) acid phosphatase (ACP) activities were measured in the homogenate of kidneys. The results of the present study revealed that, GM markedly elevates nitrites, phospholipids, MDA levels and SOD activity by 53, 99, 95 and 72%, respectively in respect to control animal. On contrast, GM decreased the GSH level and catalase, GPx, GST, NAG, ACP glucuronidase and PAF-AH activities by 45, 39, 46, 45, 59, 41, 44 and 44%, respectively compared to control group. BGM treatment keeps the mentioned parameters at values similar that of normal rats. In conclusion, BFT elicit ROS scavenging properties, therefore, it can protect renal tissues against the oxidative damage induced by GM.

Key words: Benfotiamine, gentamicin, nephrotoxicity, glutathione, nitric oxide, platelets activating factor acylhydrolase

INTRODUCTION

The kidneys are the main organs in the process of blood filtration. Therefore, abnormal renal functions often lead to chronic kidney disease. The repeated use of therapeutic agents in combination with antibiotic poses the additional risk of renal failure (Nourani *et al*., 2006). Environmental pollutants and therapeutic agents elicit numerous undesirable side effects in biological systems. The kidney is the most targets for these agents, due to its capacity to extract and concentrate substances (Martinez-Salgado *et al*., 2007).

Aminoglycosides antibiotics are commonly used in the treatment of urinary tract infections. GM is a member of these antibiotics commonly used in the treatment of bacterial infections. The long term therapy with GM induces nephrotoxicity and ototoxicity as major side effects of this antibiotic (Tulkens, 1989). GM is accumulated in the renal proximal convoluted tubules leading to metabolical and functional changes as well as kidney tubular necrosis (Pedraza-Chaverri *et al*., 2000). GM interacts with cellular molecules resulted in disruption the functions of mitochondria, lysosomes as well as endoplasmic reticulum. It also, inhibits lysosomal

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enzymes particularly leading to phospholipidosis (Martinez-Salgado *et al*., 2007).

However, oxidant/antioxidant imbalance is involved in the pathogenesis of many diseases (Mohammadirad and Abdollahi, 2011). GM can induce oxidative stress and decrease antioxidant capacity in the kidney (Martinez-Salgado *et al*., 2002). Also, GM activates Inducible Nitric Oxide Synthase (iNOS) (Martinez-Salgado *et al*., 2007). Moreover, Platelet-Activating Factor (PAF) production is increased in glomeruli by high GM administration (Barbero *et al*., 1995).

PAF is a potent lipid mediator synthesized by a variety of animal tissues; it is activator of platelets, leucocytes and endothelial cells. The alteration in PAF levels was detected in several diseases (Martinez-Salgado *et al*., 2007). It has been reported that, PAF synthesis is increased by the glomerular during GM toxicity (Lopez-Novoa, 1999). PAF is hydrolyzed by platelet-activating factor acetylhydrolase (PAF-AH). PAF-AH exists in the circulation associated with lipoprotein. This enzyme not only breaks PAF but also it degrades oxidized phospholipids that formed in response to oxidative stress (Noto *et al*., 2003).

Nitric Oxide (NO) elicits an abnormal divergence of action, working as either a physiological signaling molecule or as toxic agent (Valdivielso and Blantz, 2002). NO induce cellular damage through formation of peroxynitrite that provoke oxidative modification of lipids, proteins and DNA in biological systems. This may happen when high NO concentrations are generated by iNOS induction (Gordge, 1998). It has been reported that there an increased in iNOS activity by GM administration (Martinez-Salgado *et al*., 2004).

Agents with free radicals scavenging ability can inhibit and/or improve the alteration in renal structures induced by drug (Abdelmeguid *et al*., 2010). Several agents with antioxidant activity have been effectively used to ameliorate GM-induced nephrotoxicity (Abdel-Raheem *et al*., 2010). Moreover, treatment with certain antioxidants decreased the levels of proinflammatory cytokine accompanied by suppression of iNOS activity and markers of oxidative stress associated with glomerulonephritis (Yeh *et al*., 2010). Depletion of water soluble vitamins has been suggested to contribute to GM toxicity. The combination of thiamine; riboflavin and pyridoxal exert an important role against drug induced nephropathy (Bello and Chika, 2009). Benfotiamine (BFT) is thiamine precursor having much higher bioavailability than true thiamine (Wu and Ren, 2006). BFT improve the diabetic associated complications such as neuropathy, nephropathy and retinopathy (Stirban *et al*., 2006). Also, BFT has direct free radical scavenging activity (Schmid *et al*., 2008).

However, until now, the protective effect of BFT against GM-induced nephrotoxicity has not been fully investigated. Based on the reported antioxidant properties of vitamin B complex, this hypothesis was made that BFT could ameliorate GM-induced renal damage. Therefore, the major objective of this study was to investigate the possible protective effect of BFT against GM-induced nephrotoxicity, which was evaluated by measuring kidney some biochemical parameters such as urinary excretion of total protein, BUN and CRE. Moreover, the effect of BFT on plasma nitrites as index for NO production and PAF-AH activity were investigated. Also, effect of BFT and GM on glucuronidase, NAG, ACP was measured in homogenate of kidneys. Additionally, reactive oxygen species (ROS) scavenging properties of BFT were investigated by measuring oxidative stress biomarkers such as GSH, SOD, GST, GPx, catalase activities and MDA levels in kidney homogenate.

MATERIALS AND METHODS

Drugs and chemicals: Benfotiamine was provided by Polpharma, Poland, Gentamicin sulfate was obtained as gift from Memphis Company for Pharmaceutical and Chemical Industry (Cairo, Egypt). Fine chemicals were purchased from Sigma-Aldrich. All other chemicals used of good quality and analytical grade.

Animals: Thirty two male Wistar albino rats weighing 200-250 g were chosen for this study. The rats were obtained from animal house, department of pharmacology, College of pharmacy, Al-Azhar University (Cairo Egypt), which were fed standard diet and water *ad-libitum*. During the study, rats were maintained with a 12 h light/dark cycle in stainless steel metabolic cages to collect urine. Experimental protocols were approved by scientific research practice committee at Al-Azhar University, Egypt.

Experimental protocol: Thirty two rats were divided into 4 groups each of 8 rats: Group 1: Rats in this group were injected with phosphate buffered saline, intraperitoneally and served as a control. Group 2: Rats in this group were orally treated with 100 mg/kg/day of BFT for 15 consecutive days (Katare *et al*., 2010). Group 3: Rats in this group were injected intraperitoneally with 80 mg/kg/day of GM sulfate for 8 day (Abdel-Raheem *et al*., 2010). Group 4: (BGM) group, rats in this group was received BFT for 7 day before GM injection, afterward, they simultaneously treated with combination of BFT and GM at the same previous doses for 8 days.

Total protein levels were estimated in 24 h urine samples every day. At the end of the experiment, the rats were sacrificed by cervical dislocation. Blood samples were collected into heparin containing tubes and left to

stand at room temperature. The plasma samples were separated by centrifugation at 3000 rpm for 15 min and used for determination of BUN, CRE, phospholipids, nitrites and PAH-AH.

Both of the kidneys were collected in 0.15 M KCl (10% w/v) and homogenized using a homogenizer at 4°C, then centrifuged at 10000 xg for to remove unlysed particles. Then, the homogenates were stored at -70°C in liquid nitrogen till analysis.

Biochemical analysis: Determination of total protein contents in 24 h urine samples: Rats were placed in metabolic cages; urine was collected for 24 h every day. Protein concentrations were measured by 3% sulfosalicylic acid method and the resultant turbidity was determined by measurement of absorbance at 450 nm (Nishi and Elin, 1985).

Determination of BUN: Firstly, urea is hydrolyzed in the presence by urease enzyme to produce ammonia and carbon dioxide. The ammonia reacts with hypochlorite and salicylate to form green dye (2, 2 dicarboxylindophenol). The color of the dye was measured at 580 nm (Fawcett and Scott, 1960).

Determination of plasma CRE: CRE level in plasma was determined according to the method of Henry (1974) after protein precipitation. CRE in alkaline media reacts with picrate to form organ colored complex. The color of the complex was measured at 492 nm.

Determination of plasma phospholipids: Samples were deproteinated with trichloroacetic acid in a ratio 1:3, left for 10 min and then centrifuged at 2000 xg for 10 min. The precipitate was used to quantitate total phospholipids (Khan *et al*., 2009).

Assessment of renal oxidative stress parameters: The catalase (CAT) activity was determined according to the method of Aebi (1984). The H_2O_2 decomposition rate was followed by monitoring absorption at 240 nm. One unit of catalase activity is defined as the amount of enzymes required to decompose 1 mmol of hydrogen peroxide in 1 min. The enzyme activity was expressed as mmol H_2O_2 consumed/min/mg protein.

Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler (1984). The enzyme activity was expressed as nmol of GSH oxidized/min/mg protein.

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich (1971). The developed blue color was measured at 560 nm, the activity was expressed as U mg^{-1} protein.

Glutathione S-transferase (GST) activity was assayed by using 1-chloro-2, 4-dinitro benzene (CDNB) (at 340 nm) as described by Habig *et al.* (1974). The formation of a thioether by the conjugation of CDNB to GSH was monitored at 340 nm using a spectrophotometer. The GST activity was expressed as μmoles of thioether formed/mg protein/h, where one unit of enzyme activity is defined as one μmole of thioether formed/min/mg protein.

Reduced glutathione levels (GSH) were determined by the method of Ellman (1959) based on the development of a yellow color when DTNB (5, 5-dithiobis-2 nitrobenzoic acid) was added to compounds containing sulfhydryl groups. Five hundred milliliters of tissue homogenate were added to 3 mL of 4% sulfosalicylic acid. The mixture was centrifuged at 1600 xg for 15 min. Five hundred milliliters of supernatant were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as mg g^{-1} tissue.

Lipid peroxidation: Concentration of MDA in tissues, an index of lipid peroxidation was determined spectrophotometrically (Draper and Hadley, 1990). A 0.5 mL aliquot of kidney supernatant was mixed with 1 mL of trichloroacetic acid solution and centrifuged at 2500 xg for 10 min. One milliliter of a solution containing 0.67% thiobarbituric acid (TBA) and 0.5 mL of supernatant were incubated for 15 min at 90 °C and cooled. Absorbance of TBA-MDA complex was determined at 532 nm using a spectrophotometer. Lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substances (TBARS), using 1,1,3,3-tetra-ethoxypropane as standard. Total protein content was determined by the method of Lowry *et al.* (1951).

Assay of lysosomal enzymes

Assay of acid phosphatase (ACP): ACP activity was estimated spectrophotometrically using *p*-nitrophenylphosphate as substrate (Rosenblit *et al*., 1974).

Assay of glucuronidase: Spectrophotometric method was followed using *p*-nitrophenyl β-D-glucuronide as substrate (Kawai and Anno, 1971).

Assay of N-acetylglucosaminidase (NAG): NAG activity was estimated spectrophotometrically using p-nitrophenyl N-acetylglucosaminidase as substrate (Kawai and Anno, 1971). For all assays, the yellow color developed was read against the blank at 405 nm and *p*-nitrophenol was used as the standard.

PAF-AH assay: PAF-AH activity was measured using the colorimetric method (Wojcicka *et al*., 2010). The assay

uses 2-thio-PAF, which serves as a substrate for PAF-AH. Upon hydrolysis of the acetyl thioester bond by PAF-AH, free thiols are released which react with DNBT, to form 5-thio-2-nitrobenzoic acid detected at 414 nm., the absorbance was read once every minute to obtained 5 time points. Absorbance values were plotted as a function of time, and the PAF-AH activity was calculated from the linear portion of the curve. Results were represented as μmol min^{-1} mL^{-1}.

Nitrite-nitrate assay: Plasma nitrite levels were determined by a colorimetric method based on the Griess reaction (Green *et al*., 1982). Nitrate and nitrite levels were measured by the same assay after enzymatic reduction of nitrate to nitrite with nitrate reductase. The nitrate concentration was calculated from the difference between total nitrite concentrations after and before the reduction.

Statistical analysis: Statistically differences between the studied groups is determined using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post analysis test to compare all groups. The data were expressed as Means±SD, $p \leq 0.05$ were considered significant. GraphPad Prism® was used for statistical calculations (Version 5.00 for Windows, GraphPad Software, San Diego California USA).

RESULTS

Kidney function tests: The results of the present study revealed that supplementation of rats with BFT alone had no effect on urinary excretion of total protein, BUN and CRE levels. In contrast, administration of GM caused a significant elevation of these parameters after 8 days treatment. In the animal concurrently treated with BFT and GM, the elevated urinary content of total protein was significantly reduced by 48%. Moreover, GM produced a significant elevation in BUN, as well as CRE levels when compared to control rats. On the other hand, simultaneous administration of BGM significantly decreases the elevated BUN and CRE by 29 and 38%, respectively compared with GM-treated rats (Table 1).

Renal GSH, CAT, GPx, GST, SOD and MDA: GM produced a decrease in GSH renal content by 45% in comparison with control rats. BFT protect the GM-induced decreased in GSH level by 71% compared to GM treated group. Moreover, CAT, GPx and GST activities were decreased in the homogenate of renal tissues in GM treated rats by 39, 46 and 45%, respectively, when compared with those of the normal animal. Treatment of rats with combination of BFT plus GM keeps the activities of these enzymes at values similar that of control.

SOD activity was significantly elevated in GM treatment group by 72% in respect to control group. On the other hand, supplementation of rats with combination of BFT and GM preserves the SOD activity at values similar rats without any treatment. Furthermore, GM treatment caused an elevation in MDA in renal tissues by 95% compared to normal animals. Treatment of rats with BFT and GM simultaneously normalize the elevated MDA level. Treatment of rats with BFT alone keep these oxidative stress parameters at values like control rats (Table 2).

Renal lysosomal enzymes: The activity of NAG was decreased by 59% after treatment with GM .With respect to glucuronidase; the activity was decreased 44% after treatment with GM. Additionally, the activity of ACP decreased by 41% after treatment with GM. All compared with either control or BFT treated rats. In contrast, treatment of rats with BFT plus GM ameliorates the activity of these enzymes by 84, 58 and 33%, respectively in regarding to GM consumption. Treatment of rats with BFT alone keep the activity of these enzymes at values like control rats (Table 3).

Plasma phospholipids, PAH-AH and nitrites: GM treatment to rats resulted in significant increase of plasma phospholipids 100% treatment alone. Whereas, its plasma significantly deceased by 28% after consumption of combination between the 2 drug. Figure 1 display these results.

In GM group the significant decrease in plasma PAF-AH activity was observed compared to the control group (40%). Consumption of GM plus BFT by the rats caused significant increase in PAF-AH activity versus GM untreated rats by 52%. Treatment of control rats with BFT had no effect on the plasma PAF-AH activity (Fig. 2).

In the current work intake of GM induced significant increase of plasma nitrites level as index for NO

Table 1: Changes in urinary excretion of total protein (UPE), blood urea nitrogen (BUN) and creatinine (CRE), by exposure to BFT, GM and their combination (BGM) compared with control group

Treatment	UPE mg/day	CRE mg dL^{-1}	BUN mg dL^{-1}
Control	10.65±2.08	0.31±0.05	24.37±3.74
BFT	10.13±2.03	0.27±0.05	22.56±3.50
GM	83.80±7.56[a]	2.60±0.60[a]	54.02±5.25[a]
BGM	43.48±5.71[a,b]	1.62±0.30[a,b]	38.07±4.44[a,b]

Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8), Tukey's post test was performed to determine differences between mean values, within a row, statistically different values are marked with a, b, superscript letters when a significant was observed ($p \leq 0.05$), a: Significantly increased from either control or BFT treatment, b: Significantly decreased from GM treated group

Table 2: Effects of BFT, GM and their combination BGM on renal tissue contents of GSH and MDA as well as CAT, SOD, GPx and GST activities compared to control group

Parameter	Control	BFT	GM	BGM
GSH (μg g^{-1} tissue)	123.7±5.04	126.5±4.23	67.47±11.1[a]	115.9±7.27[b]
CAT (nM of H_2O_2 consumed/min /mg protein)	121.2±8.11	125.3±5.98	73.77±6.41[a]	107.7±8.77[ab]
GPx nM GSH oxidized/min /mg protein)	27.66±5.17	28.54±5.16	14.58±3.44[a]	21.21±3.68[ab]
GST(μM thioether formed /min/mg protein)	0.584±0.12	0.635±0.13	0.321±0.078[a]	0.514±0.089[b]
SOD (U mg^{-1} protein)	239.5±20.6	242.2±15.0	412.6±18.0[c]	279.9±14.90[b]
MDA (nM g^{-1} tissue)	58.96±7.67	54.60±8.00	116.6±11.3[c]	71.60±9.17[b]

Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8), Tukey's post test was performed to determine differences between mean values, within a row, statistically different values are marked with a, b and c superscript letters when a significant was observed at $p \leq 0.05$, a: Significantly decreased from either control or BFT treatment group, b: Significantly decreased from GM treated group, c: Significantly increased from control group

Table 3: Effects of BFT, GM and their combination BGM on renal tissue activity of NAG, ACP and glucuronidase compared to control group

Factor	Control	BFT	GM	BGM
NAG (U/g tissue)	1.23±0.19	1.17±0.18	0.51±0.11[a]	0.94±0.15[b]
ACP (U g^{-1} tissue)	4.83±0.77	4.67±0.71	2.85±0.46[a]	3.79±0.65[ab]
Glucuronidase (U mg^{-1} tissue)	105±12.7	102±12.4	58.7±11.2[a]	92.9±9.05[b]

Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8), Tukey's post test was performed to determine differences between mean values, within a row, statistically different values are marked with a and b superscript letters when a significant was observed at $p \leq 0.05$, [a]Significantly decreased from either control or BFT treatment group, [b]Significantly increased from GM treated group

production by 53% in regarding to normal healthy animals. On the other hand, BFT plus GM improve the nitrates level in comparison with injection of GM alone by 36%. The statistical data of these results were represented in (Fig. 3). BFT consumption by the rats keep the nitrites level the same as normal control group.

DISCUSSION

Numerous agents were utilized to prevent GM nephrotoxicity in experimental animal model; however, their use to treat human subjects in clinical practice could not be achieved (Khan *et al*., 2009). BFT reduces free radical formation by activation of pentose phosphate pathway, which regenerates NADPH, such molecule is important for replenishing the major cellular antioxidants (GSH).This attributed to actions of BFT in improvement of transketolase activity as well as it direct antioxidant effect

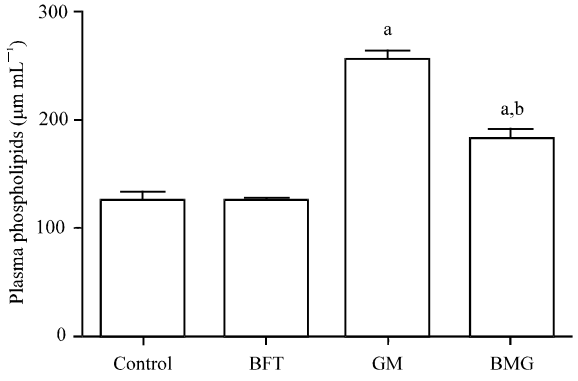

Fig. 1: Changes in plasma phospholipids by exposure to BFT, GM and their combination (BGM) compared with control, Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8), Tukey's post test was performed to determine differences between mean values, statistically different values are marked with a, b, superscript letters when a significant was observed ($p \leq 0.05$), [a]Significantly increased from either control or BFT treatment, [b]Significantly decreased from GM treated group

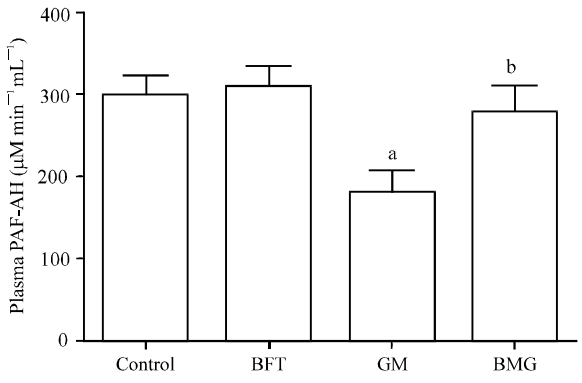

Fig. 2: Changes in plasma PAF-AH activity by exposure to BFT, GM and their combination (BGM) compared with control, Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8), Tukey's post test was performed to determine differences between mean values, statistically different values are marked with a, b, superscript letters when a significant was observed ($p \leq 0.05$), [a]Significantly decreased from control or BFT treatment, [b]Significantly increased from GM treated group

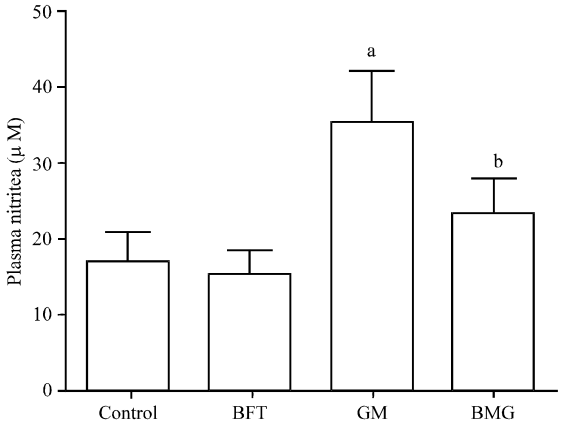

Fig. 3: Changes in plasma nitrite level by exposure to BFT, GM and their combination (BGM) compared with control Data were tested by one-way analysis of variance and represented as Mean±SD, eight rats in each group (N = 8). Tukey's post test was performed to determine differences between mean values, statistically different values are marked with a, b, superscript letters when a significant was observed ($p \leq 0.05$), [a]Significantly increased from control or BFT treatment, [b]Significantly increased from GM treated group

(Marouf *et al*., 2010). Therefore, BFT has beneficial effects in the treatment of oxidative stress induced nephropathy. As well, BFT elicit cardioprotective, neuroprotective and antidiabetic as well `as other health benefits (Katare *et al*., 2010). In the present study we have tested the hypothesis that BFT consumption would ameliorate GM-induced nephrotoxicity.

In this study, GM administration to control rats produced a typical pattern of nephrotoxicity which was manifested by marked increase in urinary protein excretion, CRE and BUN. These observations are similar to the finding of Dehghani *et al.* (2011) and Lakshmi and Sudhakar (2010), who reported that GM was induced tubular necrosis, increases BUN, CRE and kidney atrophy. Additionally, Dodiya *et al.* (2011) confirmed that nephrotoxic treated rats showed proteinuria and microalbuminuria. The increases in plasma phospholipids in the current study run in parallel to the study of Banday *et al.* (2008).

In contrast to GM, BFT consumption keeps CRE and BUN at values similar to those of normal rats. These finding are similar to the demonstration of previous study stated that antioxidant can protect against GM induced renal damage (Mohamadin *et al*., 2005). Consumption of BFT with GM by the rats significantly lowered GM-elicited increased levels of CRE, BUN and urinary protein excretion. Similarly, Bello and Chika (2009) reported that supplementation of vitamins B complex has beneficial effect against GM induced nephrotoxicity. As well, phospholipids level was increased upon GM treatment and in BFT consuming rats. However, phospholipids are essential membrane components and their significant increase by BFT may facilitate repair and regulations of various membranes as required after GM treatment. Equally similar study reported that free radical scavenger ameliorate phospholipids level (Szachowicz-Petelska *et al*., 2005).

Intracellular organelles such as mitochondria and lysosomes are known GM target toxicity (Banday *et al*., 2008). Lysosomes are reported to play an important role in cell death and tissue damage due to GM (Whiting and Brown, 1996). Therefore, the structural/functional integrity was assessed by the status of their respective biomarker enzymes. In the present study GM caused significant decrease in the activities of ACP, NAG and glucuronidase in renal homogenates. BGM consumption resulted in the reversal of GM-induced alterations in the studied renal lysosomal enzymes activities. Alterations in ACP activity demonstrate GM-induced damage to lysosomes (Banday *et al*., 2008). BFT consumption by its antioxidant properties prevented this loss either by lessening the damage caused by GM or by increasing the regeneration process. This is supported by the study of Ohtani *et al.* (1974) demonstrated that thiamine prevent aminoglycosides induced toxicity. The decrease in the activity of lysosomal enzymes may contribute to GM-induced abnormal accumulation of proteins and lipids resulted in renal damage. BFT ameliorate GM induced abnormality in these enzymes.

Most of the antioxidant enzymes become inactive in response to oxidative stress. The decrease of catalase, GPx and GST activities in the kidney tissues by GM administration in the present investigation run in parallel to the study of Pedraza-Chaverri *et al.* (2005). However, Free radicals are causative factors for aminoglycosides induced renal toxicity (Dwivedi *et al*., 2009). It has demonstrated that GM generates ROS that mediate biomolecules oxidation in the kidney (Walker *et al*., 1999). The excessive ROS can damage the protein sensitive thiols. Therefore, GM inhibits the activities of antioxidant enzymes, catalase and GPx and depletes thiol cellular content (Dean *et al*., 1991). In the current study GM significantly enhanced MDA in renal tissues. This indicating GM induced oxidative damage in renal cells. On the other hand, the BFT consumption with GM improves GH content and catalase, GPx as well as GST activity. Moreover, BFT consumption with GM attenuates the MOA level. This may be attributed to free radical scavenging property of BFT as well as direct antioxidant

action (Schmid *et al*., 2008). Furthermore, BFT increase NADPH by activation of pentose phosphate pathway which replenishes GSH. However BFT is coenzyme for the transketolase (Marouf *et al*., 2010). These were supported by the finding of Adaramoye (2009) who demonstrated that antioxidant vitamins are able to enhance recovery from renal oxidative damage.

In contrast to the above mentioned antioxidant enzyme, SOD is significantly increase by GM consumption. Thus, enhanced SOD level may be a compensatory mechanism against oxidative stress (Salomon *et al*., 2002; Soudani *et al*., 2010). BGM, treatment preserve SOD activity at values near to that control rats. These results demonstrated that BFT significantly enhanced antioxidant defense against GM induced oxidative damage in renal tissues.

PAF-AH may play a role as a scavenger of oxidized phospholipids which are thought to be involved in diverse pathological processes. In this work, plasma PAF-AH activity was decreased in GM treated group compared to control one. The activity PAF-AH is attenuated in oxidative stress situation (Ambrosio *et al*., 1994). Oxidative stress elicited by GM could inactivate the enzyme by oxidative reaction. However, the influence of GM on antioxidant/oxidant balance is fully established. Consumption of BGM ameliorates PAF-AH activity versus GM untreated, this may due to free radicals scavenging capabilities of BFT. On contrast there is other study reported that PAF-AH was increased in response to oxidative stress (Wojcicka *et al*., 2010).

In the current work intake of GM induced marked increase nitrites as index for NO production. This supported by the finding of Abdelaziz and Kandeel (2011), who demonstrated that NO was increased by aminoglycosides induced renal toxicity. Moreover, Kandeel *et al*. (2011) reported that there are an increase in the levels of oxidative stress biomarkers including NO in response to GM toxicity. As well, there is an increased in iNOS activity by GM administration (Martinez-Salgado *et al*., 2004). This is the probable cause for increase NO production by GM. NO induce renal cellular damage through formation of peroxynitrite that provoke the damage to the cellular structural molecules. On the other hand, BGM improve the nitrite level due to minimization of oxidative stress induced by GM consumption. However, Abdelaziz and Kandeel (2011) demonstrated that the renal toxic effect of certain aminoglycosides mediated by increase of NO is ameliorated by antioxidants.

The results of the present study suggest that decrease in the activity of lysosomal enzymes may contribute to GM-induced abnormal accumulation of proteins and lipids resulted in renal damage. BFT ameliorate GM induced abnormality in these enzymes these results demonstrated that BFT significantly enhanced antioxidant defense against GM induced oxidative damage in renal tissues.

In conclusion, GM-induced nephrotoxicity by increase ROS, NO formation as well as that decrease renal GSH content and inactivate GPx, catalase and GST and PAF-AH. Moreover, GM increases the cell membrane damage by increasing lipids peroxidation. BFT protects renal tissues against the oxidative damage induced by GM, which may be attributed to ROS scavenging properties. The present results suggest that BFT consumption can be an option for the long term clinical use of GM as an antibacterial drug without nephrotoxic and other harmful side effects.

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