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AMELIORATIVE EFFECT OF VOLVARIELLA VOLVACEA AQUEOUS EXTRACT (BULLIARD EX FRIES) SINGER ON GENTAMICIN INDUCED RENAL DAMAGE

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ABSTRACT

Mushrooms are used widely in Asian cuisines. *Volvariella volvacea*, commonly known as the paddy straw mushroom is an edible fungi. Mushrooms are found to be rich in phytochemicals that function as potent antioxidants. Aminoglycosides like Gentamicin, used to treat infections are reported to cause renal damage. The present study was undertaken to evaluate the capacity of the aqueous extract of *Volvariella volvacea* on Gentamicin induced renal damage in rats. The extract at 500 and 1000mg/kg body weight was observed to normalize the urine volume, serum and urine levels of creatinine, urea and uric acid. The mushroom extract was observed to improve the depressed antioxidant status and reduce the lipid peroxidation levels. The present study thus reveals the capacity of the extract to reverse the alterations imposed by Gentamicin.

KEYWORDS : Mushrooms, *Volvariella volvacea*, Gentamicin, Nephroprotective

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INTRODUCTION

Gentamicin (GM), an aminoglycoside antibiotic, is widely used in clinical practices for the treatment of life threatening gram-negative infections. This antibiotic generally causes drug-induced dose-dependent nephrotoxicity in 10–20% of therapeutic courses (Karahan *et al*., 2005). GM induced nephrotoxicity is characterized by direct tubular necrosis (Eisenberg *et al*., 1987; Cuzzocrea *et al*., 2002). GM generates hydrogen peroxide (H_2O_2) in rat renal cortex mitochondria and can also enhance the generation of reactive oxygen species (ROS) (Yanagida *et al*., 2004; Karahan *et al*., 2005). Abnormal production of ROS, induced cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Parlakpinar *et al*., 2005). The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of GM mediated nephrotoxicity (Karahan *et al*., 2005). GM also acts as an iron chelator and the iron–GM complex is a potent catalyst of radical generation (Yanagida *et al*.,2004). Several compounds with antioxidant activity have been successfully used to prevent or ameliorate GM-induced nephrotoxicity (Pedraza-Chaverrí *et al*., 2003; Yanagida *et al*., 2004; Maldonado *et al*., 2003).

Mushrooms accumulate a variety of qualitatively good protein, crude fibre, minerals and vitamins but are poor sources of lipids and are rich in secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Mushrooms have long been attracting a great deal of interest in many areas of foods and biopharmaceuticals. They are well known for their nutritional and medicinal values (Gao *et al*., 2004).

Volvariella volvacea , the paddy straw mushroom is being cultivated throughout East and Southeast Asia and used extensively in Asian cuisines.The study was undertaken to explore the effect of aqueous extract of *Volvariella volvacea* on GM induced renal damage.

2. MATERIALS AND METHODS

Chemicals

Gentamicin Sulphate i.p was purchased from NATCO Pharma Ltd, Hyderabad, India. All chemicals used for the assay were of analytical grade and were purchased from Sigma-Aldrich, Bangalore, India.

2.1 Plant sample

The mushroom was obtained from Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. The fruiting bodies were shade dried and powdered. 10g of the powder was extracted with 100 ml of water at 100^0C for 4 hours, centrifuged at 5000rpm for 15 minutes and filtered through Whatman No.1 filter paper. The residue was extracted twice with 100ml portions of water, as described above. The extracts were combined and vacuum evaporated. The extract obtained after vacuum evaporation was freeze dried and stored at 4^0C until further use.

2.2 Experimental Animals

Female Sprague Dawley rats, weighing, 160g-180g were purchased from, Small Animal Breeding Centre, College of Veterinary and Animal Science, Mannuthy, Kerala, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (AVM Cattle Feeds, Coimbatore, Tamilnadu) 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.

2.3 Experimental Design

The animals were divided into 5 groups each of 6 rats:

Group I: Rats in this group served as a control. Group II:
with 80 mg/kg/day of gentamicin as i.p for 7 days (Silan *et al*., 2007; Soliman *et al*., 2007). Group III: Rats in this group were simultaneously treated with the 500mg/kg b.wt of aqueous extract of *V*. *volvacea* one hour prior to each GM injection. Group IV: Rats were simultaneously treated with the 1000mg/kg b.wt of aqueous extract of *V*. *volvacea* one hour prior to each GM injection. Group V: Rats were simultaneously treated with the 250mg/kg b.wt of vitamin E one hour prior to each GM injection.
After dosing on the day 7, individual rats were placed in separate metabolic cages for 24h for urine collection .The animals were sacrificed on the 8th day, 24h after the last GM injection.

2.4 Biochemical Analysis

10% homogenate of the kidney tissues was prepared with 0.1 M Tri-HCl buffer, pH 7.4.
Serum was prepared from whole blood. The homogenates were centrifuged at 3000 rpm for 15 min at 4ºC for cytosolic separation.
Urine volume, protein, urea, uric acid and creatinine levels were analyzed in serum and in urine. The levels of serum and urine creatinine were determined by the method of Owen et al., (1954). The levels of uric acid in the serum and urine were analyzed by the method of Caraway, (1963) and urea levels were determined using Erba® kit based on the method described by (Kerscher and Ziegenhorn, 1990). Protein levels were determined by Lowry et al (1957).
The enzymatic activity of renal superoxide dismutase (SOD) was assessed according to the method of Das et al (2000) and Catalase (CAT) by the method of Sinha (1972). The activity of Glutathione –S- transferase (GST) was determined by the method of Moron et al.(1979). The activity of the enzyme Glutathione peroxidase (GPx) and the Glutathione (GSH) content of kidney tissues homogenate were assessed using Ellman's reagent according to the method described by Ellman (1959). Renal vitamin C was determined by Omaye et al. (1979).
Rat kidney homogenate lipid peroxide (LPO) levels were measured by colorimetric determination of TBARS according to the method of Niehus and Samuelsson, (1968).Hydroperoxide levels were assessed by Jiang et al. (1992).

2.5 Histopathological Examination

The kidneys of each animal were dissected out then fixed
in buffered formalin for 12 hours and processed for histopathological examination. Four µm-thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol (Allen, 1992).

2.6 Statistical analysis

The data are expressed as mean ± S.D. Statistical comparison was done at significance level, P<0.05 using SPPS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

3. RESULTS

3.1 Biochemical analysis

Table 1 depicts the effect of the aqueous extract of *V .volvacea* on urine volume, and the levels of urea, uric acid and creatinine in serum and urine of control and experimental animals.
The urine volume was found to be reduced significantly (p<0.05) in the GM control animals (group II) as compared against group I animals. The urine output was observed to be increased in the group III and IV animals that were treated with aqueous extract of *V .volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt respectively. In the group V rats that were treated with vitamin E, the urine output was also significantly (p<0.05) increased as compared to GM alone induced, group II animals.

In the present study there was observed a significant ($p<0.05$) decrease in the levels of urea, uric acid and creatinine and increase in protein levels in the urine, whereas there was found to be significant increase ($p<0.05$) in the serum urea, uric acid and creatinine levels and a marked decrease ($p<0.05$) in serum protein levels in the GM induced group II animals as compared to normal group I animals. Treatment with aqueous extract of *V.volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt significantly ($p<0.05$) restored the altered levels in the serum and in the urine to near normal values in a dose dependent manner in line with the standard antioxidant vitamin E treated group V rats as compared to the GM alone induced group II rats.

Table 1

Effect of aqueous extract of V .volvacea on urine volume and levels of total protein , urea, uric acid and creatinine in the serum and urine of control and GM induced experimental animals

Parameters	Control	GM (80mg/kg bwt)	GM+ VVAE (500mg/kg bwt)	GM+ VVAE (1000mg/kg bwt)	GM+ Vit E (250 mg/kg bwt)
Urine					
Urine volume (ml/24h)	6.5 ± 0.30 [b]	2.25 ± 0.10 [a]	4.10 ± 0.15 [ab]	5.70 ± 0.30 [b]	6.10 ± 0.25 [b]
Protein (g/dl)	0.97 ± 0.04 [b]	3.90 ± 0.16 [a]	2.14 ± 0.08 [ab]	1.15 ± 0.03 [b]	1.21 ± 0.08 [b]
Urea(mg/dl)	40 .73 ± 2.31 [b]	13.16 ± 1.12 [a]	27.24 ± 2.16 [b]	34.75 ± 1.40 [b]	39.24 ± 1.15 [b]
Uric aicd(mg/dl)	1.47 ± 0.04 [b]	0.86 ± 0.02 [a]	1.01 ± 0.03 [ab]	1.34 ± 0.09 [b]	1.13 ± 0.02 [b]
Creatinine(mg/dl)	1.60 ± 0.06 [b]	0.45 ± 0.02 [a]	0.98 ±0.03 [b]	1.08 ± 0.06 [b]	1.17 ± 0.04 [b]
Serum					
Protein (g/dl)	5.74 ± 0.20 [b]	4.02 ± 0.13 [a]	4.81 ± 0.22 [ab]	5.65 ± 0.36 [b]	5.60 ± 0.19 [b]
Urea(mg/dl)	39.81 ± 1.91 [b]	58.07+ 2.60 [a]	46.39 ± 2.42 [b]	41.70 ± 1.29 [b]	40.24 ± 2.46 [b]
Uric aicd(mg/dl)	2.63 ± 0.09 [b]	7.01 ±0.27 [a]	5.33 ± 0.21 [b]	3.22 ± 0.16 [b]	3.01 ± 0.08 [b]
Creatinine(mg/dl)	0.71 ± 0.03 [b]	1.46 ± 0.05 [a]	1.12 ± 0.06 [ab]	0.80 ± 0.03 [b]	0.73 ± 0.04 [b]

Values are expressed as mean ± SD, n=6; VVAE- aqueous extract of V. volvacea: Vit E – vitamin E
Group comparison and statistical significance at p<0.05: [a]: Group I vs. II, III, IV, V[b:] Group II vs. I, III, IV, V

The effect of the aqueous extract of *V .volvacea* on the activity of the antioxidant enzymes (SOD, CAT, GPx and GST) GSH and vitamin C in the kidney of the control and experimental animals is presented in Table 2. In the present study there was a marked ($p<0.05$) decline in the activities of the antioxidants, SOD, CAT, GPx, and GST and levels of GSH, and vitamin C in the kidney of the GM induced , group II rats as compared against group I control animals. In the group III and group IV rats that were treated with aqueous extract of *V .volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt, there was observed a significant elevation ($p<0.05$) in the antioxidant status in a dose dependent manner. Treatment of the group V rats with vitamin E also resulted in a marked elevation in the antioxidant status as against group II rats.

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Table 2
Effect of aqueous extract of V .volvacea on activity of the enzymic and non- enzymic antioxidants in the kidney of control and GM induced experimental animals

Parameters	Control	GM (80mg/kg bwt)	GM+ VVAE (500mg/kg bwt)	GM+ VVAE (1000mg/kg bwt)	GM+ Vit E (250 mg/kg bwt)
Kidney					
SOD	3.21 ± 0.14 [b]	1.05 ± 0.07 [a]	2.46 ± 0.11 [a b]	3.17 ± 0.16 [b]	3.19 ± 0.21 [b]
CAT	7.21 ± 0.43 [b]	2.44 ± 0.08 [a]	5.80 ± 0.05 [b]	7.12 ± 0.32 [b]	7.09 ± 0.24 [b]
GPx	3.29 ± 0.04 [b]	0.69 ± 0.03 [a]	1.99 ± 0.06 [b]	2.95 ± 0.04 [b]	3.10 ± 0.10 [b]
GST	124.8 ± 2.87 [b]	58.10 ± 3.90 [a]	107.40 ± 4.21 [b]	120.30 ± 6.10 [b]	121.10 ± 4.26 [b]
GSH	13.70 ± 0.55 [b]	7.26 ± 0.32 [a]	11.69 ± 0.71 [a b]	12.55 ± 0.26 [b]	12.27 ± 0.29 [b]
Vitamin C	1.48 ± 0.03 [b]	0.54 ± 0.02 [a]	1.03 ± 0.05 [b]	1.23 ± 0.03 [b]	1.36 ± 0.04 [b]

Values are expressed as mean ± SD, n=6. VVAE- aqueous extract of V .volvacea ; Vit E- vitamin E.

***Units**:*
SOD- inhibition of 50% nitrite formation/min/mg protein; CAT- µmoles of H_2O_2 consumed/min/mg protein; GPx- µmoles of glutathione oxidized/min/mg protein; GST - µmoles of CDNB-GSH conjugate formed/min/mg protein; GSH, Vitamin C - µg/mg protein Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V [b]: Group II vs. I, III, IV, V Table 3 represents the lipid peroxidation levels as TBARS and hydroperoxides in the control and experimental animals. The levels of TBARS and hydroperoxides was observed to be increased significantly ($p<0.05$) in the renal tissue of the group II rats as compared against normal control rats. Marked decline ($p<0.05$) in the levels of peroxides was observed in the group III and group IV rats that were treated with aqueous extract of *V volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt respectively. Treatment with standard antioxidant vitamin E to the group V rats also resulted in a significant decline in the lipid peroxidation levels and hydroperoxide levels as against group II rats.

Table 3
Effect of aqueous extract of V. volvacea on TBARS and hydroperoxides in the kidney of control and GM induced experimental animals

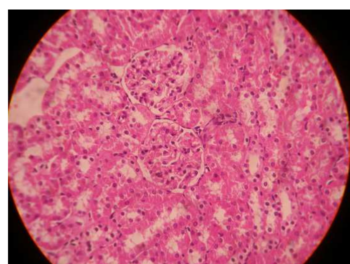
Parameters	Control	GM (80mg/kg bwt)	GM+ VVAE (500mg/kg bwt)	GM+ VVAE (1000mg/kg bwt)	GM+ Vit E (250 mg/kg bwt)
Kidney					
TBARS (nM of MDA formed/min/mg protein)	1.12 ± 0.05 [b]	2.99 ± 0.15 [a]	1.73 ± 0.04 [b]	1.19 ± 0.03 [b]	1.14 ± 0.02 [b]
Hydroperoxides (Mm H_2o_2 /g tissue)	3.92 ± 0.11 [b]	9.42 ± 0.20 [a]	6.75 ± 0.22 [b]	4.01 ± 0.14 [b]	4.06 ± 0.19 [b]

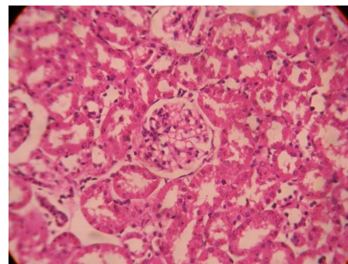
Values are expressed as mean ± SD, n=6 ; VVAE- aqueous extract of V volvacea ; Vit E- vitamin E
Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V
[b]: Group II vs. I, III, IV, V

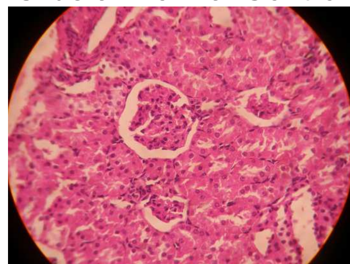
3.2 Histopathological studies

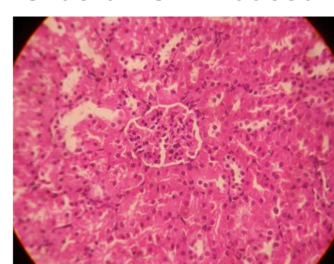
The photomicrograph represents the protective effect of the aqueous extract of *V.volvacea* against hitopathological alterations induced by GM in the kidney tissue of different experimental groups. (Figure 1- slide a-e).

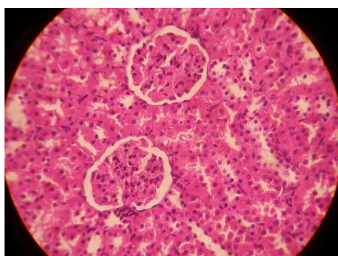
Figure 1
Effect of the aqueous extract of Volvariella volvacea on the histology of the kidney of experimental animals.


Slide a: Normal Control


Slide b : GM induced. .


Slide c: GM + aqueous extract of *V.volvacea at* 500mg/kg b.wt


Slide d : GM + aqueous extract of *V.volvacea* at 1000mg/kg b.wt



Slide e :GM + Vitamin E(250mg/kg b.wt)

Figure 1 (a) Histological structure of renal tissue of the normal control animals, presenting normal glomeruli, proximal and distal convoluted tubules. (b) Renal tissue sectioning of the GM control group animals , the architecture presenting inflammation, tubular nerosis and interstitial nephritis.(c) Renal tissue sectioning of animals treated with 500mg/kg b.wt of aqueous extract of *V.volvacea* and GM induced, the architecture presenting, mild inflammation and tubules and glomeruli appearing near normal histology. (d) Renal tissue sectioning of animals treated with 1000mg/kg b.wt of aqueous extract of *V.volvacea* and GM induced, the architecture presenting negligible inflammation, the tubules and glomeruli appearing normal.

4. DISCUSSION

Drug-induced nephrotoxicity is an important cause of renal failure (Perazella, 2003). Aminoglycosides, such as GM, are a class of clinically important antibiotics used extensively in the treatment of infections, particularly against aerobic gram-negative bacteria (Nagai and Takano, 2004). However, in nephrotoxicity and ototoxicity there are serious side effects in the use of aminoglycosides.

It has been reported that GM-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. (Pedraza-Chaverrí *et al.*,2003). GM undergoes more extensive accumulation in kidneys, and it is trapped by cells of renal cortex via different mechanism (Wiland and Szechcinski, 2003). Because of the obvious responsibility of ROS in GM induced renal damage, antioxidants could be used to block GM nephrotoxicity.

Marked reduction in the urine output observed in the GM induced group could be due to the tubular damage and necrosis caused due to GM. The significant improvement in the urine output observed in the rats that were treated with the aqueous extract of *V .volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt suggests the protective nature of the extract. This improvement could be due to the capacity of the extract in protecting the renal cells against the toxic insult of GM.

Safa et al. (2010) reported a significant decrease in the urine volume in the rats that were induced with GM .Treatment with grape seed extract was found to increase the urine output, indicating protective effect on the kidney glomeruli and tubules. *Sida rhomboidea.*Roxb extract was found to increase the output of urine and thereby ameliorate the toxic effects of GM (Thounaojam *et al*., 2010).

The observed decrease in the levels of urine urea, creatinine and uric acid and increase in levels of serum urea, creatinine and uric acid in GM induced rats suggests the renal damage caused by the aminoglycoside.

GM reduces the glomerular filtration rate which is shown by an increase serum creatinine. Results from many studies (Silan *et al*., 2007; Soliman *et al*., 2007) have shown that GM produced an elevation in the concentrations of biochemical indicators of kidney function such as urea, creatinine in serum and total protein excretion in urine. Consistent with the data from the study of Pedraza-Chaverrí et al. (2004) we observed in

our study that urinary excretion of total protein was increased after GM injection indicating tubular damage. On the other hand, serum urea and serum creatinine levels were augmented indicating glomerular damage.

The combined administration of aqueous extract of *V.volvacea* at both the doses significant reduction in the elevated levels of urinary total protein concentrations, serum urea, uric acid and creatinine and increased the serum total protein levels and improved the excretion of urea, uric acid and creatinine in urine .

These results are in accordance with several other researches, which reported that, compounds with antioxidant properties like s-allylmercaptocysteine (Pedraza- Chaverrí *et al*., 2004) and diallyl sulfide (Pedraza- Chaverrí *et al*., 2003) inhibited the increased urinary excretion of total protein induced by GM in rats. Other compounds like resveratrol (Silan *et al*., 2007), carnosine (Soliman *et al*., 2007) or garlic extract (Maldonado *et al*., 2003), Quercetin (Abdel-Raheem *et al* ., 2009) partially prevented the increase in serum urea and serum creatinine levels induced by GM.

The extracts of mushroom *Pleurotus porrigens* was able to ameliorate the effects of GM (Moghaddam *et al*., 2010).The results obtained were in line with Kore et al (2011), who reported that *Abutilon indicum* extract was able to significantly restore the alterations induced by GM.

The capacity of the mushroom extract in normalizing the biochemical alterations and the urine output indicates the protective nature of the mushroom extract against ROS formed from GM metabolism.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in renal damage induced by the antibiotic GM (Maldonado *et al.,* 2003; Cuzzocrea *et al.,* 2002). In the present study there was found to be a marked ($p<0.05$) decline in the activities of the antioxidants, SOD,CAT,GPx, and GST and levels of GSH, and Vitamin C in the kidney of the GM induced , group II rats as compared against group I control animals. In the group III and group IV rats that were treated with aqueous extract of *V.volvacea* at 500mg/kg bwt and 1000mg/kg bwt, there was observed a significant elevation ($p<0.05$) in the antioxidant status. Treatment of the group V rats with Vitamin E also resulted in a marked elevation in the antioxidant status as against group II rats.

The decrease in SOD activity after GM administration might be due to the loss of copper and zinc which are essential for the enzyme activity (Badary *et al*., 2005). The decreased SOD activity is insufficient to scavenge the superoxide anion produced during the normal metabolic process and could cause the initiation and propagation of lipid peroxidation in the GM alone treated group II animals. The decrease in the activity of CAT and GPx, in turn increases H_2O_2 concentration and enhances the lipid peroxidation.

GM induced nephrotoxicity was associated with low activity of GPx, CAT, SOD and levels of GSH in the renal tissue. The conversion of GSSG to GSH is mediated through GR. GR may interfere with the recycling of GSSG into GSH by inhibition of GR. Quercetin was reported to potentiate the activity of GR under stress condition, an effect that may lead to enhancement of recycling of GSSG back to GSH. Levels of GSH were found to improve on treatment with Quercetin (Abdel rahem *et al*., 2009).

These decreases in renal antioxidant enzymatic protection could aggravate the oxidative damage. The increased production of ROS in GM induced nephrotoxicity may cause inactivation of antioxidant enzymes

(Karahan *et al*., 2005). Treatment with the extract enhances activity of SOD, CAT,GST and GPx compared to the GM alone treated animals.

One of the most important intracellular antioxidant systems is the GSH redox cycle. Glutathione is one of the essential compounds for maintaining cell integrity (Conklin, 2000). The decreased concentration of GSH increases the sensitivity of organ to oxidative and chemical injury. The role of GSH, a non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites most often formed by Cytochrome P-450 linked monooxygenase is well established (Rana *et al*., 2002). A number of studies reveal that the metabolism of xenobiotics often produced GSH depletion (Mitchell *et al.,* 1973; Jollow *et al*., 1974).

In the present study, the levels of GSH in rat kidney tissues were significantly reduced after GM injection compared with control group. This result is confirmed by other studies, which have pointed to reduction of GSH levels after GM administration ((Silan *et al*., 2007; Soliman *et al*., 2007). An explanation to GSH depletion after GM treatment is increased consumption of GSH in non-enzymatic removal of oxygen-radicals. In addition, oxidation of GSH to GSSG by the oxidant stress, with efflux of GSSG being the major factor responsible for maintenance of the redox ratio (Eberle *et al*., 1981)**.** The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the GM treated animals.

Circulating antioxidants such as vitamin C (ascorbic acid) and vitamin E (tocopherol) are non-enzymatic scavengers of free radicals. (Arivazhagan *et al.* 2000).

The decrease in the levels of vitamin C depicts ROS damage. Concomitant treatment with the aqueous extract of *V.volvacea* and standard , vitamin E rendered protection due to the increase in GSH and vitamin C concentration and could protect the renal cells from oxidative attack.

The result of the present study was found to be in accordance with Nitha and Janardhanan,(2008) who reported that treatement of the GM induced rats with the extract of the mushroom *Morchella esculenta* was found to significantly elevate the levels of SOD,CAT ,GPx and GSH. Treatment with resveratol was found to significantly improve the activities of GST in GN induced renal toxicity Silan et al (2007). Abdel-Raheem et al (2009) observed the increase in the activities of SOD and CAT upon treatment with Quercetin to GM induced rats.

A large body of *in vitro* and *in vivo* evidence indicates that ROS are important mediators of GM nephrotoxicity (Walker *et al*., 1988;1999) These changes of membrane lipid composition may be induced by free radical-initiated lipid peroxidation (Sandhya and Varalakshmi 1997). This view is supported by increased MDA levels, one of the products of lipid peroxidation, in GM treated rats kidney (Parlakpinar *et al*., 2005).

Abnormal production of ROS, induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage, and this is believed to be involved in the etiology of many xenobiotics toxicity (Kehrer, 1993; Baliga *et al*., 1999; Parlakpinar *et al*., 2005). The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of GM nephrotoxicity.

The levels of lipid peroxides and hydroperoxides was to be increased significantly($p<0.05$) in the renal tissue of the group II rats as compared against normal control rats. Marked decline ($p<0.05$) in the levels of peroxides was observed in the group III and group IV rats that were treated with the aqueous extract of *V.volvacea* at 500mg/kg b.wt and 1000mg/kg b.wt respectively. Treatment with standard antioxidant vitamin E to the Group V rats alos resulted in a significant decline in the lipid peroxidation levels as against group II rats.

This increase in lipid peroxides observed could be due to the ROS generated by GM. It had been shown that GM treatment causes enhanced generation of superoxide anion and hydrogen peroxide (Baliga *et al*., 1999; Walker *et al.,* 1999)

The observed decrease in the levels of peroxides in the kidney tissue after GM induction is suggestive of the protective role of the mushroom extract. This could be due to the antioxidant capacity of the extract in neutralizing the ROS produced by the GM metabolites.

Previous reports suggested the elevated levels of TBARS in kidney tissue of the rats induced with GM (Nitha and Janardhanan, 2008; Abdel-Raheem *et al.,* 2009).. Karahan et al(2005) reported the protective effects of lycopene in gentamicin induced nephrotoxicty, lycopene was able to reduce the levels of LPO. Tahira et al., (2012) reported that treatment with lipoic acid and selenium was found to significantly decrease the levels of LPO in the rats induced with GM, indicating the protective role.

5. CONCLUSION

The observed results of the study suggests the treatment with the aqueous extract could prevent cell damage such as tubular vacuolization, glomerular congestion and interstitial edema as evidenced by the levels of urea, uric acid and creatinine and urine volume. The mushroom extract was found to improve the antioxidant status and suppress peroxides. The results of the biochemical and histopathological studies suggests the protective effect of the aqueous extract of *Volvareilla volvacea* against degenerative injury caused by GM.

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REFERENCES

1. Karahan, I., Atessahin, A., Yilmaz, S., Ceribasi, A.O., Sakin, F.2005. Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicol. 215, 198–204.
2. Eisenberg, J.M., Koffer, H., Glick, H.A., Connell, M.L., Loss, L.E., Talbot, G.H., Shusterman, N.H., Strom, B.L.1987. What is the cost of nephrotoxicity associated with aminoglycosides? Ann. Intern. Med. 107, 900–908.
3. Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Di Paola, R., Britti, D., De Sarro, A., Pierpaoli, S., Caputi, A., Masini, E .and Salvemini, D.2002. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol. 450: 67-76.
4. Yanagida, C., Ito, K., Komiya, I. and Horie, T. 2004. Protective effect of fosfomycin on gentamicin-induced lipid peroxidation of rat renal tissue. Chem. Biol. Interact. 148: 139-147.
5. Parlakpinar, H., Tasdemir, S., Polat, A., Bay-Karabulut, A., Vardi, N., Ucar, M. and Acet. A 2005. Protective role of caffeic acid phenethyl ester (cape) on gentamicin induced acute renal toxicity in rats. Toxicol*,* 207**:** 169-177.
6. Pedraza-Chaverrí, J., González-Orozco, A.E., Maldonado, P.D., Barrera, D., Medina-Campos, O.N. and Hernández-

Pando, R.2003. Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats. Eur. J. Pharmacol. 473: 71-78.
7. Maldonado, P.D., Barrera, D., Medina-Campos, O.N., Hernández-Pando, R., Ibarra-Rubio, M.E and Pedraza-Chaverrí, J .2003. Aged garlic extract attenuates gentamicin induced renal damage and oxidative stress in rats. Life Sci. 73: 2543-2556.
8. Gao, Y., Eli, C. and Shufeng, Z. 2004. Immunomodulating activities of *Ganoderma*, a mushroom with medicinal properties. Food Rev. Int. 20, 123-161.
9. Silan, C., Uzun, O., Comunoğlu, N.U., Gokçen, S., Bedirhan, S and Cengiz, M.2007. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol. Biol. Pharm. Bull. 30: 79-83.
10. Soliman, K.M., Abdul-Hamid, M and Othman, A.I. 2007. Effect of carnosine on gentamicin-induced nephrotoxicity. Med. Sci. Monit. 13: 73-83
11. Owen, J.A., Iggo, J.B., Scandrett, F.J. and Stemart, J.P. 1954. Determination of creatinine in plasma or serum, a critical examination. Biochem. J. 58, 426-431.
12. Caraway, W.T. 1963. Uric acid. In: Seligson, D. (Ed.), Standard methods of Clinical Chemistry. Academic Press, New York. 4, 239-247.
13. Kerscher, L. and Ziegenhorn, J. 1990. Urea, In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Third Edition, VCH Publishers Ltd., Cambridge, UK. pp. 444-453.
14. Lowry, O.H., Roseobrough, N.J., Farr, A.L. and Randall, R.J. 1957. Protein measurement with the Folin’s phenol reagent. J. Biol. Chem. 193, 265-275.
15. Das, S., Vasight, S., Snehlata, R., Das, N. and Srivastava, L.M. 2000. Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. Curr. Sci. 78, 486-487
16. Sinha, A. K. 1972. Colorimetric assay of catalase. Anal. Biochem. 47, 389-394
17. Moron, M.S., Defierre, J.W. and Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem. Biophys. Acta. 582, 67-68.
18. Ellman, G.C. 1959. Tissue sulfhydryl groups. *Arch.* Biochem. Biophys. 82, 70-77
19. Omaye, S.T., Turnball, T.D. and Sauberlich, H.E. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods. Enzymol. 62, 1-11.
20. Niehius, W.G. and Samuelsson, D. 1968. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem. 6, 126-130.
21. Jiang, Z. Y., Hunt, J.V. and Wolft, S. D. 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem. 202, 384-389.
22. Allen, C.T.1992. Laboratory Methods in Histochemistry, 1st ed., American Registry of Pathology, Washington, pp.53-56.
23. Perazella, M.A.2003. Drug-induced renal failure: update on new medications and unique mechanisms of nephrotoxicity. Am J Med Sci. 325, 349-62.
24. Nagai,J. and Takano,M.2004. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet 19, 159-70.
25. Wiland, P. and Szechcinski, J. 2003. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol. J. Pharmacol. 55, 631–637.
26. Safa, J., Hassan,A., Bahar, B., Nariman, N.,,5 BabakR.A., Amir Ghorbanihaghjo, Hassan, K.,Akbar, A., Mehran, M. and

Jafar S. R.2010. Protective Effect of Grape Seed Extract on Gentamicin- Induced Acute Kidney Injury. Iranian Journal of Kidney Diseases. 4:285-91.
27. Thounaojam, M.C., Jadeja, R.N., Devkar, R.V. and Ramachandran, A.V. 2010. Sida rhomboidea.Roxb leaf extract ameliorates gentamicin induced nephrotoxicity and renal dysfunction in rats. J Ethnopharmacol. 28;132(1):365-7.
28. Pedraza-Chaverrí, J., Barrera, D., Maldonado, P.D., Chirino, Y.I., Macías-Ruvalcaba, N.A., Medina-Campos, O.N., Castro, L., Salcedo, M.I. and Hernández-Pando, R.2004. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicininduced oxidative and nitrosative stress and renal damage in vivo. BMC. Clin. Pharmacol. 4: 5-18.
29. Abdel-Raheem, I.T., Abdel-Ghany, A.A. and Mohamed, G.A .2009. Protective Effect of Quercetin against Gentamicin-Induced Nephrotoxicity in Rats Biol. Pharm. Bull. 32:1, 61—67.
30. Moghaddam, A. H., Javaheri, M., Nabavi, S. F., Mahdavi, M. R., Nabavi, S. M. and M. A. Ebrahimzadeh.2010. Protectiverole of Pleurotus porrigens (Angel's wings) against gentamicin-induced nephrotoxicity in mice. Eur. Rev. Med. Pharmacol.14: 1011-1014
31. Kore,K.J.,Shete,R.V.,Kale,B.N. and Borade,A.S.2011.Protective role of hydroalcoholic extract of *Ficus carica* in gentamicin induced nephrotoxicity in rats. Int.J.Pharm.Life Sci. 2, 978-982.
32. Badary, O.A., Abdel-Maksoud, S., Ahmed, W.A.and. Owieda, G.H.2005. Naringenin attenuates cisplatin nephrotoxicity in rats. Life Sci. 76, 2125–2135.
33. Conklin, K.A. 2000. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr. Cancer. 37, 1–18.
34. Rana, S.V.S., Allen, T., Singh, R.2002. Inevitable glutathione then and now Indian. J. Exp. Biol. 40, 706–716.
35. Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R. and Brodie, B.B.1973. Acetaminophen induced hepatic necrosis. Protective role of glutathione. J. Pharmacol. Exp. Ther. 187, 211–215.
36. Jollow, D.J., Mitchell, J.R., Zampaglione, N. and Gillete, J.R.1974. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacol. 11, 151– 154.
37. Eberle D, Clarke R and Kaplowitz ,N .1981. Rapid oxidation in vitro of endogenous and exogenous glutathione in bile of rats. J. Biol. Chem. 256, 2115-2117.
38. Arivazhagan, S., Balasenthil, S. and Nagini, S. 2000. Garlic and neem leaf extracts ehhance hepatic glutathione and glutathion dependent enzymes during *N*-methyl-*N*-nitrosoguanidine (MNNG)-induced gastric carcinogenesis. Phytother. Res. 14, 291-293.
39. Nitha,B. and Janardhanan,K.K.2008. Aqueous-ethanolic extract of morel mushroom mycelium *Morchella esculenta*, protects cisplatin and gentamicin induced nephrotoxicity in mice. Food Chem.Toxicol. 46, 3193–3199.
40. Walker, P.D. and Shah, S.V. 1988. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest. 81:334-41.
41. Walker,P.D., Barri, Y. and Shah, S.V.1999. Oxidant mechanisms in gentamicin nephrotoxicity. Ren Fail. 21,433-42.
42. Sandhya, P .and Varalakshmi ,P .1997. Effect of lipoic acid administration on gentamicin-induced lipid peroxidation in rats. J. Appl. Toxicol. 17: 405-408.
43. Kehrer, J.P., 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol. 23, 21–48.

44. Baliga,R., Ueda,N., Walker,P.D. and Shah,S.V. 1999. Oxidant mechanisms in toxic acute renal failure. Drug Metab Rev. 31, 971-97.
45. Tahira, A., Saleem, U., Mahmood, S., Hashmi, F.K., Hussain, K., Bukhari, N.I., Ahmad B. 2012. Evaluation of protective and curative role of α-lipoic acid and selenium in gentamicin-induced nephrotoxicity in rabbits. Pak J Pharm Sci. 2012 .25(1):103-10

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