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EFFICACY OF GALLIC ACID ON OXYTETRACYCLINE INDUCED NEPROTOXICITY IN RATS

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ABSTRACT

Oxytetracycline (200 mg/kg body weight,ip) was administered in 0.5 ml of sterile physiological saline for 15 days, resulting in significant increase in serum urea, creatinine and reduction in creatinine clearance. A significant increase in lipid peroxidation markers (TBARS and lipid hydroperoxides) and, decrease in enzymic antioxidants (superoxide dismutase,catalase,glutathione peroxidase and glutathione-s-transferase) and non-enzymic antioxidant (vitamin C,vitamin E and reduced glutathione) levels were also observed in oxytetracycline-treated rats. The oral administration of gallic acid (200 mg/kg body weight) attenuated the oxytetracycline induced nephrotoxicity by significantly decreased levels of serum urea and creatininie with the significant normalization of creatinine clearance. Upon the depleted renal antioxidant defense system (enzymic and non-enzymic antioxidants) was significantly increased in rats treated with oxytetracycline. These biochemical observations were supplemented by histopathological examination of kidney section. These results indicate that the antioxidant gallic acid might have a protective role against oxytetracycline induced nephrotoxicity and oxidative stress in rats.

KEYWORDS

Gallic acid,Oxytetracycline, Antioxidant, Lipid peroxidation.

INTRODUCTION

Drug toxicity remains an important cause of acute kidney injury. Oxytetracycline generally acts as bacteriostatic antibiotic[1]. Severe adverse effects causes hepatotoxicity, nephrotoxicity[2].

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as well as valuable components of seasonings, beverages, costemics, dyes and medicines[3]. Phenolic pytochemical due to their phenolic ring and hydroxyl substituents can function as effective antioxidants because of their ability to quench the free radicals and prevent celluar damage.

Gallic acid (3,4,5-tri hydroxy benzoic acid) (Fig.1) is one of the most well-known extractive components of plants. It can be isolated, for instance, from some hardwood species such as oak trees, chestnut, grape, different berries, fruits as well as wine[4]. Gallic acid is one of the most abundant phenolic compound, may be potentially useful in the prevention of cancer, hepatotoxicity, nephrotoxicity. Gallic acid exhibits radicals scavenging, antioxidative[5],anti-fungal[6],anti-inflammatory[7] properties.

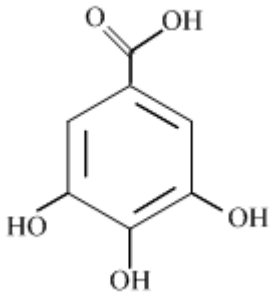

Figure.1
Chemical structure of gallic acid ($C_7 H_6 O_5$)

MATERIALS AND METHODS

Chemicals:

Gallic acid was purchased from sigma chemicals company, USA. Oxytetracyline was procured by Pfizer, India. All other chemicals used for this experiments were of analytical grade obtained from local firms.

Animals:

The studies were performed on adult male albino rats of Wistar strain weighing 180-220 g. The experimental protocol was approved by the committee for research and animal ethics (vide No: 525, 2008) Annamalai university. The animals were housed in and maintained in 24 ± 2 normal temperature and a 12 hr light /dark cycle, and were fed on pellet diet (lipton India ltd. Mumbai) and water ad libitum.

Experimental design:

The animals were randomly divided into six groups of six rats in each group.

Group 1: Normal rats.

Group 2: Normal rats received gallic acid (200 mg/ kg body weight) in aqueous solution daily using intragastric tube for 15 days.

Group: 3: Rats intraperitonially (i.p) administered with oxytetracycline (200 mg/kg) in 0.5 ml sterile physiological saline[8].

Group 4: Rats received oxytetracycline (200 mg /kg, ip) and orally received gallic acid (50 mg /kg) for 15 days.
Group 5: Rats received oxytertracycline (200 mg / kg, ip) and orally received gallic acid (100 mg /kg) for 15 days.
Group 6: Rats received oxytetracycline (200 mg / kg, ip) and orally received gallic acid (200 mg /kg) for 15 days.

At the end of the experimental period, the animals were killed by cervical decapitation. Blood was collected and centrifuged (1000 x g for 15 minutes) for serum separation. The liver was dissected out, weighted and washed using chilled saline solution. Tissue was minced and homogenized (10% w/v) in appropriate buffer (pH-7.4) and centrifuged (3000 x g for 10 min). The resulting supernatant was used for enzyme assays.

Biochemical assays:

Activities of serum renal marker: the activities of serum urea, creatinine were estimated spectrophotometrically according to the standard procedures using commercially available diagnostic kits(Sigma Diagnostics(I) Pvt Ltd, Baroda,India). Creatinine clearance was calculated from the values of urinary and serum creatinine, time(last 24h) and body weight.

Assay of lipid peroxidation:

The Lipid peroxidation indices namely thiobrabituric acid-reactive substances and lipid hydroperoxides were measured

Determination of antioxidant:

The levels of non enzymatic antioxidant reduced glutathione (GSH). Vitamin C (ascorbic acid), vitamin E (α - tocopherol) and enzymatic antioxidant superoxide dismutase, catalase, glutathione-s- transferase were assayed.

Histopathlogical investigation:

The liver sample was fixed for 48 hr in 10% formal – saline and then dehydrated by passing successfully in different mixture of ethyl alcohol and water, cleaned in xylene and embedded in paraffin. sections of liver (4-5 µm thick) were prepared and then stained with heamatoxylin and eosin dye, which mounted in neutral Depex polystyrene medium for microscopic observations.

Statistical analysis:

All data were expressed as mean $\pm$ SD (n=6). The statistical significance were analysed by analysis of variance (ANOVA) and the individual comparisons were obtained by Duncan's multiple range Test (DMRT), values were considered statistically significant at $P < 0.05$.

RESULTS

Table 1 shows the levels of serum renal markers in normal and experimental rats. Intraperetonial administration of oxytetracycline caused significant increase in the levels of serum urea and creatinine and decrease in the creatinine clearance. Treatment with gallic acid at 200 mg/kg significantly decreased the levels of serum urea and creatinine and significantly restored the creatinine clearance in oxytetracycline treated rats when compared to other doses such as (50 and 100 mg/kg). Based on this finding 200 mg/kg of gallic acid was fixed as a dose for further biochemical studies.

Table 1.
Changes in the level of kidney functional markers in serum of normal and experimental rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Creatinine clearance (mg/min)
Normal	43.16 ± 3.75[a]	0.48 ± 0.03[a]	0.37 ± 0.03[a]
Normal + GA (200 mg/kg)	45.41 ± 4.13[a]	0.47 ± 0.02[a]	0.36 ± 0.03[a]
Normal + OXT (200 mg/kg)	77.25 ± 5.47[b]	0.88 ± 0.06[b]	0.19 ± 0.01[b]
OXT + GA (50mg/kg)	71.50 ± 4.42[c]	0.82 ± 0.05[c]	0.24 ± 0.01[c]
OXT + GA (100 mg/kg)	66.33 ± 4.12[d]	0.76 ± 0.05[d]	0.28 ± 0.01[d]
OXT + GA (200 mg/kg)	55.11 ± 2.86[e]	0.67 ± 0.03[e]	0.31 ± 0.02[e]

GA-Gallic acid; OXT –Oxytetracycline.Values are mean ± SD for 6 rats in each group. [a-e] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

The changes in the levels of lipid peroxidation products, non-enzymic antioxidants in normal and experiment animals are shown in table 2 and 3. The levels of thiobarbituric acid–reactive substances and lipid hydroperoxide were significantly increased (P < 0.05) in oxytetracyline – treated rats, whereas the levels of vitamin C, vitamin E and reduced glutathione were significantly decreased (P < 0.05) in rats treated with oxtetracyline. Administration of gallic acid to oxytetracycline- treated rats significantly increased (P < 0.05) the levels of nonenzymic antioxdants and also significantly decreased the level of lipid peroxidation products in kidney.

Table 2.
Changes in the levels of TBARS and Hydroperoxides in tissues (liver and kidney) of normal and experimental rats

Groups	TBARS (mmoles/100g tissue)	Hydroperoxides (mmoles/100g tissue)
Normal	1.78 ± 0.10[a]	71.96 ± 4.83[a]
Normal + GA (200 mg/kg)	1.75 ± 0.12[a]	70.22 ± 4.80[a]
Normal + OXT (200 mg/kg)	2.88 ± 0.21[b]	108.73 ± 8.28[b]
OXT + GA (200 mg/kg)	2.31 ± 0.18[c]	81.44 ± 6.46[c]

GA-Gallic acid; OXT –Oxytetracycline.Values are mean ± SD for 6 rats in each group. [a-c] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

Table 3.
Changes in the levels of vitamin C, vitamin E and GSH in kidney of normal and experimental rats

Groups	Vitamin C (μmoles/mg tissue)	Vitamin E (μmoles/mg tissue)	GSH (μg/mg protein/ g tissue)
Normal	1.12 ± 0.09[a]	0.67 ± 0.04[a]	41.75 ± 2.47[a]
Normal + GA (200 mg/kg)	1.15 ± 0.11[a]	0.69 ± 0.05[a]	43.09 ± 3.02[a]
Normal + OXT (200 mg/kg)	0.68 ± 0.05[b]	0.43 ± 0.03[b]	26.81 ± 1.82[b]
OXT + GA (200 mg/kg)	0.86 ± 0.04[c]	0.59 ± 0.04 [c]	35.68 ± 2.63[c]

GA-Gallic acid; OXT –Oxytetracycline.Values are mean ± SD for 6 rats in each group. [a-c] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

Table 4 illustrates the levels of enzymic antioxidants namely superoxide dismutase, catalase, glutathione peroxidase and glutathione-S- transferase in the kidney of normal and experimental rats. A significant decrease in the activities of enzymic antioxidants in the livers of oxytertracycline – treated rats.

Table 4.
Changes in the activities of SOD, CAT, GPx and GST in kidney of normal and experimental rats

Groups	SOD (Units[#]/mg protein)	CAT (Units[#]/mg protein)	GPx (Units[#]/mg protein)	GST (Units[#]/mg protein)
Normal	12.54 ± 0.77[a]	56.97 ± 4.97[a]	7.20 ± 0.48a	6.25 ± 0.37[a]
Normal + GA (200 mg/kg)	13.32 ± 1.19[a]	57.91 ± 5.56[a]	7.39 ± 0.54a	6.51 ± 0.31[a]
Normal + OXT (200 mg/kg)	7.98 ± 0.64[b]	32.23 ± 3.10[b]	4.37 ± 0.26b	4.73 ± 0.26[b]
OXT 200mg/kg) + GA (200 mg/kg)	10.70 ± 0.87[c]	50.37 ± 4.88[c]	6.29 ± 0.42c	5.21 ± 0.28[c]

GA-Gallic acid; OXT –Oxytetracycline.Values are mean ± SD for 6 rats in each group.
[a-c] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).
[#] Units of enzyme activities are expresses as:
SOD - One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute.
CAT - μ moles of hydrogen peroxide consumed / minute.
GST - μ moles of CDNB-GSH conjugate formed / minute.
GPx - μ moles of glutathione consumed / minute.

Histopathological studies showed that treatment with oxytetracycline caused focal area of hemorrage (Fig.5c) of kidney damage as compared with normal kidney. The above changes were reduced in kidney of rats treated with gallic acid and oxytetracycline. Normal rats treated with gallic acid did not show any histological alterations.

NEPHROPROTECTIVE EFFECT OF GALLIC ACID

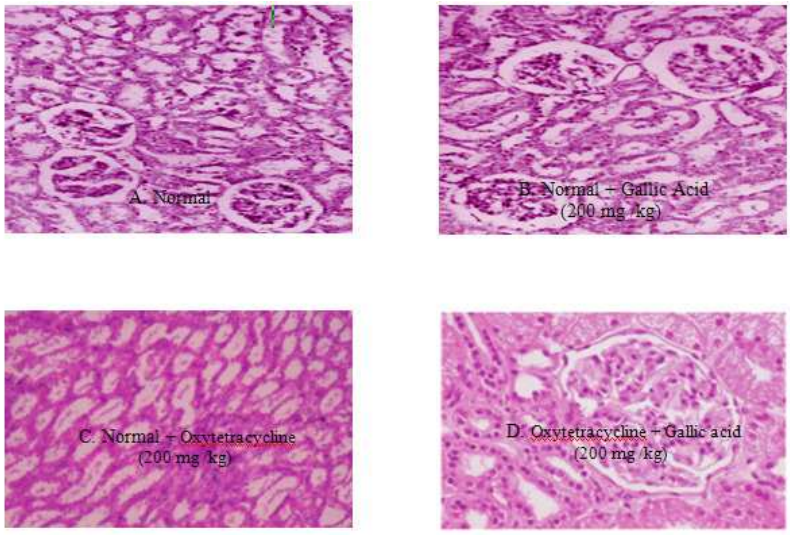

Figure 5
.Histological investigation.

A. normal rat liver. Haematoxylin & eosin, Magnification X 20. Normal glomeruli and tubules.
B. Normal + Gallic Acid (200 mg /kg)-treated rat liver. Haematoxylin & eosin, Magnification x 20. Normal appearance of tubules
C. Normal + Oxytetracycline (200 mg /kg)- treated rat liver. Haematoxylin & eosin, magnification x 20. Focal areas of hemorrhage.
D. oxytetracycline + Gallic Acid (200 mg / kg)-treated rat liver Heamatoxylin & eosin, magnification x 20. Almost normal appearance of glomerulai and tubules

DISCUSSION

Nephrotoxicity is one of the major side effects of drug therapy in clinical practice frequently leading to acute renal failure[8].The main mechanism of nephrotoxicity are vasoconstriction, altered intra glomerular hemodynamics, tubular cell toxicity, interstitial nephritis, crystal deposition, thrombotic microangiopathy and osmatic nephrosis[9]. It is well documented that tetracycline inhibit the incorporation of amino acid into protein causing an increase in urea level [10]. The increased serum creatinine and lower creatinine clearance are located to diagnosis of renal failure [11]. In the present study, increased levels of serum creatinine, urea and decreased levels of creatinine clearance in oxytetracycline treated rats reflect the renal damage. Previous report also suggest that, high dose of oxytetracycline known to cause nephrotoxicity [12].

Administration of gallic acid protects the kidney function from oxytetracycline as indicated by significant decrease of serum creatinine and restoration of creatinine clearance levels. Our finding concides with earlier study in which gallic acid has been reported to protect the CCl_4 induced nephrotoxicity[13]. In this context, gallic acid also restored the kidney function against oxytetracycline induced impairment in rats.

Membrane and lipids are particularly susceptible to the oxidant process and to the peroxidative reaction induced by free radicals[14]. Oxytetracycline inhibition of mitochondrial β-oxidation disrupts the respiratory chain and can produce superoxide anion, which plays a crucial role in production of reactive oxygen and nitrogen species such as peroxynitrite and hydroxyl radical[15]. The free radicals attack the cell membrane, thus leading to destabilization and disintegration of the cell membrane as a result of lipid peroxidation[16]. The increased level of lipid peroxide and decreased level of antioxidants might be due to oxidative stress in oxytetercycline-intoxicated rats. Since the antiperoxidative action trigerred by gallic acid and stimulate the excretion of reactive oxygen species from cells or activate some oxidase in plasma membrane [17]. These results suggest that binding of the gallate compounds to lipid membrane is a principle determining factor of the antioxidants action[18].

Antioxidant defense system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites. vitamins E, the major lipophilic antioxidant and vitamin C play a vital role in the defense against oxidative stress[19]. Glutathione, an important cellular reductant is involved in protection against free radicals, peroxides and other toxic compounds[20]. The decrease in the levels of GSH, vitamin C, vitamin E in kidney of oxytetracycline treated rats might be due to increased utilization to reduce the oxytetracycline-induced oxidative threats. Gallic acid exhibit an interesting antiradical potential using free radical scavenging activity and lipid peroxidation inhibitory activity, which may lead to significantly increased levels of vitamin C, vitamin E and GSH levels in oxytetracycline –treated rats.

Superoxide dismutase, catalase, GSH peroxidase and glutathione-s-transferase constitute a mutually supportive team of defense against reactive oxygen species. SOD, a chain breaking antioxidant,play an important in protection against the deleterious effects of lipid peroxidation [21]. CAT is found to b a major determinant of cellular resistance to hydrogen peroxide toxicity. A high level CAT activity has been shown to be protective against oxidant damage[21]. GPX is a selenoenzyme that inactivates hydrogen peroxide as well as wide range of lipid hydroperoxides[22].

Glutathione-s-transferase are phase II drug – metabolizing enzymes responsible for the glutathione conjugation of a variety of xenobiotics such as carcinogens, therapeutic drugs and highly reactive lipid peroxidation products[23]. A decrease in antioxidants status in oxytetracycline –treated groups in our study may be due to their increased utilization to counteract the lipid peroxidative end products. Administration of gallic acid increased the activities of SOD, CAT, GPX and GST. Normally phenolic compounds act by scavenging free radicals and quenching the lipid peroxidative chain. The hydroxyl and phenoxy groups of phenolic compounds donate their electron to the free radicals and quench them[24]. Gallic acid may directly combine with free radicals and lead to inactivate them which may suppress the intracellular concentration of free radicals[25]. The histopathological examination in oxytetracycline treated rats showed significant alteration in kidney. This could be due to the accumulation of free radicals as the consequence of increased lipid peroxidation in oxytetracycline – treated rats. In the present study reported that gallic acid significantly reduces the histological changes caused by oxytetracycline.

CONCLUSION

In conclusion, gallic acid increases the regenerative and reparative capacity of the liver and kidney. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against renal damage. Gallic acid possesses an antioxidant activity and protection against oxidative damage in oxytetracycline-induced nephrotoxicity.

REFERENCES

1. Susan K, Mikoto DVM and Donald C., Elephant care international,2003.
2. Dollery C. Therapeutic drugs, 2nd edition, Churchill livingstone. Vol: 2, 055-058, (1999).
3. AM J Clin Nutr, 70 (Suppl),491s-92. Printed in USA, (1999).
4. Singh J, Rai GK, Upadhyay K, Kumar R, Singh KP., Ind.J.Agric.Sci, 74(1):3-5, (2004).
5. Yeh CT, Yen GC., Effects of phenolic acids on human phenol sulfo transferases in relation to their antioxidant activity: J. Agric. Food chem,51, 1474-1479,(2004).
6. Kubo I, Xiao P, Fujita K, Antifungal activity of octyl gallate. Structural criteria and mode action: Bio Org Med Chem. Lett, 11,347-350.
7. Krores BH, Van den berg AJ, Auarles van ufford HC, Van Dijk H, Labadie RP, Anti-inflammatory activity of gallic acid: planta med, 58, 499-504, (1992).
8. Sharifi AM, Akbarloo N, Sasani F. Nephrotoxicity of isosorbide nitrate and chloestosis in rat. The possible role of nitric oxide: Ind. J.Med. Sci. 29,75,(2004).
9. Schetz M, Dasta J, Goldstein S, Golper T. Drug induced acute kidney injury: Curr Opin Crit Care. 11(6),555-65, (2005).
10. Davies DM. Text book of adverse drug reactions 4th edition. Oxford. Oxford medical publication:(1991).
11. Donadio C, Lucchesi A, Tramonti G, Bianchi C. Creatinine clearance predicted from body cells mass is a good indicator of renal function: kindey Int. 52, 166-168,(1997).
12. Naseer F, Alam M. The Protective effect of ascorbic acid on oxytetracycline induced nephrotoxicity and hepatotoxicity: J. Pak. Med. Assoc. 37, 73-75,(1987).
13. Jadon A, Bhaduria M, Shukal S. Protective effect of Terminalia belerica Roxb and gallic acid against carbon tetrachloride induced damage in albino rats: J. Ethropharmacology. 109, 214-218,(2006).
14. Rahaman K. Garlic and aging, New insight into an old remedy. Aging Research reviews: 2, 39-56,(2003).
15. Gibson BW. The human mitochondrial proteome; oxidative stress, protein modification and oxidative phosphorylation. Int. J. Biochem: Cell Biol. 37, 927-934,(2005).
16. Skakun NP, Vysotski I.I.U. Effect of tetracycline antibiotics on lipid peroxidation. Antibiotiki: 27, 684-687, (1982).
17. Makoto Inoue, Ric Suruki, Tatsuo Koide, Nahoko Sakaguchi, Yukio Ogihea, yoshisada yabu. Antioxidant, gallic acid, induces apoptosis in HL-60RG cells:204, 898-904, (1994).
18. Shahrzad S, Kazumasa A, Winter A, Koyam A., Bitsch I. Pharmacokinetics of gallic acid and its relative bioavailability from tea in health humans. American society for nutritional and metabolism research communication: 1207-1210,(2001).
19. Ray G, Hussain SA. Oxidants, antioxidants and carcinogenesis: Ind J Exp Biol. 42, 1213-1232, (2002).
20. Gerster H. β-carotene, vitamin E and vitamin C in different stages of experimental carcinogenesis: Eur J clin Nutr. 49, 155-168, (1995).
21. Dinkova- Kostova H, Talalay P. Relation of structure of curcumin analogs to their potencies as inducers of phase–2 detoxification enzymes: Carcinogenesis. 20, 911-914,(1999).
22. Ji LL , Stratman FW, Lardy HA. Antioxidant enzyme systems in rat liver and skeletal muscle: Archives of Biochemistry and biophysics. 263, 150-160, (1988).
23. Wang YC, Bachrach U. The specific anti-cancer activity of grene tea (-)-epigallocatechin – 3- gallate (EGCE). Amino acids: 22, 131-141,(2002).
24. Anderson C, Mosialou e, Weinander R, Morgenstern R. Enzymology of Microsomal

Glutatathione S-transferase: Advances in pharmacolog. 27, 19-35, (1994).

25. Gill MI, Francisco A, Barberan T, Pierce BH, Holgoft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenoilc compostion and processing: J Agric food chem. 48, 4581-4589, (2004).