

Full Length Research Paper

Chemopreventive role of vitamin C and E on potassium bromate induced renal oxidative damage in rat

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Studies have shown that potassium bromate induces acute renal damage arising from oxidative stress in man. The present study was undertaken to examine the effect of vitamin C and E on potassium bromate induced toxicity in rat kidney. Wistar albino rats were grouped into four (A, B, C and D) and administered 10mg/kg bw potassium bromate daily for 21 days (Group A serves as the control and received distilled water instead of potassium bromate). Group C and D were treated with intraperitoneal administration of vitamin C (40mg/kg bw) and vitamin E (20mg/kg bw) respectively while group B were left untreated. Antioxidant and prooxidant levels were measured in the kidney in addition to analysis of kidney function parameters in the serum. Potassium bromate caused significant depletion in antioxidant enzymes in the kidney as well as elevation in thiobarbituric acid reactive substances (TBARS). Serum urea and creatinine were also elevated in rats administered with potassium bromate indicative of nephritic malfunction. Rats treated with vitamin C and E showed significant improvement in renal antioxidant capacity and restoration of kidney function. This study suggests that vitamin C and E has protective role on potassium bromate induced renal oxidative damage in rat.

Keywords: Potassium bromate, vitamin C, vitamin E, oxidative stress, nephrotoxicity.

INTRODUCTION

The prevalence of kidney failure is on the increase across the globe with limited treatment options thereby causing a major financial and emotional burden on the population (Jaffar, 2007; Lynda, et al., 2009). Kidney disease is the ninth leading cause of death in the United States (Arias et al., 2003). A recent data suggest that more than 200,000 people in the US suffer from kidney failure representing almost 0.2% of the adult population (Coresh et al., 2007). African countries including Nigeria have also witness a similar prevalence due to increasing incidence of its risk factors (Lynda et al., 2009). Most kidney diseases are caused by oxidative damage from chemical poisoning and they attack the nephrons, causing them to lose their filtering capacity.

Potassium bromate is a food additive used primarily as a dough conditioner for flour (Diachenko and Warner, 2002). Several reports on safety evaluation of potassium bromate shows that it is highly toxic as it causes lipid peroxidation and oxidative DNA damage in rat kidney (Kurokawa et al., 1990). The chemical was considered unsafe and banned from the list of food additives by

many countries including Nigeria based on these reports. Potassium bromate induced formation of 8-hydroxydeoxyguanosine and α 2u-globulin in rat kidney which are capable of initiating carcinogenesis in this organ (Kurokawa et al., 1990).

A variety of naturally occurring enzymatic and non enzymatic mechanisms are available to protect cells against oxidative damage (Yu 1994). Some of these mechanisms are impaired during consumption of certain drugs and may therefore contribute to damage to the kidney which is the principal organ responsible for drug excretion (Ali et al., 2005).

Vitamin E (α -tocopherols) are multifaceted antioxidants, that scavenge oxygen free radicals, lipid peroxides and singlet oxygen (Diplock et al., 1989). They act as membrane stabilizers by their positive influence on membrane lipid organization. Vitamin C (ascorbic acid) is a good free radical scavenger and is known to represent the first line of antioxidant defense in the living cell (Loewus, 1988; Halliwell, 1999). It reacts with activated oxygen more readily than any other aqueous component

and protects critical macromolecules from oxidative damage. This study intends to investigate the antioxidant potential of vitamin C and E on potassium bromate induced renal oxidative damage in rat.

MATERIALS AND METHODS

Chemicals/Reagent kits

Potassium bromate salt is a product of British Drug House Limited, Poole, England. Vitamin E and D were obtained from May and Baker Ltd. Dagenham, England. Urea and creatinine diagnostic kits are products of Randox Chemical Ltd. England.

Experimental protocol

The study was performed on thirty two (32) wistar albino rats of both sexes (average weight 160g) housed in ventilated cages in the Animal House of Biochemistry Department, Osun State University, Osogbo, Nigeria. They were acclimatized for one week before administration of the drugs. Animals were divided into four groups of eight rats each. Group A served as the control and received distilled water. Group B, C and D were administered 10mg/kg bw potassium bromate daily. Rats in group C and D were treated daily with intraperitoneal administration of vitamin C and E respectively while group B were left untreated. Animals were kept at optimum temperature with a 12 h light/dark cycle and given rat feed and water *ad libitum*. The experimental protocol was approved by the Institutional Research Ethical Committee. The period of drug administration lasted for 21 days.

Preparation of serum

Rats were sacrificed at the end of the 21 days experimental period by cervical dislocation. Blood samples were collected through the jugular vein into sterilized dry test tube. It was allowed to clot followed by centrifugation at 3000rpm for 20 minutes. The clear supernatant (serum) was separated from the pellet and kept in the refrigerator for the estimation of urea and creatinine.

Preparation of kidney homogenate

The animals were quickly dissected, the kidneys removed and placed on a blotting paper to remove blood stains. It was rinsed in cold physiological saline, cleaned of gross adventitial tissues and blotted dry. 10% kidney homogenate were then prepared in 6.7mM potassium

phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 4°C to obtain a clear supernatant which was stored at 8°C and used for measurement of antioxidant contents.

Biochemical Assay

Catalase (CAT) activity was determined in the kidney homogenate by the method of Johansson and Borg (1988) based on its peroxidatic activity. The method of Kakkar et al. (1984) was employed in the determination of superoxide dismutase (SOD) activity. Glutathione-S-transferase (GST) activity was measured using the method of Habig et al. (1974) based on its ability to form conjugate with 1,2-dichloro 4-nitrobenzene (CDNB) which can be monitored at 340nm. The procedure described by Tappel (1978) was used to estimate kidney glutathione peroxidase (GSH-Px) activity while reduced glutathione (GSH) concentration was measured by the method of Ellman (1959). Thiobarbituric acid reactive substances (TBARS) was assayed in the kidney as described by Ohkawa et al. (1979) by measuring lipid peroxide product of the reaction between malondialdehyde and thiobarbituric acid. Protein concentration in kidney homogenate was estimated using the biuret method. Serum creatinine level was measured by the method of Henry (1974) while the diacetylmonoxime method of Wybenga et al. (1971) was employed for the estimation of serum urea.

Statistical analysis

Values obtained were expressed as mean \pm SD and subjected to statistical analysis using SPSS window version 9.0. Comparison was done using one-way analysis of variance (ANOVA). P values of <0.05 were considered statistically significant.

RESULT

The results of renal antioxidants and serum metabolites in rats administered potassium bromate, vitamin C and E at the end of 21 days are indicated in Table 1. The results showed that potassium bromate induced a significant reduction ($p < 0.05$) in levels of renal CAT, SOD, GSH, GST and GSH-PX in the kidney as well as significant elevation of TBARS level compared with the control. There was also a significant elevation in serum urea and creatinine in the rats following potassium bromate administration. Treatment of rats with Vitamin C and E significantly raised the levels of renal antioxidants and also reduced the TBARS levels in the rats close to that obtained in the control. Vitamin C and E also reversed

Table 1. Renal antioxidants and serum metabolites in rats administered potassium bromate, vitamin C and E for 21 days

Parameters	Normal Control	Potassium bromate	Potassium bromate + Vitamin C	Potassium bromate + Vitamin E
CAT ($\mu\text{mol}/\text{mg}$ protein)	84.69 \pm 5.50 ^a	45.37 \pm 3.20 ^b	66.53 \pm 4.22 ^{ab}	68.32 \pm 3.58 ^{ab}
SOD ($\mu\text{mol}/\text{mg}$ protein)	123.21 \pm 6.62 ^a	74.82 \pm 4.45 ^b	95.47 \pm 5.02 ^{ab}	98.23 \pm 5.75 ^{ab}
GST ($\mu\text{mol}/\text{mg}$ protein)	2.16 \pm 0.33 ^a	1.31 \pm 0.21 ^b	1.75 \pm 0.31 ^{ab}	1.73 \pm 0.28 ^{ab}
GSH ($\mu\text{g}/\text{mg}$ protein)	31.80 \pm 2.76 ^a	20.68 \pm 2.21 ^b	26.55 \pm 2.81 ^{ab}	25.89 \pm 2.44 ^{ab}
GSH-Px ($\mu\text{mol}/\text{mg}$ protein)	40.51 \pm 3.55 ^a	27.96 \pm 2.58 ^b	33.82 \pm 3.87 ^{ab}	35.31 \pm 3.69 ^{ab}
TBARS (nmol/mg protein)	102.74 \pm 5.48 ^a	149.66 \pm 6.24 ^b	124.20 \pm 5.59 ^{ab}	121.48 \pm 6.22 ^{a b}
Urea (mg/dl)	30.55 \pm 1.89 ^a	58.78 \pm 3.24 ^b	41.35 \pm 2.05 ^{ab}	40.73 \pm 2.16 ^{ab}
Creatinine (mg/dl)	12.58 \pm 0.30 ^a	24.66 \pm 0.46 ^b	17.32 \pm 0.41 ^{ab}	18.38 \pm 0.39 ^{ab}

Values are the Means \pm SD. n=6. Values with different alphabetical superscript along a row are significantly different at $p < 0.05$

the elevated concentrations of urea and creatinine caused by potassium bromate administration. There was no significant difference between results obtained for vitamin C and vitamin E.

DISCUSSION

The significant reduction in endogenous antioxidant and elevation of TBARS by potassium bromate indicates its prooxidant effects in rat kidney. CAT, SOD, GST, GSH and GSH-PX represents an armoury of antioxidants produce by the body to neutralize or 'mop up' free radicals that can harm the cells and hence defend it against oxidative stress. The ability of the body to produce these antioxidants is controlled by genetic makeup and influenced by exposure to environmental factors such as diet and chemicals (Halliwell, 1999).

The significant elevation of serum urea and creatinine by potassium bromate also indicates its adverse effects on kidney function in rats. Creatinine is a break-down product of creatine phosphate in muscle, and is usually filtered out of the blood by the kidneys with little or no tubular reabsorption (Rhodes et al., 1995). If the filtering capacity of the kidney is deficient, creatinine blood levels rise. Measuring serum creatinine is the most commonly used indicator of renal function. A rise in blood creatinine level is observed only with marked damage to functioning nephrons (Al-Qarawi et al., 2008).

Urea is the major end product of protein catabolism and is primarily produced in the liver and secreted by the kidneys. It is the primary vehicle for removal of toxic ammonia from the body. Urea determination is very useful for the medical clinician to assess kidney function of patients (Harlalka et al., 2007). In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extrarenal diseases. The observed nephrotoxicity brought about by potassium bromate in this study is similar to earlier observations (De Angelo et al., 1998; Akanji et al., 2008).

The significant recovery of renal antioxidant content and reversal in the enhancement of blood urea and creatinine by vitamin C and E suggest that they are potent chemopreventive agents against oxidative stress and may suppress potassium bromate-mediated renal oxidative damage in rat (Agarwal, 2005). Vitamin C (ascorbic acid) is an important vitamin in the human diet and is abundant in plant tissues. It functions as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress (Halliwell, 1999). Ascorbic acid can directly scavenge oxygen free radicals with and without enzyme catalysts. It reacts with activated oxygen more readily than any other aqueous component thus protects critical macromolecules from oxidative damage (Loewus, 1988).

α -Tocopherols occur in both photosynthetic and non-photosynthetic plant tissue and have been studied extensively because of its dietary importance (Hess, 1993). They are membrane stabilizers and multifaceted antioxidants that scavenge oxygen free radicals, lipid peroxides, and singlet oxygen (Diplock *et al.*, 1989). Although some of this activity is due to its influence on membrane lipid organisation, at least part of this activity is the result of its ability to complex free fatty acids (Fryer, 1992). Free fatty acids act as detergents in membranes causing disruption of the bilayer, membrane aggregation and fusion. The association between the carboxyl group of the fatty acid and the ring of tocopherol reduces this destabilization (Fryer, 1992).

CONCLUSION

Potassium bromate is a potent nephrotoxic agent as it enhances lipid peroxidation with significant reduction in the activities of renal antioxidant capacity. It also caused renal dysfunction as revealed in marked increase in serum urea and creatinine. Vitamin C and E has chemopreventive benefit on potassium bromate mediated renal oxidative damage in rat as they significantly re-

duced the extent of antioxidant loss and restoration of renal dysfunction caused by potassium bromate in rat.

REFERENCE

- Agarwal SK (2005). Chronic kidney disease and its prevention in India. *Kidney Int. Suppl.* 98:41-45.
- Akanji MA, Nafiu MO, Yakubu MT (2008). Enzyme and histopathology of selected tissues in rats treated with potassium bromate. *Afr. J. Biomed. Res.* 11:87-95.
- Ali BH, Al-Wabel N, Mahmoud O, Mousa HM, Hashad M (2005). Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. *Fundam. Clin. Pharmacol.* 19:473-477.
- Al-Qarawi AA, Rahman HA, Mousa HM, Ali BH, El-Mougy SA (2008). Nephroprotective action of *Phoenix dactylifera* in gentamicin induced nephrotoxicity. *Pharmaceut. Biol.* 46:227-230.
- Arias E, Anderson RN, Kung HC, Murphy SL, Kochanek KD (2003). Deaths: final data for 2001. *Natl. Vital Stat. Rep.* 52:111-115.
- Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers (2007). Prevalence of chronic kidney disease in the United States. *JAMA.* 298:2038-2047.
- De Angelo AB, George MH, Kilburn SR, Moore TM, Wolf DC (1998). Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats. *Toxicologic Pathology.* 26 (5): 587-594.
- Diachenko GW, Warner CR (2002). Potassium bromate in bakery products. In: *Bioactive Compounds in Foods*. Lee TC and Ho CT eds. American Chemical Society, Washington DC. p 218
- Diplock AT, Machlin LJ, Packer L, Pryor WA (1989). Vitamin E: Biochemistry and health implications. *Ann. N.Y. Acad. Sci.* 570: 555-563
- Ellman GL (1959). Tissue sulfhydryl group. *Arch. Biochem. Biophys.* 82: 70-77.
- Fryer MJ (1992). The antioxidant effects of thylakoid vitamin E (α -tocopherol). *Plant Cell Environ.* 15:381-392.
- Habig WA, Pabst MJ, Jacoby WB (1974). Glutathione transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249: 7130-7139.
- Halliwell B (1999). Antioxidant defense mechanisms: From the beginning to the end. *Free Radical Research.* 31:261-272.
- Harlalka GV, Patil CR, Patil MR (2007). Protective effect of *Kalanchoe pinnata pers.* (Crassulaceae) on gentamicin induced nephrotoxicity in rats. *Indian J. Pharmacol.* 39:201-205.
- Henry RJ (1974). Determination of serum creatinine. In: *Clinical Chemistry: Principle and Technics.* 2nd Edn. Harper and Row p 525.
- Hess JL (1993). Vitamin E (α -tocopherol) In: *Antioxidants in higher plants*. Alscher R.G. and Hess J.L. (eds) CRC Press. Boca Raton, pp.111-134.
- Jaffar SA (2007). Commentary on acute renal failure in Asian region. *Nephrology.* 2:213 219.
- Johansson LH, Borg LA (1988). A spectrophotometric method for the determination of catalase activity in small tissue samples. *Anal. Biochem.* 174:331-336
- Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. *Ind. Biochem. Biophys.* 21: 130-132.
- Kurokawa Y, Maekawa A, Takahashi M (1990). Toxicity and carcinogenicity of potassium bromate: a new renal carcinogen. *Environ. Health perspect.* 87:309-355.
- Loewus FA (1988). Ascorbic acid and its metabolic products. In: *The Biochemistry of Plants*, Vol. 14. Academic Press, New York, pp 85-107.
- Lynda AS, William H, Thomas HH, Paul EK, Neil RP, John RS (2009). World Kidney Day 2009: problems and challenges in the emerging epidemic of kidney disease. *J. Am. Soc. Nephrol.* 20: 453-455.
- Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95:351-358.
- Rhodes C, Stryer L, Tasker R (1995). *Biochemistry* (4th ed.). San Francisco: W.H. Freeman. pp. 280-297.
- Tappel AL (1978). Glutathione peroxidase and hydroperoxides. *Methods Enzymol.* 52: 506-513.
- Wybenga DRD, Giorgio J, Pileggi VJ (1971). Determination of serum urea by diacetylmonoxime method. *J. Clin. Chem.* 17: 891-895.
- YU RP (1994). Cellular defenses against damage from reactive oxygen species. *Physiological reviews.* 74:139-162.