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Full Length Research Paper

Effect of a natural antioxidant fruit – tomatoes (*Lycoperscion esculentium*) as a potent nephro-protective agent in lead induced nephrotoxicity in rat

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The toxicity of lead (Pb) to the kidney amongst other organs is not in doubt, in that various devices and interventions, aimed at mitigating its toxic effect, have also been described. Effect of lycopene (the active constituent of tomatoes) as a natural antioxidant in modulating Pb toxicity and its attendant reduction of reduced glutathione (GSH) levels in Pb poisoned kidney tissue of Wister rats is the main focus of this work. Blood lead concentration and GSH activity were investigated using standard laboratory methods on blood and homogenised kidney tissue of Wister rats, respectively. Expectedly, rats with induced lead poisoning (positive control group) showed significantly high blood Pb level (23±2.1 µg/dl), relative to the negative control (10.7±0.92 µg/dl) ($p<0.05$). Although there was no significant decrease in mean blood Pb concentration levels between test group and positive control rats (22±2.1 µg/dl versus 23±2.1 µg/dl, respectively; $p>0.05$), following tomato supplementation, the mean activity of reduced GSH in experimental rats was found to be 8.2±0.57 µM relative to that of the control (5.9±2.1 µM). This difference was found to be significant ($p<0.05$). This experimental work strongly suggest the use of lycopene-rich tomatoes as regular diets to improve reduced glutathione activity especially in subjects occupationally exposed to lead as an intervention mechanism against Pb nephrotoxicity.

Key words: Lycopene, nephrotoxicity, antioxidant, lead toxicity and lead acetate.

INTRODUCTION

Lead (Pb) has been commonly used for thousands of years because it is widespread, easy to extract and easy to work with. It is highly malleable and ductile as well as easy to smelt. Metallic lead beads, dating back to 6400 BC, have been found in modern-day Turkey (Heskel and Dennis, 1983). Lead is a common environmental contaminant like other commonly found persistent toxic metals - mercury, arsenic and cadmium-lead (Pb), which damage cellular material and alters cellular genetics. The mechanisms that all these toxic metals have in common involve oxidative damage.

Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. Data now indicate that low-level exposure to lead, resulting in blood levels previously considered normal may cause cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension and renal impairment. Lead toxicity leads to free radical damage via two separate, although related, pathways: These are (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen and hydrogenperoxide, and (2) the direct depletion of antioxidant reserves (Ercal et al., 2001). In any biological system where ROS production increases, antioxidant reserves are depleted. One of the effects of lead exposure is on glutathione metabolism: glutathione reductase. The enzyme responsible for recycling of glutathione from the oxidised form (glutathione disulfide-GSSG) to the reduced form (GSH) is deactivated by lead (Sandhir et al., 1994). The mechanism of lead related pathologies, many of which are a direct result of the

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Table 1. Blood lead concentration of the different experimental groups.

Group	Group 1 (Negative control group)	Group 2 (Positive control group)	Group 3 (Test group)
Blood lead (Mean±SD) concentration (µg/dl)	10.7±0.87	23±2.14	22.2±2.1

Table 2. Total glutathione, oxidized glutathione (GSSG), reduced glutathione (GSH) level and GSH/GSSG ratio.

Group	Negative control group	Positive control group	Test group
Total GSH	14.07	13.9	14.1
Reduced Glutathione (µM) Mean ±SD	8.2±1.52	5.95±2.1	8.2±0.578
Oxidized Glutathione (µM) Mean ±SD	5.67±1.5	7.9±2.59	5.94±1.7
GSH/GSSG Ratio (µM)	>1	<1	>1
Plasma creatinine (mg/dl)	10.0±2.3	18.0±4.6	15.1±3.5

oxidant effect of lead on tissues and cellular component, may be mitigated by improving availability of antioxidants. One of such antioxidant is the active antioxidant constituent of tomato fruit called lycopene (Di Mascio et al., 1989). This work is thus an attempt to investigate the effect of this natural antioxidant in preserving and improving the antioxidative function of GSH in experimental lead poisoning.

MATERIALS AND METHODS

Animals

Thirty healthy adult white Wister rats weighing 187 g (averagely) were used. The rats were obtained and kept in the animal house unit of Ladoke Akintola University of Technology, and were allowed to acclimatize with the house for a period of two weeks.

Preparation of tomato powder

Fresh and ripened tomato fruits were collected, sun dried and ground into powder form. The powdered tomato fruit was given with the dose of 33 g powder per kg animal weight based on USDA National Nutrition Database (USDA National Nutrient Database for Standard Reference, 2006).

Lead acetate for induction of lead poisoning

The lead acetate model described by Rader et al. (1981) was used for scheduling the dose regimen (Rader et al., 1981). A total of 200 µg lead acetate/ml was employed for inducing lead poisoning in the adult Wister rats. Lead toxicity was established by estimating blood lead concentration in the rats used.

Experimental design

Group 1 = Negative Control: Normal feed and water daily.
Group 2 = Experimental Group I: Normal feed and water daily, and 200 µg lead acetate/ml orally once per week.
Group 3 = Experimental Group II: Normal feed, 200 µg/l of lead acetate, 33 g powdered tomato and water daily.
Statistical Analysis of Results: ANOVA was used to analyse the data obtained.

Biochemical analysis

The determination of blood lead concentration was done by Atomic Absorption spectrophotometry based on the modified method of Hessel (1968). GSH and GSSG were done using glutathione kit based on Ellman method (1995).

RESULTS

The blood lead level, total glutathione, reduced glutathione (GSH), oxidised glutathione (GSSG) levels and GSH/GSSG ratio of the different experimental group are presented in Tables 1 and 2.

The mean value of blood lead concentration was 10±0.8 µg/dl for the negative control group, while for the positive control group that was induced with lead acetate, the mean value was 23±0.68µg/dl and that of the experimental group (animals treated with 200µg/l of lead acetate and 33g of powdered tomato fruit) was 22±0.69 µg/l. As a consequence, there was no significant difference between the blood Pb concentration in the positive group and the experimental group ($p>0.05$).

Mean concentration of oxidized glutathione in the negative control group was 5.67±1.587 µM, while in the positive control animal, the mean value was 7.92±2.589 µM, and in the experimental group, the mean value was 5.94±1.70 µM. As such, there was a significant mean difference between the positive group and the experimental group ($P<0.05$). The mean concentration of reduced glutathione for the negative control group was 8.4±1.525 µM, while the positive control group had a mean value of 5.95±2.134 µM. However, the experimental group had a mean GSH activity of 8.2±0.578 µM.

As such, there was a significant difference between the positive and experimental groups ($P<0.05$).

DISCUSSION

The pathogenesis of lead (Pb) toxicity is multifactorial as it directly interrupts enzyme activation, completely inhibits trace minerals absorption, binds to sulfhydryl protein, alters calcium homeostasis and lowers the level of available sulfhydryl antioxidant reserve in the body (Heskel and Dennis, 1983). The fact that Pb binds to enzymes that have functional sulfhydryl groups, rendering them non-functional and further contributing to impairment in oxidative balance, has been severally documented (Ahamed et al., 2005). The concentration of two specific sulfhydryl containing enzymes that are inhibited by Pb -delta aminolevulinic acid dehydrogenase (ALAD) and glutathione reductase (GR)-have been demonstrated to be depressed in both animal and human Pb-exposure studies (Gurer-Orhan et al., 2004).

Lots of works have been published on the mechanism of lead related pathologies, many of which are a direct result of the oxidant effect of lead on tissues and cellular component, which may be mitigated by improving the cellular availability of antioxidant; example of such antioxidant includes N-acetylcysteine (NAC). In a classical research by Patrick in 2006, it was shown that following the administration of NAC to lead-exposed animals, the ratios of reduced glutathione/oxidized glutathione were normalized. So, there was a decrease in MDA levels in brain and liver tissue, and an increase in cell survival rates (which had been significantly reduced by lead toxicity). Also, zinc is known to compete with lead binding by metallothionein-like transport protein in the gastrointestinal tract (Flora et al., 2006). As a result, zinc appears to have a mitigating effect on lead toxicity. When lead-exposed rats were given zinc, previously depressed level of SOD in the testes returned to normal and ALAD inhibition was reversed (Batra et al., 1998). So also, vitamin C, a known free radical scavenger, has been shown to inhibit lipid peroxidation in liver and brain of lead-exposed animals. Selenium, a required mineral for metalloenzyme and glutathione peroxidise has also been found to have antioxidant properties. Following intramuscular injection of sodium selenite, increased levels of SOD, glutathione reductase and reduced glutathione occurred in both liver and kidney (Othman and El Missiry, 1998). Other antioxidants used in mitigating the effect of lead are vitamin B_6 and vitamin E (Hsu et al., 1998), taurine (Selvaraj et al., 2006) and Alpha lipoic acid (Packer et al., 1995). The search for a chemical that will prevent or modulate the toxicity of lead and other toxic metals especially their antioxidant activity is continuous.

In the present study, the effects of a natural antioxidant-tomato fruit, rich in lycopene, in mitigating changes in antioxidant reserve in the kidney tissues of rats experimentally fed with high concentration of lead acetate was investigated. Expectedly, the mean blood lead concentration in the negative control group was substantially lower than among lead-exposed groups (10.7±0.8 µg/dl), 23.01±2.1 and 22.2±2.1 µg/dl, respectively. Similarly, blood Pb level was observed to be high in the experimental and positive control groups. Although the only kidney function marker studied (plasma creatinine level) was found to be similar and the differences found to be statistically insignificant in the three groups. The presence of kidney damage, probably due to nephrotoxicity, can not be ruled out especially since it has been documented by several authors that nephrotoxicity usually remain assymptomatic clinically until the chronic stage when end stage renal disease (ESRD) would have set in. This could explain why emphasis was on other early markers of antioxidant depletion especially as it is the main focus of the work (Morgan et al., 1966; Ball and Sorensen, 1969; Klinenberg, 1969).

On supplementation of their diet with powdered tomatoes fruit, there was no statistically significant difference between the mean blood Pb level obtained from the control and the test group supplemented with tomatoes powder (23.01±2.1 and 22.2±2.1 µg/dl, respectively) ($P>0.055$). On the other hand, reduced glutathione (GSH) was preserved and enhanced by tomato supplement in the experimental rats as observed in the increased mean GSH activity of 8.2±0.57 µM in the group of rats exposed to lead combined with tomato supplement relative to the other group without tomatoes fruit 5.9±2.1 µM. This difference was found to be statistically significant when compared to the mean GSH activity obtained for the negative control group (8.4±1.5 µM; $P<0.05$). As earlier stated, production of ROS and therefore depletion of the antioxidant level of the kidney cells due to Pb poisoning has been copiously reported, and GSH is one of these antioxidants that is directly affected by the toxic effect of Pb. Glutathione reductase, the enzyme responsible for recycling of glutathione from the oxidized form (glutathione disulfide-GSSG) to the reduced form (reduced glutathione-GSH), is deactivated by lead (Sandhir et al., 1994). Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase were found to correlate with depressed glutathione levels in occupationally-exposed workers (Hunaiti et al., 1995). The sulfhydryl complex of glutathione also directly binds to toxic metals that have a high affinity for this radical. Thus, mercury, arsenic and lead effectively inactivate the glutathione molecule, so that it is unavailable to act as an antioxidant or as a substrate in liver metabolism (Christie and Costa, 1984).

GSH level was depleted in the group of rats fed lead acetate only, while the activity of this powerful antioxidant was shown to be preserved and enhanced in the experimental group supplemented with tomatoes powder

along with lead acetate. That the preservation and enhancement of the GSH activity could be adduced to the tomatoes supplement was further shown by a significant GSSG level of 7.92±2.5 µM compared to GSSG level of 5.6±1.58 µM in the positive control and the test groups, respectively. Also, the normal ratio of GSH/GSSG was >1, but when mammalian cells were exposed to increased oxidative stress, the ratio of GSH/GSSG decreased as a result of GSSG accumulation. In this study, lead-exposed rats fed tomato supplement had GSH/GSSG ratio of >1 (1.38). This increase in GSH/GSSG ratio could not be unconnected with the free radical scavenging activity of the active constituent of tomato (Lycopene) with consequent reduction in GSSG level (5.94±1.7 µM) compared to what was obtained in Pb exposed rats not fed tomato supplement (7.9±2.59 µM).

Conclusion

In conclusion, as previously mentioned, the search for a remedy to the problem of lead toxicity in the vulnerable groups is still on. The need for solution to the problem of lead toxicity is underscored by its ubiquitous nature and extensive application in most industries and homes; although the current scope of work on antioxidant status may bring the necessary solution. That Pb affects the mammalian system by directly lowering antioxidant reserves and generating ROS resulting in oxidative damage is not in doubt. However, it has been shown in this study that lycopene from tomato interrupts the damaging effects of Pb and preserves and enhances the level of reduced glutathione, while reducing the level of oxidised glutathione. Therefore, supplementing the diet of lead occupationally exposed workers and other vulnerable group with tomatoes fruit, rich in lycopene, can be of help to prevent lead toxicity and increase the level of available sulfhydryl antioxidant reserve in the body.

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