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Modification by garlic extract against atrophic changes in lead induced nephrosis

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ABSTRACT

Lead is one of the oldest environmental contaminant, which is a known mutagenic agent or carcinogen vin cases of high exposure. The present experimental study is designed to observe the gradual antimutagenic effects of crude garlic extract on the atrophic changes in the nephron of albino rats due to excessive ingestion of lead. Twelve (12) male albino rats were used and were divided into three groups A, B and C with four animals in each group. Animals in group A, B and C were treated with 100mg/ml distilled water, 2.5mg/ml lead acetate and 25mg/ml garlic extract with 2.5mg/ml lead acetate respectively for 30-days. The animals were fed with normal diet (21% protein) and their body weights monitored and recorded weekly as well as change in their physical appearances. The animals were later sacrificed after 30-days by cervical dislocation and pathological examination of the kidneys was carried out. The results showed that group A animals had 1.41g mean weight/paired kidney and 369.91g mean body weight as well as average tissue ratio of 0.0038 and appeared active with normal feeding behavior. Animals in group B had 1.15 mean weight/ paired kidney, reduced mean body weight of 314.07g and average tissue of 0.0032 while group C animals fed with the extract in combination with lead showed fairly normal feeding behavior, active with sharp agility and were sensitive to their immediate environment having 1.36g mean weight/paired kidney, 361.24g mean body weight and 0.0036 average tissue ratio respectively. Hence, crude garlic extract is a viable dietary supplement with high antioxidant properties that could offer protection against heavy metal induced nephrosis.

Keywords: nephrosis, kidney, anti-mutagenic, atrophic-change, lead acetate, garlic.

INTRODUCTION

Lead is known generally as one of the oldest environmental contaminants with high molecular weight (207.2). It is a ubiquitous element in the environment causing oxidative stress in exposed individuals, leading to tissue damage. However, human beings are exposed to various environmental contaminants at different stages of life in which majority are very harmful. Lead in particular is harmful as it had been shown in various research findings as a carcinogen as well as a mutagen as the case may be (Fairhall and Sayers, 2001). Evidences have shown that about 93% cases involving both sexes in Pakistan with high blood lead levels resulted from lead contaminated public water supply, fish ponds and other sea foods via effluents from industrialized cities (Grandjean, 2001). In addition, association between lead poisoning and renal disease in human has been recognized for more than a century (Wedeen and Heddle, 1995). Numerous epidemiological, mortality, as well as experimental studies in animals have reported lead nephrotoxicity as being on the high side at high levels of exposure. However, studies on the action of lead on renal function at low levels of exposure have produced mixed pattern of findings (Payton et al, 1994).

To date, only a few cross-sectional studies and one notable longitudinal study (Kim, 1996) had reported significantly, association between elevated blood lead levels and reduced renal function measured by creatinine. Blood lead, which mostly reflects relatively recent exposure is an inadequate measure of total blood lead burden, this however, explains why most of the previous observational studies failed to find a significant association between low lead level exposure and renal dysfunction. In comparison with 95% of adult body lead burden that has a biologic half-life ranging from years to decades, now a better biologic marker for studying chronic toxicity of accumulated exposure and blood lead burden has been established (Hu, 1996: Korrick, 1999; Lin, 2003). In addition, bone lead may be a risk factor for impaired renal function by serving as cumulative exposure of the kidney to lead. Also aging in both men and women is associated with the release of bone lead into the circulation and this is a potentially important for soft-tissue lead exposure and toxicity (Ford, 2001). The present study was designed to evaluate the degree of damage done by lead to the tubules in the nephron of the kidney with its consequential effects as well as protection afforded by crude garlic extract.

MATERIALS AND METHODS

Experimental design: Twelve (12) male albino rats were used and were divided into three groups A, B and C with four animals in each group. Animals in group A, B and C were treated with 100mg/ml distilled water, 2.5mg/ml lead acetate and 25mg/ml garlic extract with 2.5mg/ml lead acetate respectively for 30-days. The animals were fed with normal diet (21% protein) their body weights monitored and recorded weekly as well as change in their physical appearances. The animals were later sacrificed after 30-days by cervical dislocation and pathological examination of the kidneys was carried out. Blood was collected in heparinized bottles (vials) for serum separation (Fahim and Griffin, 2002). The paired kidneys were removed, washed free of extraneous materials and weighed. Average tissue ratio was then determined using the formula:

$$\text{Average tissue ratio} = \frac{\text{Weight of paired kidney (g)}}{\text{Body Weight (g)}}$$

Procedure: The kidneys were cut into small pieces less than 5cm in size and the uniferous tubules were plucked out with forceps to see the plucking phenomena and were later fixed in formalin for 12hrs where they were later observed for stringing process. The tissues were later processed and parafin blocks prepared. 5cm thick sections were cut and stained with PAS-sulphuric acid haematoxylin. The stained sections were observed with compound microscope for qualitative assessment of the pathophysiology of the uniferous tubules as well as atrophic change in the kidney morphology. Diameter of the uniferous tubules was measured with occular micrometer and observations were recorded from three different sections of the slides chosen at random. Four stained slides from each of the twelve animals were examined with respect to body weight and the weight of paired kidneys were analyzed to quantify the average tissue ratio.

Table 1: Animal treatment

Group	Treatment Administered	No of rat
A	100mg/ml distilled water	4
B	2.5mg/ml lead acetate	4
C	25mg/ml garlic extract + 2.5mg/ml Lead acetate	4

Table 2: Average tissue ratio of the treated rats

Group	Mean Weight of Paired Kidneys (g)	Mean Body Weight after Experiment (g)	Average Tissue Ratio
A	1.41	369.91	0.0038
B	1.15	314.07	0.0032
C	1.36	361.24	0.0036

Group	1st week	2nd week	3rd week	4th week
A	(n = 363.005 ± 39.71	363.706±43.66	371.192±36.00	369.91±34.00
B	(n = 358.379±47.70	340.57±48.81	324.970*±41.41	314.07**±47.00
C	(n = 360.002±39.68	361.715±43.57	369. 198±35.91	369.88±33.97

*{Mean ±S.D : Students 't'- test * p<0.05, ** p<0.01}*

RESULTS

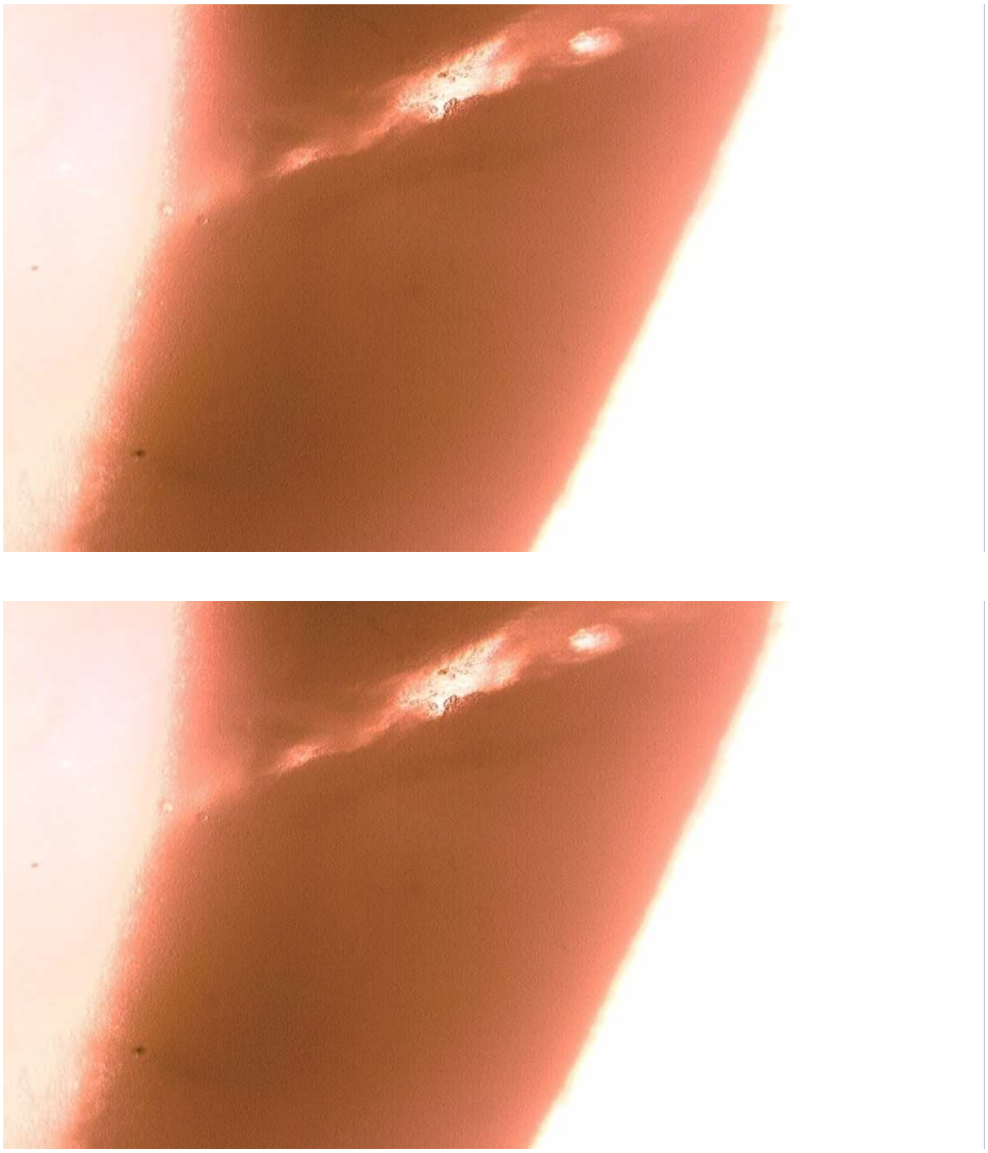

FIGURE A: Modified Kidney by garlic

FIGURE B: Nephritic Kidney

DISCUSSION

The nephroprotective potential of Allium sativum extract was tested in the kidneys of rats administered the pro-oxidant lead acetate and the extract which later showed the antioxidant effect was effective. From the experiment, all the rats in group A serving as control were active, healthy with normal feeding behavior. Their mean body weights (MBW) at the end of the experiment was 369.91 as shown in Table 2. The group B animals that were administered the pro-oxidant (lead acetate) only, were less active, appeared more irritable and lost considerable degree of weights. However, animals in group C fed with the extract (garlic) in combination with the pro-oxidant showed fairly normal feeding behavior, active with sharp agility and were responsive to their environment (sensitive) prior to sacrifice. After 30-day, the animals were sacrificed and the following observations were made on the kidneys of the animals. In group A, the kidneys were normal in size, shape, colour and texture and were less resistant on cutting with clear basement membrane. Besides, the plucking and stringing phenomenon of the uriniferous tubules were normal. The mean weight and average tissue ratio of the paired kidneys were 1.41 and 0.0038 respectively. While animals in group B administered the pro-oxidant only, had their kidneys acutely reduced in size, pale looking, and tough in consistency and showed resistance on cutting. Besides, it was difficult to pluck out any tubule from the kidney nephrotic mesh and stringing out phenomenon was absolutely lacking. The mean weight of the paired kidney was 1.15g and average tissue ratio was 0.0032. The group B animals under the 30-day exposure, lost average of 16% body weight and this significant drop in weight may be due to loss of appetite and gastrointestinal disturbances (Harvey, 2002). The kidneys were reduced in size and weight and the mean weight of paired kidneys of the rats was 5.6% less than that of control in group A. However, there was a slight modification over the mutagenic effects of the pro-oxidant in group C animals administered the garlic extract in combination with the pro-oxidant (lead acetate). It was observed that their kidneys were averagely normal in size, shape, colour with fairly soft texture and were also less resistant on cutting but to a lesser degree compared with the control in group A. besides, the tubules were easily plucked out and well flexible with free elasticity which proved that the plucking and stringing phenomenon of the uriniferous tubules were normal. The group C animals however, lost average of 4% under the 30-day exposure which was considered insignificant and this could be attributed to the antimutagenic effect of the Allium sativum extract administered in combination with the pro-oxidant into the animals.

In addition to this effect, average tissue ratio calculated for the group C animals (0.0036) which serves as parameter of assessment of kidney tissue relative to body weight was significantly reduced from that obtained in group B (0.0032) and slightly less than that of control animals(0.0038) in group A as shown in Table 2. However, the degeneration of the uniferous tubules, thickening of the basement membrane, pale colour of the paired kidneys which resulted from reduced vascular from collapsed blood vessels are all indications of atrophic changes that occurred in the kidneys of the experimental animals as a result of the administered pro-oxidant (lead acetate) while

the modification and amelioration of these effects were observed in group C animals fed with the extract due to the nephroprotective antimutagenic and anticlastogenic as well as antioxidant potentials which are the inherent properties of the Allium sativum extract exerted on the induced pro-oxidant in the kidney tissue. The mutated kidney and its pathological defect is represented by the diagram in Figure B (nephrotic kidney) while the averagely normal kidney modified by the extract *(Allium sativum)* is represented by the diagram in Figure A. The present research also reflects so much on the degenerative renal diseases which often lead to hypertension or heart failure as far as ultrafilteration by the kidney is concerned. The kidney altrafilteration which normally occurs in the nephron where present mesh of uniferous tubules with re- absorption of minerals, ions, amino acids and water via active transport powered by ATP energy. However, lead accumulates in the mitochondrion of the proximal convoluted tubules and inhibits the oxidative respiration in the mitochondrial cell that provides ATP energy for the active transport and then reduces the surface area of microvilli for absorption of these molecules, thereby obstructing smooth ultrafilteration process and consequently, causes congestive heart failure or hypertention as regards feed back inhibition system (Marsell and Reckless, 1999).

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