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PROTECTIVE POTENTIAL OF SUGAR BEET PULPEXTRACTS (SBPE) AGAINST HEAVY METAL INDUCED HEPATO-RENAL TOXICITY IN RATS

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ABSTRACT

The current study was aimed to assess renal and hepatic protective potential of sugar beet pulp extracts(SBPE) against toxicity in rats induced by lead acetate (Pb $(C_2H_3O)_2$). A total of 30 rats were pretreated with methanol and sodium hydroxide SBPE (100mg/kg/day) for 15 days, orally followed by intra-peritoneal lead acetate (40mg/kg/day). All rats were sampled on the 16th day for blood and organs. The results revealed significant ($p<0.05$*) protective effect by reversing fluctuation in opted biomarkers due to lead acetate such as AST (203±14.24 U/L), ALT (95.6±4.36U/L), CR (1.29±0.09mg/dl), BUN (40.1±2.61mg/dl), MDA (265±18.8n,mol/g) and CAT (21.5±0.75μ/g). The histopathological examination of SBPE treated groups indicated protective ability by alleviating morphological alteration due to heavy metal into more or less normal morphology. Methanol extract of Sugar beet pulp (SBPME) possesses more antioxidant ability against lead-induced oxidative stress than aqueous extract of Sugar beet pulp (SBPAE) in rats.

Keywords: Hepato-renal toxicity, Lead acetate, Sugar beet pulp, Antioxidant, Methanol Extract, Aqueous extract

INTRODUCTION

The metals are elements having high electric conduction, pliability, or gleam which intentionally allows electron to lose resulting in formation of cations. In earth's crust, metals are present naturally and abundantly. The composition of different metals varies according to their different areas leading to variation in surrounding concentrations. The different environmental factors and specifications of metal are the monitoring parameters for distribution of metals in our atmosphere (Khlifi & Hamza-Chaffai, 2010). The most significant and persistent environmental pollutants are heavy metals as their poisoning accounts multiple sectors like ecology, evolution, nutrition and environment (Jaishankar, Mathew, Shah, & Gowda, 2014). There are numerous heavy metals which are present in waste water and can be crucial for human health along with environment such as lead, arsenic, cadmium, nickel, zinc, copper, and, chromium. (Lambert, Leven, & Green, 2000). Heavy metals have multiple sources including erosion of soil, mining, weathering of earth's crust, industrial waste, sewage discharge, urban runoff, pesticides, and, insecticides etc (Morais, e Costa, & de Lourdes Pereira, 2012).

Lead induces toxicity in various organs of body through ionic mechanism or oxidative stress. The main reason of oxidative stress in cell is fluctuation in balance among generation of free radicals and production of antioxidants to neutralize the toxicants or reactive species and alleviation of possible cell damage. In case of lead exposure, reactive oxygen specie (ROS) production inclines while antioxidant level declines. But glutathione is present in oxidized (GSSG) or reduced (GSH) form which provides reducing equivalents ($H^+ + e^-$) from its thiol groups of cystein to reactive oxygen species to stabilize them. The glutathione disulfide (GSSG) is formed when reduced glutathione binds with another glutathione molecule by donating the electron in the presence of glutathione peroxidase enzyme. Among total glutathione, there is 90% proportion of reduced glutathione (GSH) while oxidized glutathione (GSSG) accounts 10% in normal case. On contrary, in case of oxidative stress, the GSSG concentration is much higher than that of GSH. Lipid peroxidation is another bio-indicator of oxidative stress, resulting from release of free radicals from lipids embedded in cell membrane(Wadhwa, Mathew, Jatawa, & Tiwari, 2012). The extensive cellular damage can be the result of higher concentration of ROS which cause destruction of various structures e.g. proteins, membranes, lipids, and nucleic acid (Mathew, Tiwari, & Jatawa, 2011).

Sugar beet (*Beta vulgaris*) pulp is a material which is deficient in sugar and rich in fibers which is end product of sugar beet extraction (A. A. A. Mohdaly, Sarhan, Mahmoud, Ramadan, & Smetanska, 2010). The major constituents of SBP are L-arabinose, oligosaccharide and fibre, which are recognized to hinder the intake of sucrose (Ferreira, Diez, Faulds, Soliveri, & Copa-Patiño, 2007). The various extracts of sugar beet pulp e.g. ethanolic, are rich in phenolic acids having significant antioxidant potential (Marinova & Yanishlieva, 1994).

MATERIALS & METHODS

Extraction with Methanol:

The most common solvent used for extraction of phenolic compounds from medicinal plants is methanol (Eghdami & Sadeghi, 2010). Polarity is one of the significant factors to select any solvent as ferulic acid in sugar beet pulp is a polar phenolic compound and has protective potential in methanol. For methanol extraction, 5 gram of SBP was mixed in 100ml methanol solution (99%v/v). The ferulic acid extraction was carried out at 60 C° by following reflux method for 2, 6, and 12 hours. The 6M HCl was added to adjust the pH of methanolic extract for precipitation of lignin (Bunzel, Ralph, Funk, & Steinhart, 2003). The filtrate obtained after filtration of mixture was subjected to centrifugation for 2 minutes at 9000 RMP. To remove the extra methanol, the supernatant was vacuum evaporated.

Extraction with Sodium Hydroxide:

To prepare sodium hydroxide extract, 5 gram of SBP in powder form was poured in an Erlenmeyer flask connected with a condenser, and 100 ml NaOH 0.5 molL-1 solution was added. Heat was provided up to 60°C for 2, 6 and 12 h and gradually cooled down to 20°C. Once the extraction process was completed, pH was reduced to 2.0, so that the hemicellulose would precipitate. The final mixtures were filtered off, and subsequently 150 mL ethyl acetate was added to the filtrates in a 250 ml baffled Erlenmeyer flask and was shaken in vortex (100 rpm) at room temperature for 15 min to carry out a liquid–liquid extraction. The supernatant was separated and was placed in vacuum evaporator to remove excess solvent. All the treatments were conducted in triplicate and extracts were characterized through the determination of total phenolic compounds, ferulic acid and antioxidant activity. It should be noted that all of the hydrolysates were held under nitrogen gas in a 250 ml flask covered with aluminum foil and kept in a dark place in order to avoid phenolic compounds oxidation and cis–trans isomerization (Oosterveld, Beldman, Schols, & Voragen, 1996).

Experimental Design:

Thirty male albino rats (weight=250g±5) were divided into six groups each having five rat labeled as Group 1 (Control Positive Group), Group 2 (Control Toxic Group), Group 3 (Methanolic SBPE), Group 4 (Sodium Hydroxide SBPE), Group 5 (Lead acetate + Methanolic SBPE) Group 6 (Lead acetate + Sodium Hydroxide SBPE). Following Table No. 1 is showing medication to rats of all groups including route of administration, dose along with extracts. The dosing was continued for 15 days.

Table 1: Experimenat Design

Groups	Medication
G1	Normal Saline I.P
G2	Lead Acetate 40mg/kg/day I.P + Normal Saline P.O
G3	Methanolic Sugar Beet Pulp Extract 100mg/kg/day P.O + Normal Saline 40mg/kg/day I.P
G4	Sodium Hydroxide Sugar Beet Pulp Extract 100mg/kg/day P.O
G5	Lead Acetate 40mg/kg/day I.P+ Methanolic Sugar Beet Pulp Extract 100mg/kg/day
G6	Lead Acetate 40mg/kg/day I.P+ Sodium Hydroxide Sugar Beet Pulp Extract 100mg/kg/day

I.P= Intra-peritoneal, P.O= Per-oral

Sample Collection:

The rats of all groups were weighted after overnight fasting of animals on 16th day of experimentation. All these rats were anesthetized with sodium pentobarbital (50mg/kg) to collect blood and tissue samples. Blood samples were collected at very next day of termination of dosing. The intra-cardiac route was selected to collect the blood from rats. Blood was collected in plane tubes by using 5ml syringe with 26 G needle. These samples were allowed to coagulate then stored at room temperature. For isolation of serum, tubes were centrifuged at a rate of 3000 RMP for 10 minutes. Finally, supernatant layer was separated which was preserved at 4°C for further biochemical and antioxidant analysis. These samples were subjected to spectrophotometric analysis by using commercially available kits (Randox of UK) to analyze values of various biomarkers e.g. AST (Aspartate transaminase), ALT (Alanine transaminase), CR (Creatinine), BUN(Blood urea nitrogen). Furthermore, antioxidant enzymes concentratione.g. catalase (CAT) or malondialdyhede (MDA), was also evaluated from samples by using spectrophotometric technique.

The liver and kidney of rats were quickly excised and ice-cold phosphate buffer saline (PBS) was used to wash tissues. These excised tissues were preserved in 10% formaline buffer solution for further histo-pathological study (Murakami et al. 2007).

Histopathology:

Parrafin Embedding technique was used to examine morphology of hepatic and renal tissue by H&E stain (Bancroft et al. 2013).

Antioxidant enzymes:

Spectrophotometer method was used for quantitative analysis of catalase (Hadwan et al. 2016) and malondialdehyde (Ohkawa et al. 1979) in serum.

Statistical Analysis:

The obtained data was expressed in mean ± SEM (standard error mean). ANOVA (Analysis of variance) was used for statistical analysis of data with significance of $P < 0.05$.

RESULTS

AST and ALT:

Table-2 represents significant elevation of aspartate aminotransferase (AST) 872±38.98U/L and alanine aminotransferase (ALT) 355±48.47U/Ldue to lead acetate in control toxic group (G2). On contrary, sugar beet pulp extracts treated groups showed significant ($p<5$) possible reversal in elevated amounts of ALT (95.6±4.36 U/L) or AST (203±14.24U/L).

BUN and CR:

The renal function test of G2 rats revealed significant ($p<5$) fluctuation in kidney biomarkers above their normal scale such as blood urea nitrogen (BUN) 51.7±3.02mg/dlor creatinine (CR) 1.9±0.16mg/dl. In case of G5 and G6, the significant ($p<5$) decline was observed in above biomarkers due to treatment with sugar beet pulp extracts e.g. CR (1.29±0.09mg/dl) or BUN(40.1±2.61mg/dl).

MDA and CAT:

Table-2 is also indicating the fact that in control toxic group (G2), there was significant ($p<5$) disturbance in antioxidant enzymes such as increase in melondialdehyde (MDA) 324±45.nmol/gor decrease in catalase (CAT) 19.33±1.27µ/g. This variation in antioxidant enzyme due to toxicity was reversed by sugar beet pulp extracts as in G5 or G6 e.g. MDA (265±18.8nmol/g) and CAT (21.5±0.75µ/g).

Histopathological Examination:

Figure-1 is showing normal healthy liver morphology of G1 rats having smooth exterior, reddish brown architecture, normal hepatocytes, and portal tact with central vein. On contrary, the morphology of control toxic group (G2) rats shows significant toxic potential of lead acetate having inflammatory hepatocytes and congested central vein (Figure-2). Furthermore, in case of treated rats with sugar beet pulp extracts exhibits near to normal histology early damaged by lead acetate as shown in figure-3.

Similarly, Figure-4 is representing healthy kidney tissue morphology of G1 rats including healthy renal tubules and glomerulus. In control toxic group (G2), rats show abnormal renal histology due to lead acetate such as congested glomerulus and infiltrated renal tubules as shown in figure-5. In addition, the sugar beet pulp extracts exhibited significant protective effect by reversing histological alterations in rats to almost normal morphology in treated groups G5 & G6.

Table 2: Protective Effect of SBPE against Lead acetate toxicity

Groups	AST (U/L)	ALT (U/L)	CR (mg/dl)	BUN (mg/dl)	CAT (µ/g)	MDA (nmol/g)
G1	121±15.36	66±10.19	0.90±0.21	29.4±2.44	21.1±0.71	179±15.6
G2	872±38.98**	355±48.47**	1.9±0.16**	51.7±3.02**	19.33±1.27**	324±45.1**
G3	122±12.94*	71.2±12.41*	0.88±0.2*	40.6±1.96*	21.36±0.71	194±22.4*
G4	121±16.77*	68.2±12.82*	0.85±0.15*	31.4±1.50*	21.9±0.67	191±18.9*
G5	203±14.24**	95.6±4.36**	1.29±0.09**	40.1±2.61**	21.5±0.75**	265±18.8**
G6	216±11.69**	101.4±5.5**	1.42±0.13**	42±1.58**	20.9±0.92**	274±10.1**

Data are shown as means ± SEM (standard error mean). (n=5). Group 1 (Control Positive Group), Group 2 (Control Toxic Group), Group 3 (Methanolic SBPE), Group 4 (Sodium Hydroxide SPBE), Group 5 (Lead Acetate + Methanolic SBPE), Group 6 (Lead Acetate + Sodium Hydroxide SBPE).*(P <0.05) and **(P<0.05) when compared to G2 (Control Positive Group)

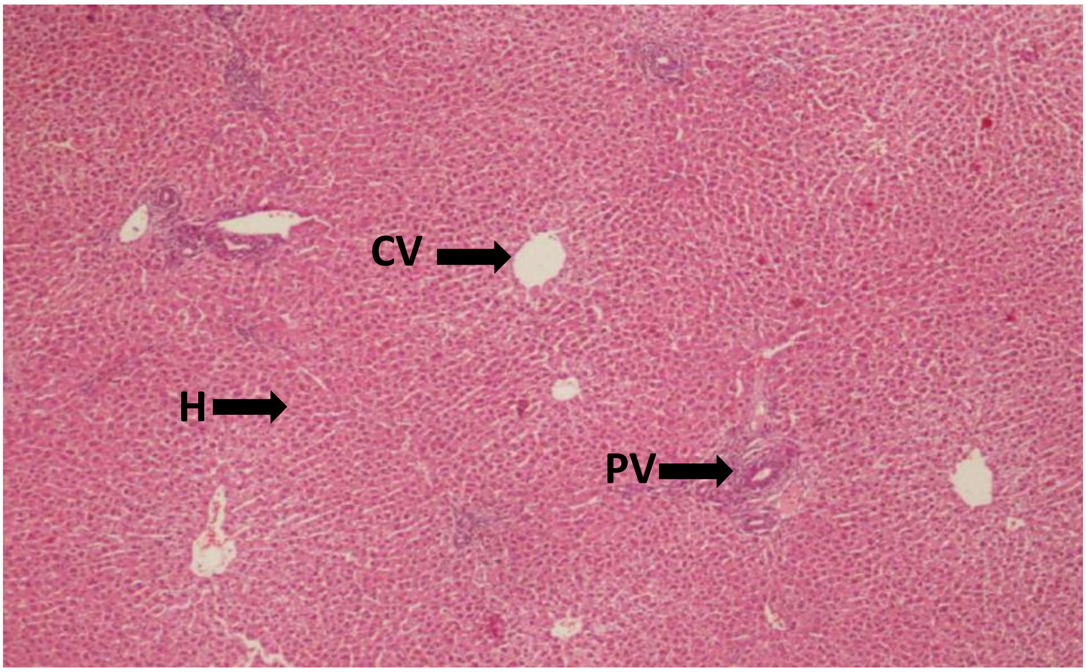

Figure-1: Photomicrograph of mouse liver showing normal morrphology of portal vein (PV) central vein (CV) and Hepatocytes (H) from Group A

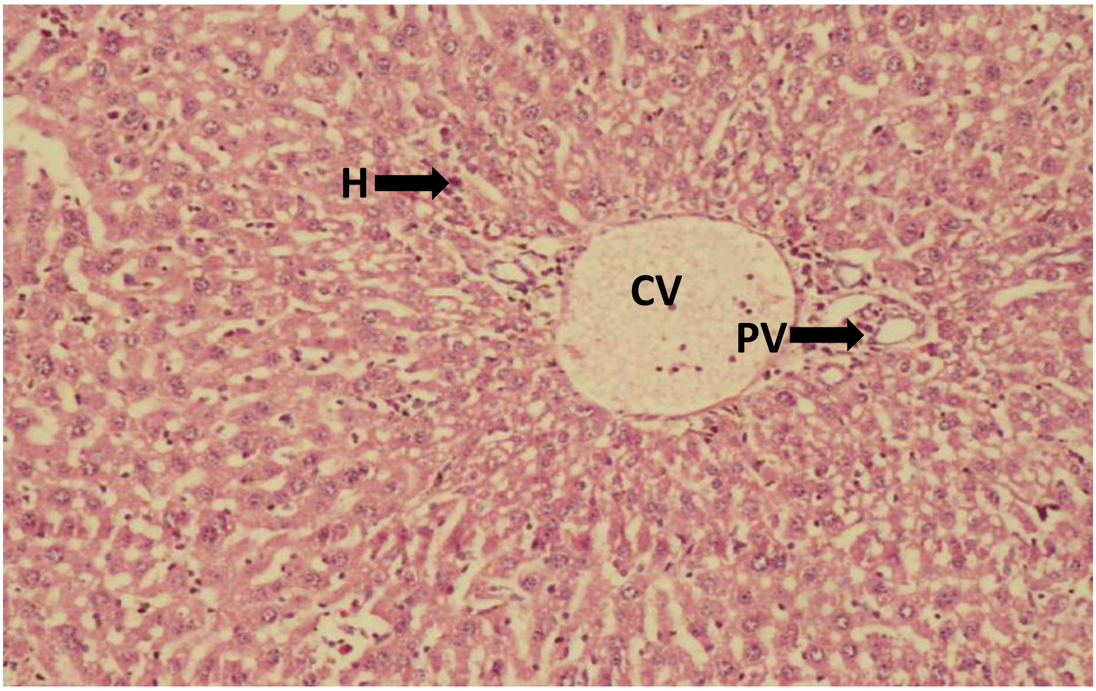

Figure-2: Photomicrograph form Group B showing severe periportal inflammation, i.e., inflammatory hepatic cells (H) around portal triad consisting of portal vein (PV)

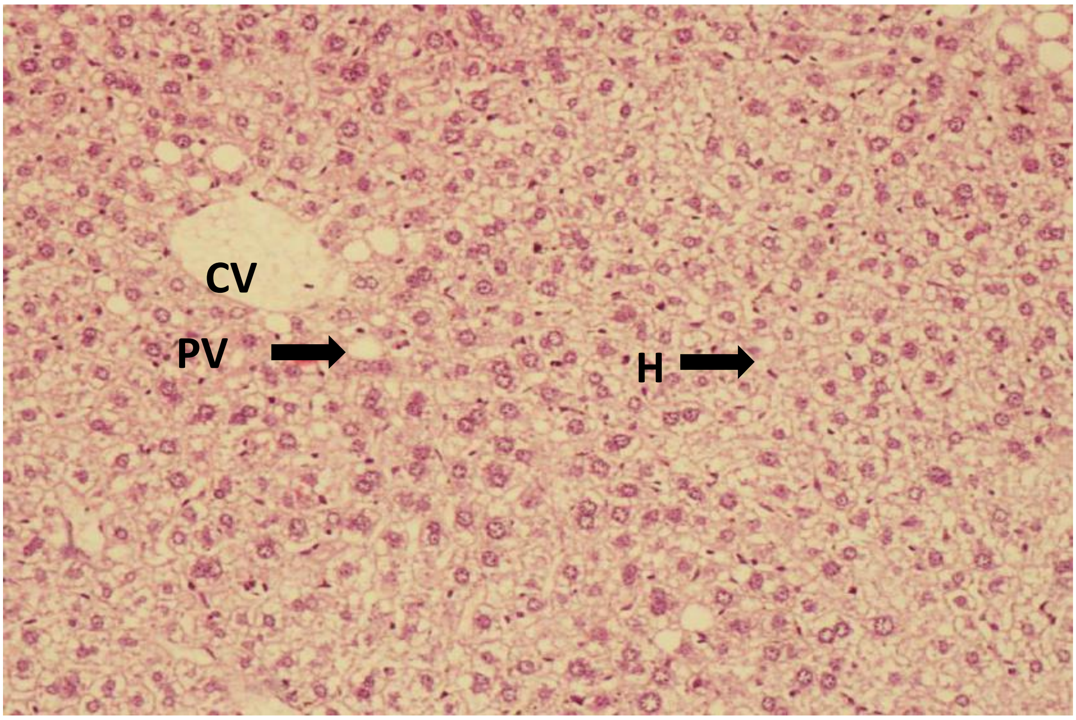

Figure-3: Photomicrograph from Group treated with sugarcane juice showing portal triad clear of inflammatory cells and hepatocytes (H) with normal morphology

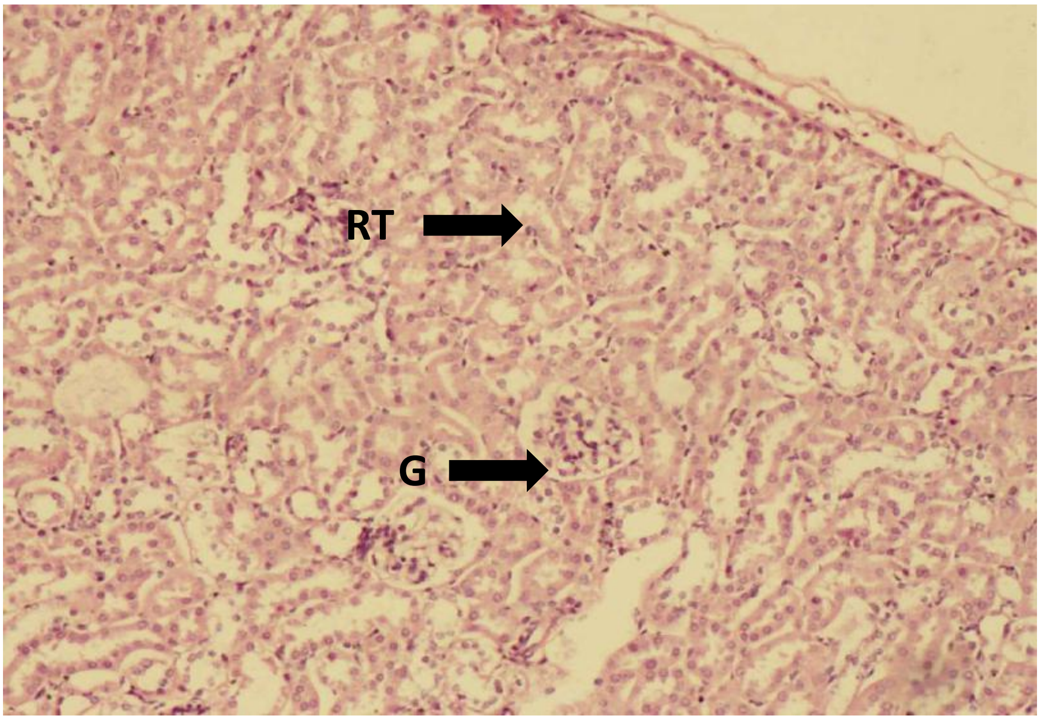

Figure-4: Photomicrograph of mouse kidney showing normal morphology of renal tubules (RT) and Glomerulus (G) from Group A

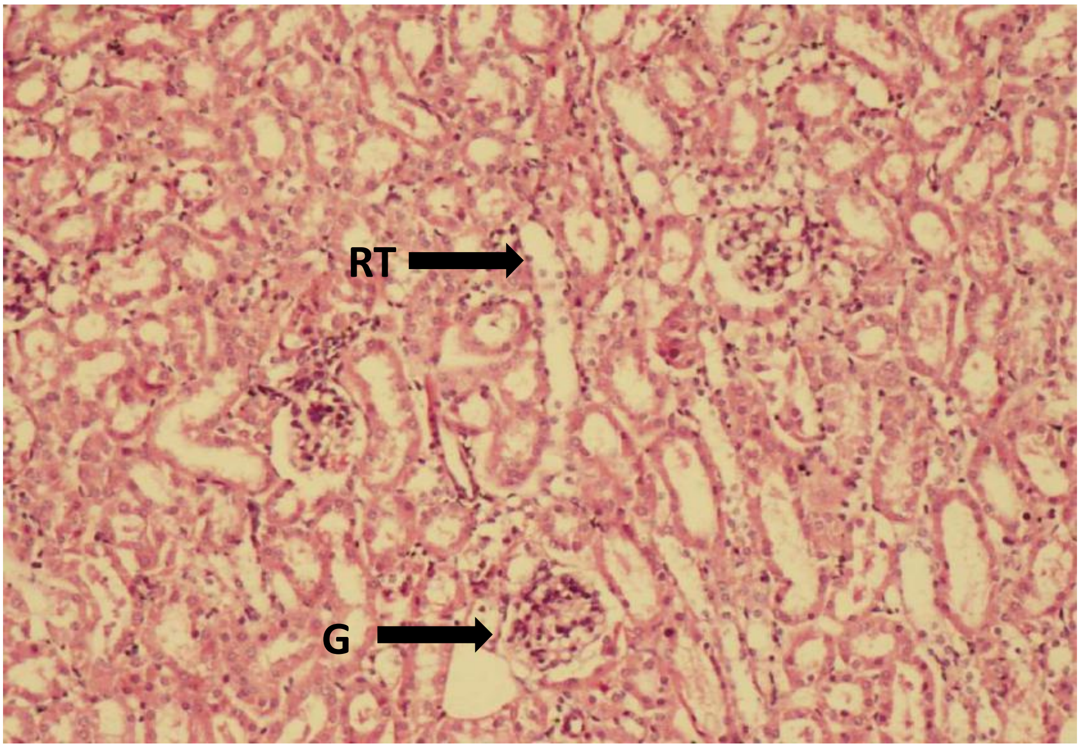

Figure-5: Photomicrograph of mouse kidney showing abnormal morphology of infiltrated renal tubules (RT) and congested glomerulus (G) from Group B

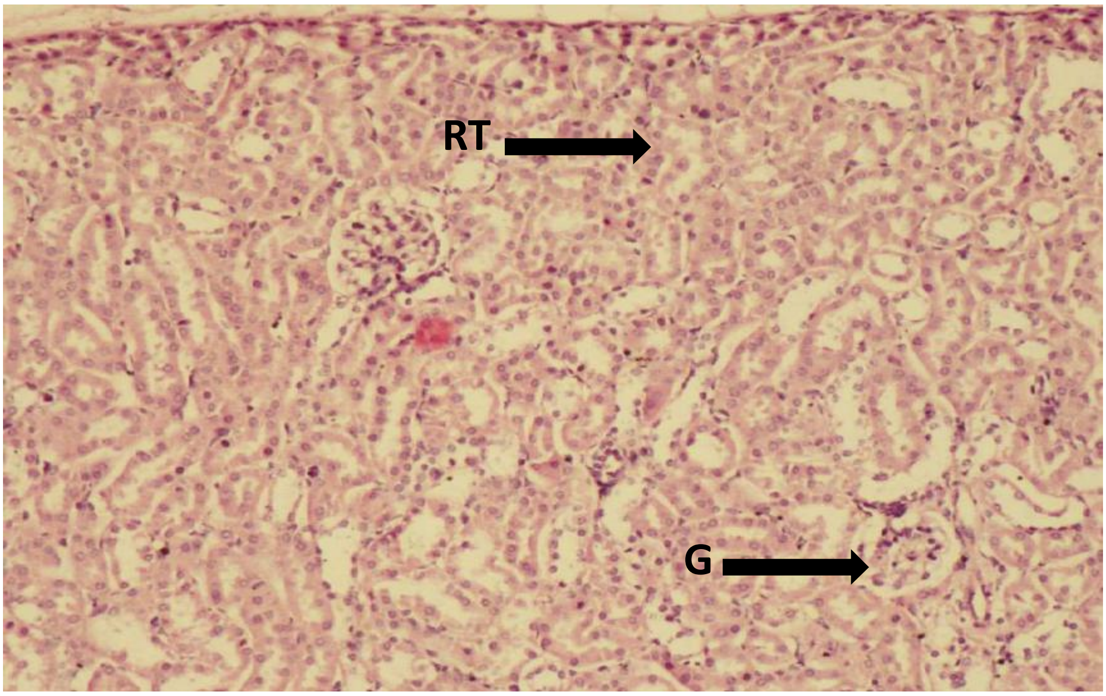

Figure-6: Photomicrograph of mouse kidney showing morphology of renal tubules (RT) and Glomerulus (G) from Group treated with sugarcane juice

DISCUSSION

The metallic elements which are denser than water are usually called as heavy metals. There is a postulation that toxicity and heaviness have direct relation therefore even minute exposure of arsenic causes toxic effects (Duffus, 2002). These heavy metals have extremely contaminated our environment resulting into escalation in ecological and global health issues in our community. These heavy metals have numerous applications in industry, agriculture technology and domestic which increasing heavy metal exposure to human. There are multiple sources of heavy metals while geology, industry, agriculture, pharmaceutics, domestic wastes and atmosphere are well documented. Mining, smelters, and industrial operations are significant regions of environmental pollution (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014).

A major recognized public health risk is lead toxicity in developing countries. To encounter this risk, number of remedies has been investigated but still some cases are reported. The numerous harmful actions of lead exposure have been noted due to oxidative stress e.g. renal, hematopoietic, nervous and reproductive complications (Flora, Gupta, & Tiwari, 2012).

The plants contain natural antioxidants which hunt injurious free radicals which counter our body. The mechanism of antioxidants involves suppression of reactive oxygen species by inhibiting enzymes or by formation of chelates with trace elements. The chemical entities having unpaired electron are free radicals which impose various beneficial effects on human health and provide support in disease condition to cure it such as lung or cardiovascular disorders or inflammation. Such radicals can also inactivate enzymes resulting in cellular damage including genetic material. These can damage cell membranes and other vital cell components, such as genetic material in the cell nucleus, and can inactivate enzymes. The free radical hunting action is widely been evaluated by using DPPH. The plants contain antioxidants e.g. ascorbic acid, phenolic, and vitamin E which have potential to reduce oxidative stress due to many complications like cataracts, diabetes, cardiovascular diseases, ageing, immune deficiency disease, atherosclerosis, and cancer (Bharti, Ahuja, Sujan, & Dakappa, 2012).

A research was conducted to evaluate antioxidant potential of sugar beet pulp to compare with antioxidant having synthetic origin. Methanol was used as solvent to extract antioxidant ingredients of sugar beet pulp by providing significant oxidative environment through sunflower or soybean at 70 C for 72 hours at various concentrations. The gallic acid and chlorogenic acid were most eminent phenolic ingredients identified in sugar beet pulp by utilization of thin-layer chromatography (A. A. A. Mohdaly et al., 2010).

The antioxidant ability of sugar beet pulp was studied by using various solvents. Among these solvents methanol extracted highest concentration of phenolic compounds such as0.81 mg gallic acid equivalent g^{-1} dry weight(A. A. Mohdaly, Sarhan, Smetanska, & Mahmoud, 2010).The protective effect of SBP was investigated by using LLC-PK_1cells in which oxidative stress was induced by high glucose. High glucose caused significant lipid peroxidation in cell which was reversed by sugar beet pulp (Song, Kim, Choi, Song, & Cho, 2012).

CONCLUSION

It was concluded that sugar beet pulp has significant protective power against heavy metal induced toxicity in rats. This project can be beneficial to curb various human health issues related with environmental toxicity. Heavy metals exposure is the prominent global health complication. However, such research projects should be conduct to reduce the toxic effects of heavy metals.

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