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Full Length Research Paper

Hepatoprotective Effects of *Portulaca oleracea* on Liver Enzymes of Potassium Bromate Induced Hepatotoxicity in Adult Wistar Rats

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Abstract

To evaluate the effects of methanolic extract of *Portulaca oleracea* on liver enzymes of Potassium bromate ($KBrO_3$) induced hepatotoxicity in wistar rats. Twenty five adult wistar rats weighing 160-280g were divided into five groups. The negative control group A was orally administered with 1ml of distilled water daily; the positive control groups B was orally administered with 75mg/kg for 14 days. Group C received 250mg/kg of extract and 75mg/kg of $KBrO_3$ after six hours orally. Group D received 500mg/kg of extract and 75mg/kg of $KBrO_3$ after six hours orally. While group E received oral dose of potassium bromate at 75mg/kg and 500mg/kg of extract after six hours. The body weights were reduced at the end of the period of administration. The liver enzymes (AST and ALT) and serum bilirubin level increased significantly ($P<0.05$) in the positive control group B, which received 75mg/kg. Administration of $KBrO_3$ six hours after the plant extracts at two dose levels (250 and 500mg/kg) resulted in prevention of hepatic injury by $KBrO_3$. Administration of the plant extracts six hours after $KBrO_3$ at one dose level (500mg/kg) resulted in recovery as indicated by the decrease in the serum parameters, produced by $KBrO_3$. Our findings suggest that methanolic extracts of *Portulaca oleracea* has hepatoprotective effects on $KBrO_3$ induced hepatotoxicity and seems to be useful in controlling hepatic injury in drug induced hepatotoxicity.

Keywords: Liver, Potassium bromate, Hepatotoxicity, *Portulaca oleracea*.

INTRODUCTION

Once in a while one comes across a plant that is so outstanding, that one wonders how on earth it has been overlooked. Purslane (*Portulaca oleracea*) is one such plant. *Portulaca oleracea* is an annual succulent in the family of Portulacaceae which may reach 40cm in height. Approximately forty varieties currently are cultivated (Prashanth *et al*., 2005). It is commonly called Purslane in English language, ebe ehofen in Edo language, Ntioke in Igbo language, babba jibji in Hausa language and esan omode or papasan in Yoruba language (Burkill, 1997). It has an extensive old world distribution extending from North Africa through the Middle East and the Indian subcontinent to Malaysia and Australia. This half-hardy low growing plant has slightly succulent leaves and stems that are used raw or cooked. There are green and yellow leaved forms; the green type has thinner leaves, is more vigorous and possibly better flavoured (Brickell, 1992). It is used as a potherb in the Mediterranean, Central European and Asian countries. It is also referred to as the common (Purslane and Quah, 2007). It is naturalized elsewhere and in some regions, it is considered an invasive weed. It has smooth, reddish, mostly prostrate stems and alternate leaves clustered at stem joints and ends. The yellow flowers have five regular parts and are up to 6mm wide. Depending upon rainfall, the flowers appear at any

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time during the year. The flowers open singly at the center of the leaf cluster for only a few hours on sunny mornings. Seeds are formed in a tiny pod, which opens when the seeds are mature. It has a taproot with fibrous secondary roots and is able to tolerate poor compacted soils and drought (Ramesh and Hanumantappa, 2011).

Liver is the largest organ in the mammalian body. The hepatocytes have metabolic functions that deal with very essential processes such as detoxification, deamination, transamination, removal of ammonia in the form of urea, biosynthesis and release of the non-essential amino acids and plasma proteins with the exception of immuno-gamma globulins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids, synthesis of lipoproteins, phospholipids and cholesterol, oxidation of fatty acids, storage of iron in the form of ferritin as well as storage of vitamins A, D and B12. Several functional tests have been formulated to explore hepatic status (Johnson, (1995); Stryer, (1995); Ganong, (1999); Nelson and Cox, (2000)). Several enzymes have been determined to explore hepatic status such as alanine amino transferase (ALT) and aspartate amino transferase (AST). In addition some other tests include measurement of serum lactic dehydrogenase (LDH), gamma glutamyl transpeptidase (GGT), alkaline phosphatases and 5-nuc- leotidase activities are employed (Burtis and Ashwood, 1999).

Potassium bromate ($KBrO_3$) is an oxidizing agent, primarily used as a maturing agent for flour and as a dough conditioner (National Toxicology Program, 1991). It is also generated as a by-product of ozonization of surface water in treated drinking water (Cavanagh *et al*., 1992).

Although adverse effects are not evident in animals fed with bread-based diets made from flour treated with $KBrO_3$, recent studies have reported that the agent is hepatotoxic (Dimkpa *et al*., 2013) and several other studies have also reported the nephro and neurotoxicity of $KBrO_3$ in man and its carcinogenicity in animals following exposure (International Agency for Research on Cancer (1986); Kurokawa, (1990); Nakano, (1989); Kurokawa, (1983)) thus demonstrating the danger, which potassium bromate poses to health if consumed in food or water.

Therefore, this work is aimed at investigating the hepatoprotective effects of portulaca oleracea on liver enzymes of potassium bromate induced hepatotoxicity in adult wistar rats

MATERIALS AND METHODS

Breeding of Animals

A total of twenty five wistar rats weighing between 160g and 280g were obtained in the pre-clinical Animal House of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. They were acclimatized for a period of (14) days and housed under standard laboratory conditions (29 ± 2℃) temperature, 40-55% humidity, good ventilation) and had free access to water and diet (normal rat chow).

Collection of Plant Material

The fresh specimens of *Portulaca oleracea* were collected from St. Thomas Anglican Church's Compound along Ubiaja Road, Esan North East Local Government Area, Edo State and were authenticated by Dr. Orji, a botanist in the department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Preparation of Extracts

Large quantities of the fresh specimens of *Portulaca oleracea* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air-dried for eight weeks, and the dried specimens were pulverized using mechanical grinder. The pulverized specimen weighed 999g approximately 1kg. This weighted specimen was macerated and extracted with 70% methanol (1:2 wt/vol.) for 72 hours at room temperature (26℃-28℃). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.2% of the starting material.

Procurement Chemical and Kits

Potassium bromate ($KBrO_3$) and the biochemical kits for the determination of serum biomarkers of liver were purchased from Cephas Global Resources Limited (A division of Deliving Stone Int'l), E Line 444 (along Fin Bank/Eco Bank), Head Bridge Market, Onitsha, Anambra State.

Experimental Design

The animals were divided randomly into five groups, each containing five rats. The rats were also separated into male and female in each cage.

Group A received 1.0ml of distilled water orally as the negative control group.

Group B received oral dose of potassium bromate at 75mg/kg as positive control group.

Group C received 250mg/kg of extract and 75mg/kg of potassium bromate after six hours orally.

Group D received 500mg/kg of extract and 75mg/kg

of potassium bromate after six hours orally.

Group E received oral dose of potassium bromate at 75mg/kg and 500mg/kg of extract after six hours orally.

The administration lasted for fourteen days. Twenty four hours (day 15) after the last dosing of the animals, blood samples were collected for determination of serum biomarkers of the liver and histopathological studies were also done. (Table 1).

RESULTS

Figure 1 indicates the effect of the extract on AST. When compared with the negative control group (Group A), there was no significant difference between group C and D. In contrast, data indicated significant difference in AST between Group A and B and D. Although Group D and E took the same dose (500 mg/kg), there was a significant increase in AST of group D when compared with group E, indicating that the prevention was higher in group E. The positive control group (Group B) showed hepatic injury evidenced in the highly significant increase in AST.

Figure 2 indicates the effect of the extract on ALT. When compared with the negative control group (Group A), there was significant difference between group C and D. Also, data indicated significant difference in ALT between Group A and B and D. Although Group D and E took the same dose (500 mg/kg), there was a significant increase in ALT of group D when compared with group E, indicating that the prevention was higher in group E. The positive control group (Group B) showed hepatic injury evidenced in the highly significant increase in ALT.

Figure 3 indicates the effect of the extract on total bilirubin. When compared with the negative control group (Group A), there was no significant difference between group A and C. In contrast, data indicated significant difference in total bilirubin between Group A, B, D and E. Although Group D and E took the same dose (500 mg/kg), there was a significant increase in total bilirubin levels in Group D when compared with group E, indicating that the prevention was higher in group E. The highly significant increase in total bilirubin levels suggests that there is a problem with the functionality of the liver.

DISCUSSION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury or impairment of its function may lead to several implications on one's health. Management of liver diseases is still a challenge to modern medicine. Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety.

Also, studies have shown that it possesses the potential of inducing deafness, redness and pains of the eye and skin (DeAngelo *et al.,* 1998) and Office of Environmental Health Hazard Assessment (2004).

In the present study, we used $KBrO_3$ model for liver damage induction to investigate whether the plant extract could decrease efficiently the toxicity produced by the hepatotoxicant.

A reduction in body weights of the rats was observed. The reduction in weight may be due to reduced water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract (Joseph *et al.,* (1989); Hassan *et al.,* (2005)). In contrast, other studies, Watanabe *et al*. (2004), Farombi *et al.* (2002), Abuelgasim *et al*. (2008) have reported absence of $KBrO_3$ effect on body weights of rats.

The effect of the extracts at two dose levels (250, 500mg/kg) and $KBrO_3$ on serum marker enzymes (ALT and AST), and bilirubin in potassium bromate-induced hepatotoxicity showed that *Portulaca oleracea* has significant and efficient hepatoprotective activity. This agrees with previous studies (Anusha *et al.,* (2011)); Mohammed-Abdalla and Soad-Mohamed, (2010); Muneer *et al*., (2013) which reported the hepatoprotective effect of the extract.

Destruction of the hepatocytes induced by $KBrO_3$ at 75mg/kg caused significant ($P<0.05$) rise in ALT, AST, and bilirubin in group B, which served as the positive control group, when compared with the normal control group (group A). This finding agrees with a previous study (Dimkpa *et al.,* 2013) which reported increase in ALT and AST in rats administered with 100 and 200mg/kg of $KBrO_3$.

Administration of $KBrO_3$ six hours after the plant extracts at two dose levels (250 and 500mg/kg) resulted in prevention of hepatic injury induced by $KBrO_3$ as indicated by the decrease in the hitherto increase of the serum parameters, produced by $KBrO_3$.

In the same fashion, administration of the plant extracts six hours after $KBrO_3$ at one dose level (500mg/kg) resulted in recovery as indicated by the decrease in the serum parameters, produced by $KBrO_3$.

The prevention of hepatic injury induced by Potassium bromate was observed to be higher in group E, which took the extracts six hours after the induction of potassium bromate than group D, which took the extracts six hours before potassium bromate induction.

Necrosis or membrane damage releases the enzymes into circulation and hence it can be measured in the serum. The reversal of increased serum enzymes in $KBrO_3$ induced liver damage by the extract may be due

Table 1. Showing the Mean Weights of the Animals before and after Treatment

GROUP n=5	MEAN ± SEM BASELINE WEIGHT	MEAN ± SEM AFTER TREATMENT	P-VALUE
A	1.8500 ± 8.36660	1.8500 ± 8.36660	1.00
B	2.3800 ± 11.13553	2.2600 ± 8.71780	0.421
C	2.0800 ± 4.89898	1.9100 ± 5.09902	0.043
D	2.0000 ± .00000	1.8800 ± 2.54951	0.002
E	2.0400 ± 6.78233	1.9520 ± 4.74763	0.319

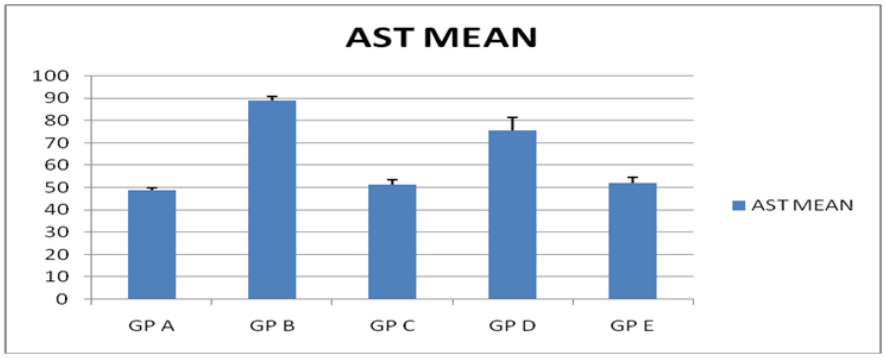

Figure 1. Showing the effect of *P. oleracea* on AST

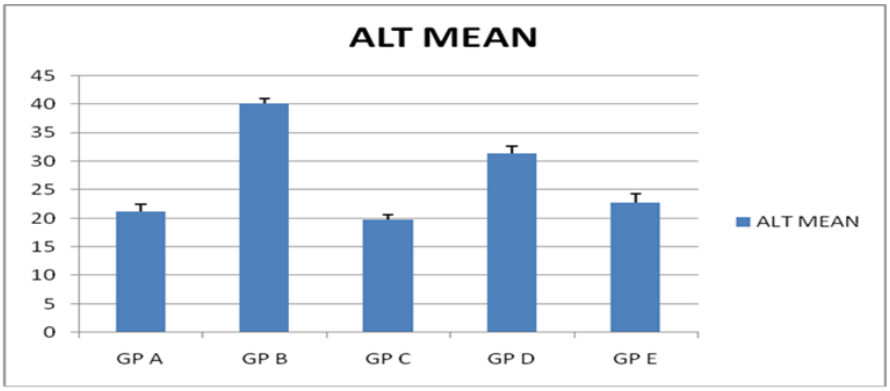

Figure 2. Showing the effect of *P. oleracea* on ALT

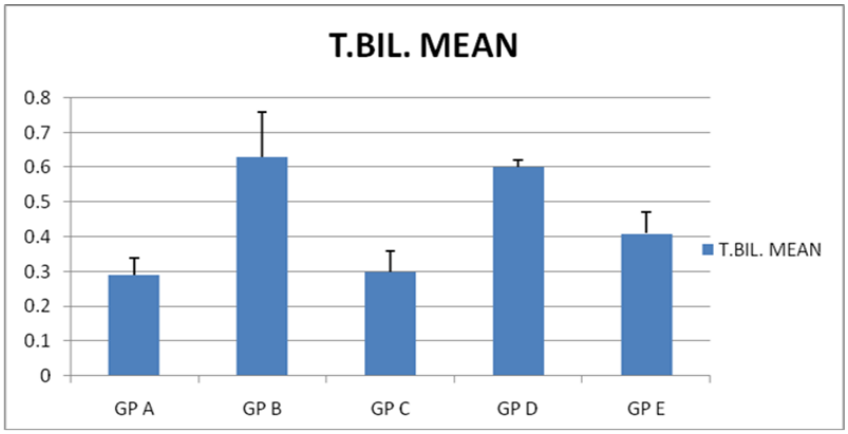

Figure 3. Showing the effect of *P. oleracea* on Total Bilirubin

to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity.

Both AST and ALT levels increase due to toxic compounds affecting the integrity of the liver cells (Subramoniam and Pushpagada, 1999). Decreased levels of transaminases indicate stabilization of plasma membrane and protection of hepatocytes against damage caused by hepatotoxin. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. The extracts decreased $KBrO_3$ induced elevated enzyme levels, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

Phytoconstituents like the flavonoids (Baek *et al*., 1996), triterpenoids (Xiong *et al.,* 2003), saponins (Tran *et al*., 2001) and alkaloids (Vijayan *et al*., 2003) are known to possess hepatoprotective activity. The presence of flavonoids in the extract may be responsible for its antioxidant and thus hepatoprotective activity.

In summary, the extracts of *Portulaca oleracea* seem to be useful in controlling hepatic injury in drug induced hepatotoxicity.

CONCLUSION

This study suggests that oral administration of *P. oleracea* significantly ameliorates potassium bromate induced hepatotoxicity in rats seem to be useful in controlling hepatic injury in drug induced hepatotoxicity.

The extracts may be protecting the liver by free radical scavenging activity and thus preventing peroxidation of lipids of the endoplasmic reticulum. And this may be due to the presence of flavonoids in the extract.

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