The ameliorative effect of grape seed extract (GSE) on sodium borate-induced kidney injury of male albino rats

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ABSTRACT

Borax (sod-borate) is a toxic compound that is implicated daily to environmental pollutant, so occupational exposure leading to adverse effects on functions of some organs causing their damage as nephrotoxicity, neurotoxicity, hepatotoxicity and testicular atrophy. In particularly, kidney is the most organ that is affected by borax exposure due to continuous exposure with slow rate of excretion leading to accumulation in the renal tissue. Supplementation with high potent antioxidant grape seed extract may alleviate the worse damage effects induced in the kidney as a result of continual exposure of borax in our daily life. The current study aimed to evaluate the ameliorative effect of grape seed extract on renal injury of male albino rats intoxicated with sod-borate. Twenty eight male albino rats were classified to 4 groups (GI & II & III & IV). GI served as a control, group GII was a group intoxicated with sod-borate for 45 days, whereas rats in GIII supplemented with GSE beside sod-borate for 45 days, GIV was a group supplemented with GSE only.

Serum and kidney samples were collected for biochemical, histopathological and DNA examinations. Significant elevation in the levels of blood urea and creatinine in GII were observed when compared to control group (GI). Significant decline were prominent in biochemical kidney functions when intoxicated group supplemented with GSE (GIII), where as non significant changes were observed between control group and group supplemented with GSE only (GIV). Significant increase in both cytokines TNF-α and IL-6 was observed in group intoxicated with sod-borate (GII) when compared to control rats (GI). Oral supplementation with high potent antioxidant GSE (GIII) caused alleviation in the kidney injury leading to the reduction of both pro-inflammatory mediator cytokines TNF-α and IL-6. DNA% fragment migration showed that worse significant migration of DNA fragments were observed in toxicated group (GII) followed by increase in tail length that was evaluated by a fluorescence microscope, but supplementation with GSE (GIII) caused significant improvement with reduction in the percentage of DNA fragments migration contributing to renal tissue recovery. Corticosteroid levels were elevated in intoxicated group (GII) when compared to control group (GI), whereas significant reduction in the level of corticosteroid were observed when intoxicated rats were supplemented with GSE (GIII). Histopathological examination showed that orally administration with sod-borate induced worse alteration with sever damage in renal tissue when compared to control. Supplementation with GSE to intoxicated group resulted in improvement in the damaged renal tissue with mild congestion when compared to intoxicated group. No histological structure alteration were observed between control group and supplemented group with GSE only. The present work concluded that GSE may act as a natural therapeutic agent to alleviate and neutralize the nephrotoxicity and oxidative stress induced by Sod-borate.

Keywords: Borax/ Sod-borate/ Nephrotoxicity/ TNF/IL-6/ GSE as antioxidant
INTRODUCTION

Borax is a chemical compound that is widely used in our daily life as a component of many detergent, cosmetic, enamel glazes and tooth bleaching, buffer solution, glass, pottery and ceramic. It is also used as fungicide, insecticide and bactericide. It can be used as food additives, household laundry, cleaning products and stain removal. Borax known as sod. borate, sod. tetra borate or disod. tetraborate. The route of exposure was via inhalation, ingestion and dermal contact. Sufficient exposure can lead to accumulation in various organs and adversely affect their functions. The most prominent effect include nephrotoxicity, neurotoxicity, hepatotoxicity and testicular atrophy with degeneration of seminiferous tubules and reduction in sperm count. The degree of borax toxicity depends on its dose and duration of exposure. Borax becomes toxic when accumulated in the body causing renal failure and adverse effect on immune cell proliferation and sister chromatid exchange in chromosomes causing cellular toxicity and genetic defect. High accidental ingestion or exposure to sod. borate may cause injury to the kidney at both tubular and endothelium locations leading to tubular necrosis and decline in renal function. Sod. borate is metabolized by enzymes of the cytochrome P450 converted to toxic intermediate metabolites causing cellular death. The main route of borax excretion from the body is via glomerular filtration so, it can be accumulated in proximal renal tubular as a result of its slow rate of excretion causing renal toxicity. The nephrotoxicity of most chemicals is more profound in patient with already suffer from renal impairment. Sod. borate decreases renal blood flow and glomerular filtration rate with increased lipid peroxidation, decreased endogenous antioxidant system, increased expression of inflammatory mediators with increased apoptosis. Grape seed extract (GSE) is a naturally dietary potent antioxidant due to its high content of several active components including Vit. E, flavonoids, polyphenol and proanthocyanins. GSE has been reported as antioxidant, anti-inflammatory and antimicrobial activities. GSE contain high concentration of Vit. E and Flavonoids which are involved in ameliorating and protect body against oxidative stress so, GSE may be a promising natural therapeutic agent in nephrotoxicity and oxidative stress and may neutralize the action of inflammatory cytokines. GS pro-anthocyanidin is highly bioavailable and provides significantly greater protection against free radicals and free radical-induced lipid peroxidation, DNA damaged, inhibition of antioxidant system and decreasing in glutathione content. In conclusion occupational exposure to chemically and structurally diverse environmental pollutant produce enormous amounts of free radicals resulting in oxidative deterioration of lipids, proteins and DNA, inhibition of antioxidant defense system and alteration in gene expression but supplementation with GSE may alleviate the toxic adverse effects by scavengers and neutralization of free radicals-inducing damage of organs.

MATERIAL AND METHODS

Twenty eight Wister male albino rats with weight ranged 120-150 mg were purchased from Helwan breeding farm and kept at the animal house of radioisotopes Dept. AEA under convential laboratory condition. They were divided into 4 groups (7 rats/one group).

GI: Served as a control group neither intoxicated nor treated.

GII: Orally intoxicated with sod. borate were purchased from EL-Gomhoria Company, Egypt (35mg/kg/day).

GIII: Orally intoxicated sod. borate (35mg/kg/day) and supplemented with GSE (5mg/kg/day).

GIV: Orally supplemented with GSE only were purchased from Kahira Pharm and Chem. Ind. Co. (5mg/kg/day).

All groups were orally administrated for 45 days then sacrificed and blood samples were collected. Serum samples were separated and kept at -20°C for biochemical and immunological investigations. Kidneys were exaggerated for DNA and histopathological examination. Serum creatinine was estimated by kinetic colorimetric method.
Serum Urea was estimated by Enzymatic determination (urease-modified berthelot reaction). Serum corticosterone was determined by coat-A-count rat for the quantitative measurement. Rat TNF-α was estimated by ELISA Kit. Serum IL-6 was determined by ELISA technique. Investigation of DNA fragmentation was performed on kidneys by Comet technique. Histopathological examination of kidneys was done.

RESULT

There were significant elevation in the levels of blood urea and creatinine when male albino rats were intoxicated with sod-borate(35mg/kg/day) for 45 days comparing to control group. There were significant decline prominent in biochemical kidney functions when intoxicated albino rat were supplemented with GSE(5mg/kg/day) for 45 days whereas non significant change were observed between control group and group supplemented with GSE only for 45 days. There were significant increase in cytokine TNF-α as a result of renal injury induced by sod-borate treated for 45 days. There were significant elevation in the levels of IL-6 contributing to elevation of TNF-α as a result of renal inflammatory tissues. Orally supplementation with high potent antioxidant GSE were alleviate the kidney injury leading to reduction of both inflammatory mediator cytokine TNF-α and IL-6.

The present work showed that worse significant migration of DNA% fragments were observed in the intoxicated group with sod-borate for 45 days followed by increase in tail length that was evaluated by a fluorescence microscope, but treatment with GSE caused significant improvement with reduction in the percentage of DNA fragments migration as a result of renal tissue recovery. Corticosteroid levels were elevated in case of group intoxicated with sod-borate compared to control group whereas significant reduction in the level of corticosteroid were observed when intoxicated rats supplemented with GSE. No significant change were noticed between group intoxicated with supplemented of GSE and group supplemented with GSE only.

Histopathological examination showed that orally administrated with sod-borate induced degeneration in the lining epithelium of the tubules with congestion in the glomerular tuft. Focal hemorrhage in between the tubules and corticomedullary portion were observed when compared with control. Treatment with GSE to intoxicated group causing improvement in the renal tissue injury with mild congestion of the glomeruli were observed. No histological structure alteration of the glomeruli and tubules were observed between control group and group supplemented group with GSE only.
Table (I): Effect of GSE on the different parameters of intoxicated and/or supplemented male albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>23.9±2.2</td>
<td>59.9±2.9</td>
<td>47.2±1.7</td>
<td>23.9±1.7</td>
</tr>
<tr>
<td>Creatinine (mg%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>0.49±0.03</td>
<td>0.65±0.03</td>
<td>0.55±0.013</td>
<td>0.47±0.013</td>
</tr>
<tr>
<td>Corticosteroid (mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>266±7.8</td>
<td>474.3±16.1</td>
<td>358±15.6</td>
<td>344.4±7.8</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>79.8±2.6</td>
<td>136.6±2.3</td>
<td>116.8±1.7</td>
<td>87.01±1.9</td>
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<tr>
<td>IL6 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>162.4±1.89</td>
<td>195.4±1.49</td>
<td>183.05±2.09</td>
<td>166.22±2.41</td>
</tr>
</tbody>
</table>

All values are expressed as mean±S.E. Values with different letters at the same row were significantly different at p≤0.05.

Table (II): Percentage of DNA migration of intoxicated and/or supplemented male albino rats by Comet technique.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>GIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail length (Um)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>1.54±0.02</td>
<td>6.6±0.08</td>
<td>3.3±0.08</td>
<td>1.6±0.02</td>
</tr>
<tr>
<td>DNA %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±St.E</td>
<td>1.55±0.02</td>
<td>4.7±0.06</td>
<td>3.5±0.07</td>
<td>1.6±0.02</td>
</tr>
</tbody>
</table>

All values are expressed as mean±S.E. Values with different letters at the same row were significantly different at p≤0.05.

Fig. (1): DNA% migration of different studies groups
Fig(2): Normal histopathological section of the kidney tissue showing glomeruli(g) and tubules(t) in the control group.

(A)                                                                  (B)

Fig(3): Histopathological sections of the kidney tissue showing focal hemorrhage at the cortex (A) and at the corticomedullary portion (B), in addition to degeneration in the lining epithelium of the tubules at the cortex (C) for the intoxicated rats with borax.

(C)
Fig(4): Histopathological section of the kidney tissue showing mild congestion in the left glomeruli for intoxicated rats supplemented with GSE.

Fig (5): Histopathological kidney tissue showing normal glomeruli (g) and tubules(t) for GSE supplemented group.

**DISCUSSION**

Borax is a chemical substance which is toxic to human and animals, so, continual exposure as a result of permanent use in our daily life lead to accumulation in organs causing adverse in their functions\textsuperscript{11,34}. Na-borate is metabolized by the enzymes of the cytochrome p\textsubscript{450} to toxic macromolecules that directly cause cellular death but when conjugated with antioxidant agent, it is converted to non toxic metabolites. Nephrotoxicity is the most common adverse effect of borax due to low glomerular filtration with slow rate of excretion. Accumulated borax in the body cause cellular toxicity and genetic defect\textsuperscript{11}. High accidental ingestion or exposure to sod. borate may cause injury to the kidney at both tubular and endothelium locations leading to tubular necrosis and decline in renal function\textsuperscript{12,13}. Sod. borate either by inhalation or ingestion increased lipid peroxidation with increased free radical-induced tissues destruction, decreased endogenous antioxidant system, increased expression of inflammatory mediators with increased in gene expression of apoptosis\textsuperscript{17}. Exposure to borate cause nephrotoxicity accompanied by decrease in albumin ,increase in urea and creatinine levels with increasing of kidney free radicals and decrease in the level of glutathione content and the activities of antioxidant enzymes. Borax can also affect on immune cell proliferation so, consumer should be careful about deal in with this substance\textsuperscript{11}. Exposure to toxins induced significantly different higher degrees of sever acute tubular necrosis and interstitial mononuclear cell infiltration \textsuperscript{35}. Treatment with grape seed extract(GSE) as a naturally dietary potent antioxidant due to its high content of several active components as Vit. E, flavonoids, polyphenol and pro-anthocyanin may alleviate the cytotoxic and injury-induced borate\textsuperscript{28,19}. Supplementation with grape seed extract may
significantly improve the kidney injury induced by borate exposure as evident on tissue histology. GSE is a natural dietary antioxidant that have an important role in their ability to protect cells from miscellaneous damage induced by sod-borate, so GSE may be promising as a natural therapeutic agent in nephrotoxicity and oxidative stress. Proanthocyanidin present in grape seed has a protective effect against toxins-induced nephrotoxicity. Treatment with GSE protect against-induced acute kidney injury as evident on tissue histology, that was accompanied by improvement in biochemical markers. The Antioxidant potential effect of GSE against oxidative stress caused by adverse effect of borate on the kidney were prominent. There were obvious significant elevation in serum levels of urea and creatinine that is attributed to background fluctuation underlying exposure to xenobiotic. There were a marked rise in the basal serum level of corticosteroid of male albino rats with disturbance in biochemical and physiological functions after repeated exposure to toxins with alteration of immune system. Renal injury as a result of exposure to toxins stimulate a cascade of events culminating in tubulointerstitial fibrosis, programmed cell death and a permanent decline in renal function so, TNF-α is an important mediator of renal fibrosis. Tumor necrosis factor-alpha(TNF-α) is a potent mediator of inflammatory function. The cytokine TNF functions as a central mediator in many inflammatory and immunological processes. IL-6 is a multifunctional cytokine that play an important roles in host defense, acute phase reactions and immune responses. Elevated serum IL-6 have been observed in a number of pathological conditions including inflammation. GSE contain mainly flavonoids which is involved in a meliorating oxidative stress by inhibition of enzyme systems that are responsible for production of free radical. Ramesh and Reeves studied the role of cytokines in kidney injury as a result of nephrotoxic agent, they concluded that there were significant elevation of serum TNF-α and IL6. TNF-α inhibitors ameliorate renal dysfunction and reduce kidney structural damage. DNA damage resulted due to nephrotoxicity, formation of ROS and either necrotic or apoptotic cell death. Oxidative stress is an activator of the necrosis factor transcription which in turn promotes the production of proinflammatory cytokines including TNF-α. TNF-α stimulates the production of ROS and decrease free radical scavengers. IL6 expression correlates with the onset and severity of kidney injury. IL6 reduces lipid peroxidation after injury suggesting that its protective effect may be largely mediated through a melioration of oxidative stress.

CONCLUSION

The present work concluded that exposure to borate causing deleterious damage in kidney with decreasing in antioxidant enzymes and increasing in lipid peroxidation but treatment with grape seed extract(GSE) showed a protective and ameliorative effects against nephrotoxicity and oxidative damage.

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