ORIGINAL ARTICLES

Hepatoprotective effect of aqueous leaves extract of Psidium guajava and Zizyphus spina – christi against paracetamol induced hepatotoxicity in rats.

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ABSTRACT

Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of some medicinal herbs have proven hepatoprotective potential. Therefore, the aim of this study was to evaluate the hepatoprotective effect of aqueous leaves extract of psidium guajava and zizyphus spina-christi on paracetamol induced hepatic damage. The hepatoprotective activities of the two extracts were compared with a known hepatoprotective drug, silymarin. For this purpose, thirty adult male albino rats Wistar strain were assigned into 5 groups. Group1: normal control group, group 2, group 3 and 4: experimentally induced hepatotoxicity by oral administration with paracetamol (400mg/kg body) followed by oral administration of silymarin, Psidium guajava and Zizyphus spina-christi aqueous leaf weight extracts, respectively, group 5: experimentally induced hepatotoxicity by oral administration with paracetamol. Oral administration of paracetamol induced liver damage in rats as manifested by a significant rise in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase(ALP), Lactate dehydrogenase (LDH), gamma glutamyl transferase (γ-GT) and total bilirubin. Oral administration of psidium guajava and Zizyphus spina-christi aqueous leaf extracts, significantly attenuated hepatotoxicity induced by paracetamol resulting in a significant decrease in serum levels of hepatic enzymes marker and total bilirubin. The results also showed that oral administration of paracetamol induced oxidative stress in liver reflected in a significant decrease in hepatic superoxide dismutase (SOD) and catalase (CAT) activities. Treatment with aqueous leaves extract of either psidium guajava or zizyphus spina-christi ameliorated the oxidative stress in paracetamol hepatotoxic rats and restored normal activities of hepatic antioxidant enzymes. It is concluded that aqueous leaf extracts of psidium guajava or zizyphus spina-christi possesses good hepatoprotective activity.

Key words: Psidium guajava; Zizyphus spina- Christi; Paracetamol; Hepatotoxicity; Silymarin; Antioxidative enzyme.

Introduction

Liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body. It has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders Rajkpoor et al. (2008). Hepatotoxicity is one of very common ailments resulting into serious debilities ranging from sever metabolic disorders to even mortality Patel et al. (2008). A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage Meyer and Kulkarini (2001). Paracetamol overdoses in both animals and man has been shown to produce hepatic necrosis Thomas. (1993) and it has been used as an animal model for hepatotoxicity Visen et al. (1993). Most of hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and oxidative stress in liver. Carbon tetrachloride (CCl4) and paracetamol get converted into reactive toxic metabolites by hepatic microsomal cytochrome P450 and cause hepatotoxicity Buwa et al. (2001).

Herbs play a major role in the management of various liver disorders. A number of plants possess hepatoprotective property Mansour et al. (2006). One of these plants used traditionally is Psidium guajava Linn. It is believed in Indian folklore that the water decoction of the leaves of this plant can cure jaundice within three days. Psidium guajava contains a number of chemical constituents which are reported to possess antioxidant activities Qian and Nihorimbere (2004). Also, Zizyphus spina-christi is known for its medicinal properties as liver protective agent, antioxidant, hypoglycemic, hypotensive, anti inflammatory, antimicrobial, antitumor and as an immune system stimulant Said et al. (2006). The aim of this study was to evaluate the hepatoprotective activities of aqueous leaf extracts of Psidium guajava and Zizyphus spina-christi against paracetamol induced hepatotoxicity in rats. The hepatoprotective activity of the two extracts were compared with a standard hepatoprotective drug, silymarin.

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Materials and Methods

Animals:

Thirty adult male Wistar strain albino rats body weight (150g ± 5 g) were purchased from Animal House of El-Salam-Farm, Giza, Egypt. After acclimatization, rats were housed individually with constant environment in controlled stainless steel cages, temperature (25°C ± 5°C) humidity (50% ± 10%), and light cycle were held constant 12/12 h. During the experimental period (4 weeks) and throughout the trial, food and water were provided ad libitum.

Animal's diet:

The animals were fed on the standard commercial pellets diet NRC, (1995) which obtained from Elandalous company, Giza. The composition of pellets diet: Protein 18%, fat 4.9% and fiber 3.2%.

Preparation of aqueous extract of psidium guajava leaves:

100g of dried ground leaves powder was steeped in 300 ml of hot distilled water then boiled in water bath for 1h. The boiled leaves extract were then filtered through whatman No 4 filter paper, then the filtrate was stored at 4°C, Roy et al. (2006)

Preparation of aqueous extract of ziziphus spina christi leaves:

100g of the dried ground leaves powder were steeped in 600 ml of hot distilled water and allowed to stand for 30 min. The extract was pooled and filtered through whatman filter paper, then the filtrate was stored at 4°C, Azdu et al. (2001)

Induction of hepatotoxicity:

Hepatotoxicity was induced by oral gavage with paracetamol (400mg/kg body weight) by intragastric tube Kanchana and sadiq (2011)

Experimental design:

The animals were randomly assigned to five experimental groups (each of 6). All international and local rules and regulation for handling animals in experiments were followed. The experimental groups illustrated as follows:
Group1: Healthy rats fed on pellet diet and served as normal controls.
Group2: Healthy rats fed on pellet diet and received paracetamol followed by silymarin (100mg/Kg of body weight/day) by intragastric tube for 4 weeks Roy et al. (2006).
Group3: Healthy rats fed on pellet diet and received paracetamol followed by aqueous extract of guava leaves (0.5 ml /day) by intragastric tube for 4 weeks.
Group 4: Healthy rats fed on pellet diet and received paracetamol followed by aqueous extract of ziziphus leaves (0.5 ml /day) by intragastric tube for 4 weeks.
Group5: Healthy rats fed on pellet diet and received paracetamol by intragastric tube for 4 weeks.

At the end of the experiment, the animals were anesthetized with diethyl ether after 12 hours fasting and whole blood samples were taken from hepatic Portal vein. The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 20 minutes, then sera were separated and kept in plastic vials at −20°C until analyses.

Biochemical assays:

Serum aspartate aminotransferase enzyme activity was determined according to the method described by Bergmeryer et al. (1978), serum alanine aminotransferase enzyme activity was determined according to the method described by Berrneryer et al. (1978), serum alkaline phosphatase activity was determined according to the method described by Bowers and McComb (1975), gamma-glutamyl transferase activity was determined in serum according to the method described by Schumann et al. (2002), Lactate dehydrogenase activity was determined in serum according to the method described by Seikman et al. (2002) and serum total bilirubin was determined according to the method as described by Domas et al. (1973). All enzymes activities and total
bilirubin were determined by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) using Dimension clinical chemistry system.

**Preparation of liver homogenate:**

The liver of the rats from individual group was dissected out, washed with ice-cold saline and weighed and homogenized in 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare 10% w/v homogenate (pH 7.4). The homogenate was centrifuged at 3000 rpm g for 30 min. The supernatants were used for measuring the activity of enzymes- superoxide dismutase (SOD) and catalase (CAT)

**Superoxide dismutase (SOD) activity:**

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome according to the method described by Saggu *et al.* (1989). To 0.05 ml supernatant, 2.0 ml of carbonate buffer and 0.5 ml of EDTA were added. The reaction was initiated by addition of 0.5 ml of epinephrine and the autooxidation of adrenaline to adrenochrome at pH 10.2 was measured by following the change in O.D at 480 nm. The change in optical density every minute was measured at 480 nm against reagent blank. The results are expressed as units of SOD activity (U/g wet tissue). One unit of SOD activity induced approximately 50% inhibition of adrenaline.

**Catalse (CAT) activity:**

The activity assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide according to the method described by Beers and Sizer (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by the reaction mixture consisted of 2 ml phosphate buffer (pH 7.0), 0.95 ml of hydrogen peroxide (0.019 M) and 0.05 ml supernatant in final volume of 3 ml. Absorbance was recorded at 240 nm every 10 sec for 1 min. One unit of CAT was defined, as the amount of enzyme required decomposing 1µmol of peroxide per min, at 25°C and pH 7.0. The results were expressed as units of CAT activity (U/g wet tissue).

**Statistical Analysis:**

The data were presented as means ±SD. One way analysis of variance (ANOVA) followed by post-hoc least .Significant difference analysis (LSD) at (p< 0.05) was performed using the statistical package for social science (SPSS) version 9 to compare all treated groups. Differences were considered to be significant when (p< 0.05).

**Results:**

Results reported in Table (1) and (Fig.1 and 2) showed that, levels of serum ALT, AST, ALP, γGT, LDH and total bilirubin were markedly increased in paracetamol -hepatotoxic rats when compared with normal control rats. Oral administration of psidium guajava and Zizyphus spina-christi leaf extracts as well as standard control drug silymarin significantly reversed these altered levels (P<0.05).

The results also demonstrated that, serum transaminase enzyme activities were significantly decreased in the rats treated with psidium guajava aqueous leaves extract as compared with rats treated with Zizyphus spina-christi aqueous leaves extract.

The results also indicated that there was no significant difference in the levels of serum ALP, LDH, γGT and total bilirubin between rat groups treated with psidium guajava and Zizyphus spina-christi aqueous leaves extract or silymarin.

Result reported in Table (2) and illustrated in Fig. 3 revealed that, the activities of hepatic antioxidative enzymes superoxide dismutase (SOD) and catalase (CAT) were significantly decreased in paracetamol hepatotoxic rats. Treatment with psidium guajava and Zizyphus spina-christi aqueous leaves extract or silymarin caused a significant increase in antioxidative enzymes activities. Also, non significant difference was observed between rat groups treated with psidium guajava and Zizyphus spina-christi aqueous leaves extract or silymarin.
Table 1: Serum levels of ALT, AST, ALP, GGT, LDH activities (U/l) and total bilirubin (mg/dl) in different experimental groups (Data are presented as mean±SD).

<table>
<thead>
<tr>
<th>Rat groups Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>61.6±1.76</td>
<td>67.3±8.8</td>
<td>71.9±5.26</td>
<td>Abc 127±3.7</td>
<td>Abcd 35.9±31</td>
</tr>
<tr>
<td>AST</td>
<td>136.6±27</td>
<td>148.4±25.3</td>
<td>155.9±19.3</td>
<td>a bc 260.2±2</td>
<td>Abcd 342±91</td>
</tr>
<tr>
<td>ALP</td>
<td>241.4±14.7</td>
<td>255.2±33.7</td>
<td>263.1±24</td>
<td>a b 272.2±26.27</td>
<td>Abcd 287.8±0.18</td>
</tr>
<tr>
<td>GGT</td>
<td>6.4±0.84</td>
<td>6.8±0.44</td>
<td>7.2±0.44</td>
<td>7.4±1.1</td>
<td>Abcd 11.2±5.71</td>
</tr>
<tr>
<td>LDH</td>
<td>117±15.4</td>
<td>121.6±21.17</td>
<td>142.5±31.3</td>
<td>144.1±11.64</td>
<td>Abcd 206.6±26.4</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.14±5.7</td>
<td>0.18±8.36</td>
<td>0.16±5.4</td>
<td>0.18±8.36</td>
<td>a 0.244±8.1</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05): compared to: (a): group 1, (b): to group 2, (c): to group 3, (d) compared to group 4

Fig. 1: Serum levels of ALT, AST, ALP and LDH (U/L) in different experimental groups.

Fig. 2: Serum levels of γ GT (IU/µL) and total bilirubin (mg/dl) in different experimental groups.

Table 2: Hepatic Catalase and SOD activities (U/g of liver homogenate) in different experimental groups.

<table>
<thead>
<tr>
<th>Rat groups Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase activity</td>
<td>17.94±4.27</td>
<td>14.6±4.5</td>
<td>17.09±8.3</td>
<td>17.4±2.05</td>
<td>abed 4.84±2.26</td>
</tr>
<tr>
<td>SOD activity</td>
<td>26.90±5.61</td>
<td>20.71±5.3</td>
<td>23.66±5.15</td>
<td>22.99±6.6</td>
<td>abc 13.11±3.5</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05): compared to: (a): group 1, (b): to group 2, (c): to group 3, (d) compared to group 4
Discussion:

Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Paracetamol toxicity like many other disease conditions is widely believed to involve the generation of Reactive Oxygen Species (ROS).

Antioxidants have been reported to play prominent roles in prevention of ROS generation Valko et al. (2006) and by extension may offer protection against paracetamol hepatotoxicity. These antioxidants are most readily available in edible vegetables and other herbal plants. Hence, the evaluation of medicinal plants or herbs with free radical scavenging potentials for protective roles against drug induced toxicity becomes relevant.

Large doses of carbon tetrachloride (CCl₄) and paracetamol induces hepatic necrosis in humans and experimental animals Bhattacharrya et al. (2003). Observation of the preventive effect of some herbs to the liver damage caused by paracetamol may give an indication of the hepatoprotective effect of these herbs in general.

From the results of the current study it is clear that oral paracetamol administration induced hepatotoxicity in rats. Paracetamol hepatotoxicity was manifested by significant increase in serum liver enzymes ALT, AST, ALP, γ GT, LDH activities and serum total bilirubin. Elevated levels of serum enzymes are inductive of cellular leakage and loss of functional integrity of cell membrane in liver. Treatment with psidium guajava and Zizyphus spina-christi aqueous leaf extracts or silymarin significantly reduced these elevated levels of the enzymes towards the respective normal values indicating stabilization of plasma membrane as well as repair of hepatic tissue damage induced by paracetamol.

Bilirubin is one of the most clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte Manokaran et al. (2008). In the present study paracetamol hepatotoxic rats showed a significant increase in the level of serum total bilirubin when compared with control rats. Oral administration of aqueous leaves extract with psidium guajava and Zizyphus spina-christi or silymarin significantly restored serum total bilirubin level near normal value. This decrease in serum bilirubin after treatment with these extracts in liver damage induced by paracetamol, indicated the effectiveness of these extracts in normal functional status of the liver.

The results of the previous studies of (Gutierrez and Solis 2009; Mayuren, 2010 and Kanchana and sadiq, 2011) supported the results of the current study which confirmed that, paracetamol administration induced hepatotoxicity and significantly altered hepatic enzyme markers and total bilirubin levels in serum.

The enzymatic antioxidant defense system is the nature protector against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage Scott et al. (1991). In the present study, it was observed that, Psidium guajava and Zizyphus spina-christi aqueous leaf extracts or silymarin significantly prevented the diminution in the activities of hepatic SOD and CAT in paracetamol induced liver damage rats. These findings confirmed that Psidium guajava and Zizyphus spina-christi aqueous leaf extracts or silymarin can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzymes.

These results are in accordance with the results of (Das et al., 2007, Farghaly and Huessien, 2010 and Marzouk et al., 2011) which demonstrated that, the activities of antioxidative enzymes SOD and CAT significantly decreased in paracetamol hepatotoxic rats.

The hepatoprotective effect of Psidium guajava aqueous leaves extract against paracetamol hepatotoxicity was reported by (Roy et al., 2006 and Roy and Das, 2010) which supported the results of this study. Also, the hepatoprotective effect of silymarin against paracetamol hepatotoxicity was confirmed by previous finding of (Kang et al., 2004 and pradeep, 2007). Moreover, results reported by (Amin and Ghoneim, 2009) are similar to the results of the current study which reported that oral administration of Zizyphus spina-christi aqueous
leaves extract ameliorated liver injury judged by reduced ALT and AST activities in serum. In addition, it retained control activities of endogenous antioxidant such as SOD and CAT in liver.

Conclusion:

In conclusion, the results of this study demonstrate that, both Psidium guajava and Zizyphus aqueous leaf extracts exhibit a potent hepatoprotective action against paracetamol-induced hepatic damage in rats. It is possible that, the mechanism of hepatoprotective action of Psidium guajava and zizyphus aqueous leaf extracts may be due to their antioxidant and free radical scavenging properties.

Reference

Amin, A and M.D. Ghoneim, 2009. Zizyphus spina-christi protects against carbon tetrachloride-induced liver fibrosis in rats. Food and Chemical Toxicology, 47: 2111-2119