HEPATOPROTECTIVE INVESTIGATIONS OF CUMINUM CYMINUM DRIED SEEDS IN NIMESULIDE INTOXICATED ALBINO RATS BY PHYTOCHEMICAL AND BIOCHEMICAL METHODS

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Received: 04 Mar 2014 Revised and Accepted: 18 Mar 2014

ABSTRACT

Objective: Nimesulide, a 4-nitro-2-phenoxy methane sulphonamide is very effective non-steroidal anti-inflammatory Drug (NSAID), but at higher doses it leads to hepatotoxicity. This study was carried out on albino rats to evaluate the hepatoprotective activity of aqueous-ethanolic extract of Cuminum cyminum (Cc.E) seeds.

Methods: Aqueous ethanolic extract of fresh dried cumin seed was prepared and was subjected to phytochemical analysis. For Biochemical investigations, the animals were divided into seven groups and hepatotoxicity was induced by oral administration of 100 mg/Kg Nimesulide suspension. After 15 days of treatment, the animals were dissected out and their livers were preserved for histopathological examination.

Results: There was a significant increase in serum glutamic-pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and serum total bilirubin (TB) level in intoxicated controls, which were restored towards normal in Cuminum cyminum (100, 200 and 300 mg/Kg, P.O.) treated animals. The results were compared with Silymarin (25 mg/Kg, P.O.) treated animals.

Conclusion: The extract significantly (p< 0.001) reduced the serum enzyme in comparison to intoxicated control group. Furthermore, histopathological examination on the rat liver tissues supported the hepatoprotection. So, we recommend for further studies to isolate the pure component and the mechanism that displayed the hepatoprotective activity for making standard drug.

Keywords: Nimesulide, Cuminum cyminum, Hepatoprotective activity, SGOT, SGPT

INTRODUCTION

Liver is one of the most important and massive visceral organ present into substantial portion of abdomen. It is also called haper and made up of hepatocytes which carry out multiple metabolic processes essential for life. They are also involved in the removal of toxic materials from blood to avoid life threatening toxicities. Many drugs and chemicals cause different types of hepatotoxicities, i.e. Type-A (intrinsic) or Type-B (idiosyncratic) reactions with prevalence of 80 and 20%, respectively. A few drugs, like troglitazone, gregafflexin, levamisole, rofecoxib and thioridazine have both anti-oxidant and free radical scavenging activities due to the presence of plenty of essential oils [6]. Cumin seed contains many chemical compounds, some of which are potent anti-inflammatory and anti-oxidant agents. It possesses both antibacterial and antifungal activities and used as an additives in the storage of foodstuff. Because of the aromatic properties Cuminum cyminum is famous for fragrances, aroma and therapeutic substances. The plant is medicinally important as anti-spasmodic agent, carminative and appetizer [4].

Cumin crop is quite limited because of numerous biological stresses and with diseases. USA is the chief importer of cumin and has developed mass strategies and regulations to stop Kaphra bug disease, which has previously created major business crisis for cumin crop [5]. C. cyminum have both anti-oxidant and free radical scavenging activities due to the presence of plenty of essential oils. Cuminoids A and B (sesquiterpenoid glucosides), two allyl glycosides as well as five additional well-known constituents are found in Cumin [7]. Different traditional spices and herbs are considered to own healing properties, like anti-thrombotic, antithrombotic, hypolipidemic and anti-inflammatory actions.

The aqueous ethanolic extract of Cuminum cyminum was used for the very first time in such studies for the hepatoprotective activity in albino rats intoxicated with Nimesulide and it was observed that the different doses of the extract showed a marked reduction in the elevated serum enzyme level and also decreased the ballooning-degeneration, fibrosis, inflammation and apoptosis of the hepatocytes.

MATERIALS AND METHODS

The approval of this study (Ref. No. 1560/Pharm) was taken from the Board of the Advanced Study and Research (BASAR), the Islamia University, Bahawalpur and the Institutional Ethical Committee, Faculty of Pharmacy and Alternative Medicine, the Islamia University, Bahawalpur.
Pharmacological materials

Different secondary metabolites are present in plant materials but this is still in practice in some developing countries [14].

Equipment

Digital electronic balance (AY 62 Shimadzu Corporation, Japan), Centrifuge machine (EBA 20 Heltich D-7853), Vortex Mixer (SLV 6 Serulim Bioscience, Korea), Grinder (National, Japan), Merck Microlab 300 (Merck Germany), Rotary evaporator (Heidolph Laborota 4000, efficient, Germany) and Microscope (Micron).

Experimental animals

Albino Sprague-Dawley rats of both sexes weighing 180-200 g were selected for study. Albino rats and mice are available in animal house of Faculty of Pharmacy and Alternative Medicine. All animals were kept in polycarbonated cages of size 47x34x18 cm. They were provided temperature controlled hygienic, neat and clean environments in animal house.

Preparation of crude extract of *Cuminum cyminum*

Dried Cumin seeds were purchased from local market of Bahawalpur. Material was then identified by the botanist and specimen was preserved in the herbarium vide Voucher No.CC-SD-04-12-046, at the Faculty of Pharmacy and Alternative medicine, the Islamia University of Bahawalpur, Pakistan. Completely dried material was then ground to coarse powder by using electric grinder (National, Japan). 1000 g of ground powder was macerated in 2 L of 70% aqueous ethanol for five days. Soaked material was thoroughly stirred thrice daily. At the end of 5th day of maceration, it was filtered through muslin cloth and then through Whatmann filters paper No. 1.

Residue was again macerated to obtain more filtrate. This was repeated thrice and filtrate obtained after three soakings was evaporated by using rotary evaporator at 30-40°C. In the end, thick, viscous, semisolid paste of golden brown color was obtained. The paste obtained was weighed out to find percentage yield. The extract was packed in air tight container and labeled as Cc.E. It was evaporated by using rotary evaporator at 30-40°C. In the end, thick, viscous, semisolid paste of golden brown color was obtained. The Paste obtained was weighed out to find percentage yield. The extract was packed in air tight container and labeled as Cc.E. It was then put in the refrigerator for future use [8].

Phytochemical Analysis

Different secondary metabolites are present in plant materials which exhibit various pharmacological activities [9]. Crude extracts were subjected to phytochemical analysis for identification of saponins, tannins, alkaloids, glycosides terpenes and sterols by using standard phytochemical procedures [10-13] and results were represented in table 1.

Induction of hepatotoxicity

Hepatic toxicity was induced by Nimesulide, administered orally on daily basis in suspension form. Nimesulide is selective COX-2 inhibitor, which inhibits leukocyte function, PAF synthesis, TNFa release and metalloproteinase activity in cartilage. Although this is very effective NSAID yet it is associated with severe adverse effects like hepato-biliary, cutaneous and gastrointestinal system. Acute hepatitis, fulminant hepatic failure, cholestatic liver injury, multiple enterolecral perforations and end stage renal failure with Nimesulide intake have been reported in various case reports of hepatotoxicity. Even fatal hepatic failure leading to withdrawal of drug in various countries but this is still in practice in some developing countries [14].

Hepatoprotectivity

For evaluation of hepatoprotective activity the animals were divided into seven groups having seven animals each. Group-I received normal saline at dose of 5ml/kg b.w. p.o. once daily. Group-II was given DMSO (DimethylSulfoxide) at dose of 5ml/kg b.w. p.o. Group-III received Nimesulide 100 mg/Kg b.w. for seven days to produce hepatotoxicity. Group IV was Standard Control given Silymarin alone for first eight days at dose of 25 mg/Kg b.w. and then along with Nimesulide (100 mg/Kg p.o.) for further seven days. Group V-VII gave crude extract alone at dose of 100, 200 and 300 mg/Kg b.w. respectively for first eight days and then Nimesulide in dose of 100 mg/Kg p.o. along with plant extract to study hepatoprototoxicity for further seven days.

24 hours after the last treatment dose, animals were given anesthesia by administration of diazepam (5 mg/Kg i.p.) and ketamine (50 mg /kg i.p.). Animals were dissected and 3ml of blood was taken by cardiac puncture from each rat. Serum was collected by centrifugation of each sample of blood and then enzyme levels were monitored by using diagnostic kits.

Histopathology

Diazepam was injected in dose of 5 mg/kg i.p. to induce hypnosis before induction of anesthesia. Then Ketamine (50 mg /Kg i.p.) was injected to induce anesthesia. After that rats were dissected and blood was withdrawn by cardiac puncture and finally livers were preserved in 10 % formalin. Liver sections were dehydrated in ethanol, cleared in xylene and then fixed in paraffin. 4-5 μm sections were cut to prepare slides and hematoxylin and eosin dye was used for staining slides [15].

Statistical Analysis of Results

Results were expressed as Mean ± SEM (n=7). Student t test was applied. P values were considered as P > 0.05 non-significant (ns), and P < 0.05 as significant.

RESULTS

Phytochemical study of Cc.E

Different secondary metabolites present in crude extract were found by phytochemical analysis of Cc.E. The results obtained after analysis are mentioned in table: 1

Effects of *Cuminum cyminum* extract (Cc.E) on Biochemical parameters

Experimental studies revealed that Serum ALP, SGOT, SGPT and TB level in normal control group was 220±7±5.6 IU/L, 112.24±5.27 IU/L, 51.60±4.35 IU/L and 0.85±0.07 IU/L, respectively, which was very close to values of these parameters in vehicle control group i.e., 219.17±15.82 IU/L, 108.81±4.22 IU/L, 51.04±4.35 IU/L and 0.86±0.08 IU/L, respectively. The level of all these four parameters was significantly (P < 0.001) elevated in intoxicated control group with values of 809.0±24.71 IU/L, 223.29±7.57 IU/L, 115.57±5.67 IU/L and 3.60±0.16 IU/L, respectively. Standard control group which was given Silymarin, reduced the values up to level of 260.16±17.81 IU/L, 116.69±5.76 IU/L, 58.03±3.34 IU/L and 0.95±0.15 IU/L for all the four parameters (Table 2).

Aqueous ethanolic extract of *Cuminum cyminum* seeds significantly (P < 0.001) reduced the parameters in all three doses. The level of ALP, SGOT, SGPT and TB in normal control group was 531.54±14.41 IU/L, 171.59±5.97 IU/L, 84.63±3.15 IU/L and 2.46±0.17 IU/L which was significantly (P < 0.001) elevated in intoxicated control group with values of 809.0±24.71 IU/L, 223.29±7.57 IU/L, 115.57±5.67 IU/L and 3.60±0.16 IU/L, respectively. Standard control group which was given Silymarin, reduced the values up to level of 260.16±17.81 IU/L, 116.69±5.76 IU/L, 58.03±3.34 IU/L and 0.95±0.15 IU/L for all the four parameters (Table 2).

Aqueous ethanolic extract of *Cuminumcyminum* seeds significantly (P < 0.001) reduced the parameters in all three doses. The level of ALP, SGOT, SGPT and TB in normal control group was 331.89±6.88 IU/L, 139.43±7.34 IU/L, 73.43±3.79 IU/L and 1.40±0.12 IU/L for all four parameters while, Cc.E 300 mg/kg showed the values as 348.19±15.20 IU/L, 149.40±5.91 IU/L, 78.67±3.71 IU/L and 1.73±0.19 IU/L. Cc.E 200 mg/kg markedly reduced the values of all the four serum enzymes while Cc.E 100 mg/Kg produced least reduction in serum enzymes level. Values of Cc.E 300 mg/Kg were close to the values of Cc.E 200 mg/kg group.
Table 1: Phytochemical constituents of *Cuminum cyminum* (Cc.E)

<table>
<thead>
<tr>
<th>Sr. #</th>
<th>Phytochemical Tests</th>
<th>Phytochemical Constituents</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Foam Test +</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Iodine Test</td>
<td>+ve</td>
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<tr>
<td>3</td>
<td>Ferric Chloride Test</td>
<td>+ve</td>
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<tr>
<td>4</td>
<td>Nitric Acid Test</td>
<td>+ve</td>
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<tr>
<td>5</td>
<td>Gelatin Test</td>
<td>+ve</td>
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</table>

**Tannins**

<table>
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<tr>
<td>1</td>
<td>Hager’s Test</td>
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</tr>
<tr>
<td>2</td>
<td>Wagner’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Mayer’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Dragendorff Test</td>
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**Alkaloids**

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<tr>
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<td>Keller-Killani test</td>
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**Cardiac glycosides**

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<tr>
<td>1</td>
<td>Libermann-Burchard test</td>
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**Terpenes and sterols**

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<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Libermann - Burchard Test</td>
<td>+ve</td>
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</table>

**Note:** (+) and (-) signs report the relative presence and absence of constituents.

Table 2: Effect of different doses of *Cuminum cyminum* extract (Cc.E) on ALP, SGOT, SGPT & TB level in Nimesulide intoxicated albino rats.

<table>
<thead>
<tr>
<th>Sr. #</th>
<th>Treatment Groups</th>
<th>Level of ALP (IU/L)</th>
<th>Level of SGOT (IU/L)</th>
<th>Level of SGPT (IU/L)</th>
<th>Level of TB (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>220.77±15.56</td>
<td>112.24±5.27</td>
<td>51.60±4.35</td>
<td>0.85±0.07</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle Control</td>
<td>219.17±15.82</td>
<td>108.81±4.22</td>
<td>51.04±4.35</td>
<td>0.86±0.08</td>
</tr>
<tr>
<td>3</td>
<td>Intoxicated Control</td>
<td>889.01±24.71***</td>
<td>223.29±7.57***</td>
<td>115.57±5.67***</td>
<td>3.60±0.16***</td>
</tr>
<tr>
<td>4</td>
<td>Standard Control</td>
<td>260.16±17.81</td>
<td>116.69±5.76</td>
<td>58.03±3.34</td>
<td>0.95±0.15</td>
</tr>
<tr>
<td>5</td>
<td>Cc.E 100 mg/kg</td>
<td>531.54±14.41***</td>
<td>171.59±5.97***</td>
<td>84.63±3.15***</td>
<td>2.46±0.17***</td>
</tr>
<tr>
<td>6</td>
<td>Cc.E 200 mg/kg</td>
<td>331.89±16.88***</td>
<td>139.43±7.34***</td>
<td>73.43±3.79***</td>
<td>1.40±0.12***</td>
</tr>
<tr>
<td>7</td>
<td>Cc.E 300 mg/kg</td>
<td>348.19±15.20***</td>
<td>149.40±5.91***</td>
<td>78.67±3.71***</td>
<td>1.73±0.19***</td>
</tr>
</tbody>
</table>

[Values are mean ± SE with 7 animals in each group]

P-values: \( >0.05, <0.05, \ll<0.01, \ll<0.001 \) vs. intoxicated control, and \( \ll<0.001 \) vs. vehicle control

**Histo pathological Observations**

Photomicrograph of liver tissue of normal and vehicle control group showed normal cellular pattern with clear nucleus. However, photomicrographs of intoxicated control group exhibited high scores of balloonning-degeneration, apoptosis, inflammation and fibrosis as shown in figures. Groups treated with Silymarin and cumin extract represented fewer score of hepatic damages as clear from photomicrographs of liver slides.

![Normal Control](image1)

![Vehicle Control](image2)

![Intoxicated Control](image3)

Normal liver slides exhibited regular liver cells containing clear cytoplasm, well-known nucleus and discernible central veins. Intoxicated liver slides represented enormous fatty changes, balloonning degeneration, necrosis, missing of cellular margins and lymphocytic broad infiltration [15]. Hepatotoxic substances (CCl4 and Paracetamol) produce histopathological changes (steatosis and fibrosis) in hepatocytes [16].
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then transportation by hepatocytes is disturbed and enzymes are leaked from cells due to change in permeability of membranes. Thus, Nimesulide has reported hepatotoxicity as enzyme level in hepatic cells is reduced and it is raised in serum and hepatotoxicity is assessed by observing the serum enzymes like ALP, SGOT and SGPT [20]. Cuminum seeds contain monoterpenes, sesquiterpenes, aromatic aldehydes and aromatic oxides etc. Terpenes, terpenols, terpenals, terpenones, terpene esters and aromatic compounds are in small fraction [21]. Hepatoprotection of Cc.E might be due to the presence of saponins, tannins, glycocides, terpenes and steroid present in plant. Tannins are well recognized due to their hepatoprotective action [22]. Saponins like saikosaponins inhibit lipid peroxidation by scavenging reactive and toxic species [23]. Phytochemical analysis indicated that cumin extract contains saponins, tannins, alkaloids, cardiac glycosides, terpenes and sterols which are responsible for hepatoprotective activity. Steroidal alkaldoids of different plant species have hepatoprotective role [24].

Aqueous ethanolic extract of dried seeds of Cuminum cyminum have better hepatoprotective potential because C. cyminum seeds contain a non specific lipid transfer protein nsLTP1. This nsLTPs play an important role in lipid transportation between different membranes [25]. Plant extract reduced significantly (p< 0.001) the level of all four liver enzyme markers (ALP, SGOT, SGPT and TB) which might be due to free radical scavenging mechanism of different constituents of plant extract. Hepatotoxic substances (CCls and Paracetamol) produce histopathological changes (steatosis and fibrosis) in hepatocytes [16]. Liver sections of intoxicated rats showed, necrosis, fibrosis and lymphocyte infiltration [15]. Photomicrograph of liver section of Cc.E treated group indicated decreased pattern of ballooning-degeneration, apoptosis, inflammation and fibrosis as shown in figure 1. The relative score of all four parameters was less than intoxicated control group which supported hepatoprotective activity of Cc.E.

Similarly, histopathological studies indicated that Nimesulide produced severe inflammation in hepatic cells along with fibrosis, apoptosis and ballooning-degeneration as shown in figure 5c. Proposed mechanism of action is that Nimesulide impaired the production of ATP from mitochondria due to uncoupling on account of the activity of its nitro group. It produces hepatic injury by induction of covalent modifications in target proteins, oxidoreductive stress, immune-mediated reactions, interference with hepatobiliary export and mitochondrial injury. Moreover, nitroarene group of nimesulide is metabolized in reactive intermediate which causes oxidative stress, covalent bonding and mitochondrial injury [14]. But there was significant reduction in changes and inflammatory cell infiltrates in diabetic rats supplemented with cumin extract.

CONCLUSION

On the basis of results, it is concluded that aqueous ethanolic extract of dried seeds of Cuminum cyminum exhibited marked hepatoprotective activity in Nimesulide intoxicated albino rats. Cc.E reduced the level of liver markers ALP, SGOT, SGPT and TB. Cc.E in dose of 200 mg/kg significantly (p < 0.001) reduced the level of all four parameters as compared to other two doses. Cc.E in 300 mg/kg showed better reduction in the level of all the parameters as compared to Cc.E 100 mg/kg but less then Cc.E 200 mg/kg. Furthermore, histopathological studies also confirmed the hepatoprotective action of cumin seeds against Nimesulide intoxicated rats.

ACKNOWLEDGMENT

The authors of this publication are thankful to the Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan for providing all the necessities used in this research.

REFERENCES


