Protective Effect of *Brassica oleracea* L. var. capitata against Simvastatin Induced Hepatotoxicity in Rats

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Abstract

The aim of this study was to investigate the hepatoprotective activities of *Brassica oleracea* L. var. capitata against simvastatin induced hepatotoxicity. The phytochemical screening was carried on the ethanolic leaves extract of *Brassica oleracea* L. var. capitata revealed the presence of some active ingredients such as Alkaloids, Amino acids, Carbohydrates, Flavonoids, Glycosides, Phenols, Proteins, Saponins, Steroids, Tannins and Terpenoids. Hepatotoxicity in rats was induced by simvastatin (20 mg/kg p.o. for 30 days) and the protective effect of *Brassica oleracea* L. var. capitata (300 mg/kg/p.o. and 500 mg/kg/p.o. either along with drug or followed by inducing hepatotoxicity) was identified by estimating marker enzymes. There was a significant changes were recorded in biochemical parameters i.e. increases in Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alanine Phosphatase (ALP), Serum bilirubin and decrease in Total proteins content and in oxidative stress markers such as GPx, GST, SOD and CAT, in simvastatin treated rats, which were restored towards normalization in *Brassica oleracea* L. var. capitata (300 mg/kg and 500 mg/kg) treated animals. Thus the present study ascertains that the leaf extract of *Brassica oleracea* L. var. capitata possesses significant hepatoprotective activity.

Keywords: *Brassica oleracea* L. var. capitata, Hepatoprotective Activity, Simvastatin, Ethanol, Antioxidant Activity and Silymarin

1. Introduction

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward & Daly, 1999). The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of haematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang et al, 1992). Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (Ross et al, 1996). The disorders associated with the liver are also numerous and varied (Wolf et al, 1999). More than 900 drugs have been implicated in causing liver injury (Friedman et al, 2003) and it is the most common reason for a drug to be withdrawn from the market. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz et al, 2002 and McNally et al, 2006). Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions (Kanathur et al, 2001 and Ricaurte et al, 2006). Simvastatin (Lipid Lowering Agent) competitively inhibits HMG-Co A (3-hydroxy-3 methylglutaryl coenzyme A) to mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels. CoQ10 acts as an antioxidant, has membrane stabilising effects, and is important for cellular mitochondrial respiration, which is
essential for energy production in organs (Frei et al, 1990 and Stocker et al, 1991). Thus, simvastatin causes oxidative stress mediated hepatotoxicity by depleting antioxidant enzymes (Vaghasiya et al, 2008). Cabbage can also be included in dieting programs, as it is a low calorie food (Danish, 2011).

The present study was directed to investigate the hepatoprotective activities of *Brassica oleracea* L. var. *capitata* against simvastatin induced hepatotoxicity.

2. Materials and Method

2.1. Plant Materials

The basic plant material of *Brassica oleracea* L. var. *capitata* used for the investigation was obtained from Mount Opera Garden, Near Ramoji Film City and Nalgonda Dist. The plant was authenticated by Department of Botany, research office (Botanist), Anwar-ul-Uloom College of Pharmacy, Hyderabad.

2.2. Preparation of Ethanolic Extract

The leaves of *Brassica oleracea* L. var. *capitata* were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder which was then used for extraction with ethanol. 250 g of powder the leaf powder was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

2.3. Phytochemical Investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc. present in ethanol extracts (Kokate et al, 1990; Trease & Evans, 2002 and Khandelwal et al, 2006)

2.4. Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of Nizam Institute of Pharmacy, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd.). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional Animal Ethical Committee).

2.5. Acute Toxicity Study

*Brassica oleracea* L. var. *capitata* in the dose range of 200-2000 mg/kg were administered orally to different groups of mice comprising of ten mice in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of Litchfield & Wilcoxon (1949).

2.6. Experimental Design for Hepatoprotective Activity (Vaghasiya et al, 2009)

Animals are divided into 5 groups, each comprising 6 rats as:

Group I: Normal control (saline)
Group II: Simvastatin (20 mg/kg. p.o. (Pre- Oral)
Group III: Simvastatin (20 mg/kg. p.o.)+*Brassica oleracea* L. var.*capitata* extract (300 mg/kg. p.o.)
Group IV: Simvastatin (20 mg/kg. p.o.)+ *Brassica oleracea* L. var. *capitata* extract (500 mg/kg, p.o.)
Group V: Simvastatin (20 mg/kg. p.o.) + Silymarin (25 mg/kg. p.o.)

Animals were divided into five different groups, each having 6 rats and treated accordingly. Group I: rats fed with a normal standard diet for 30 days. Group II rats receives Simvastatin (SMT) (20 mg/kg. p.o. alone for 30 days). Group III and IV rats receive SMT along with *Brassica oleracea* L var. *Capitata* extracts (300 mg/kg and 500 mg/kg. p.o. respectively for 30days) and Group V rats receive SMT along with silymarin (20 mg/kg/ p.o. for 30 days). On the 31st day, all the animals were sacrificed by mild ether anaesthesia.

2.7. Blood Biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (Reitman & Frankel, 1957), ALP (Walter & Schutt, 1974.) and bilirubin (Malloy & Evelyn, 1937) by standard method. Serum total protein was measured according to the method of Lowry et al (1951).

2.8. Estimation of Oxidative Stress Markers

All the animals were euthanized after blood collection with the spinal dislocation method under light ether anaesthesia and the liver was removed for the study of oxidative stress markers like Superoxide dismutase (SOD) (Moron et al,
1979) Catalase (CAT) (Takahara et al, 1960), Glutathione peroxidase (GPX) (Necheles et al, 1968) and Glutathione S transferase (GST) (Habig et al, 1974) were assayed.

2.9. Histopathology

Histopathology of liver was carried out by a modified Luna technique (Luna, 1999). In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed by bovine solution for 6 h. The livers were then paraffin embedded and 5 μ thick microtome sections (Figures 1-5) were made (Krajian, 1963). The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

2.10. Statistical Analysis

The data are represented as mean ± S.E.M. Students’ t-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes.

3. Results

The acute oral toxicity study of Brassica oleracea L. var. Capitata showed no mortality up to 2000 mg/kg. It
phytochemical screening shows the presence of Alkaloids, Amino acids, Carbohydrates, Flavonoids, Glycosides, Phenols, Proteins, Tannins and Steroids (Table 1). Its effect of ethanol extract on serum transaminases, alkaline phosphates, bilirubin and total protein level in Simvastatin intoxicated rats are summarized in Table 2. Results shows that there was a significant increase in bilirubin levels, SGOT, SGPT and ALP, in Simvastatin intoxicated group compared to the normal control group. The total protein levels were significantly decreased to 3.31 g/dl in Simvastatin intoxicated rats from the level of 6.46 g/dl in normal group. On the other hand the groups which received both *Brassica oleracea L. var. Capitata* extracts (300 mg/kg, and 500 mg/kg,) + Simvastatin (20 mg/kg. p.o) (Group III & IV) and Simvastatin (20 mg/kg. p.o.) + Silymarin (25 mg/kg, p.o.) (Group V) showed significantly decrease the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 2).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Constituents</th>
<th>Ethanol Extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Proteins</td>
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</tr>
<tr>
<td>11</td>
<td>Amino acids</td>
<td>+</td>
</tr>
</tbody>
</table>

*Where:*
+ : Present
- : Absent

The effect of *Brassica oleracea L. var. Capitata* on GPx, GST, SOD and Catalase activity is shown in Table 3. Table showed that GPx, GST, SOD and Catalase activity were significantly decreased in Simvastatin-intoxicated rats when compared with animals in normal control group. On the same time, the groups which received both *Brassica oleracea L. var. Capitata* extracts (300 mg/kg, and 500 mg/kg,) and Simvastatin (20 mg/kg. p.o) (Group III & IV), the values of above enzymatic parameters were near normal as compared to Group I animals and were significantly different from their Simvastatin (20 mg/kg. p.o) treated control group (Group II). The results are well compared with Silymarin standard drug treated group (Group V).

### 4. Discussion
Liver disorders, expressed in several forms i.e. jaundice, acute and chronic hepatitis, hepatoses and degenerative disorders resulting in fibrosis of the liver are still without appropriate therapies. Many chemicals and drugs (Leo et al, 1982) can injure the liver. During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase their concentrations (Deb, 1998). The decrease in elevated serum levels followed by simvastatin-treated animals in part may be due to the protective effect of *Brassica oleracea L. var. Capitata* extracts on liver cells follwed by the restoration of liver cell membrane permeability (Kalab & Krecschl, 1997). This protective effect indicates a reduction in enzymes present in the extra cellular milieu of the liver cell. The protective effect of the component of PHF has also been observed in several experimental studies (Mathur et al, 1994 and Sandhir & Gill, 1999).

In a previous study, it was reported that simvastatin caused oxidative stress mediated hepatotoxicity (Vaghasiya et al, 2008). The protection of liver cells against toxic materials including drugs, lipid peroxidation, and free radical injury may decrease inflammation (Yang et al, 2000). It is reported that phenols are responsible for the variation in the antioxidative activity of the plant (Cai et al, 2004). They exhibit antioxidant activity by inactivating with lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pitchaon et al, 2007 and Pokorny et al, 2001). Phenolic compounds are considered to be the most important antioxidative components of herbs and other plant materials and a good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported (Pellegrini et al, 2000 and Madsen et al, 1996).

Orhan et al (2007) reported that polyphenols can inhibit nitrosation and flavonoids have hepatoprotective activities. Since flavonoids are a group of potentially chemoprotective compounds and have similar structures that consist of 2 phenolic benzene rings linked to a heterocyclic pyre or pyrone (Aherne & O'Brien, 2002). In vitro and vivo experimental studies suggested that flavonoids influence signal transduction pathways (Friga et al, 2002) and inhibit proliferation in human cancer cell lines (Manthey & Guthrie, 2002). The obtained results suggest that *Brassica oleracea* possesses hepatoprotective capacity due to flavonoids and sulfurated compounds (Al-Howiriny, 2008).

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in simvastatin control group. Ethanolic extracts of
Brassica oleracea L. var. Capitata (300 mg/kg and 500 mg/kg p.o) prevented these histological changes, further indicating their hepatoprotective activity. Although there is insufficient information to establish the mechanism of action of Brassica oleracea L. var. Capitata protection, this could be due to its anti-oxidative of phenols.

5. Conclusion
The results of present study demonstrate that Brassica oleracea L. var. Capitata extracts (300 mg/kg and 500 mg/kg) has potent hepatoprotective activity against simvastatin induced liver damage in rats. The results also imply that the hepatoprotective effects of Brassica oleracea L. var. Capitata may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepatoprotective effect.

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