The Potential Liver Toxicity of Lepidium sativum Seeds in Albino Rats

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Abstract: Garden cress Lepidium sativum (LS) is an annual plant grown in many regions of Saudi Arabia. In the present study, LS collected from three regions of Saudi Arabia. Water suspension of seed powder was prepared from all samples and gavaged at different concentration (2, 4, 8 g/100 mL) to both adult female and male rats for 3.56 weeks, respectively. Blood samples from experimental animals were analyzed for liver functions tests and histopathology of the liver was conducted at the end of the study. Biochemical studies showed an increase in serum total protein with all doses. Albumin was increased at the high dose group however, AST, GGT were within normal levels. ALT and ALK were significantly increased after 3 weeks in males receiving 2 and 4 g kg⁻¹, respectively. Liver parenchyma in the form of vascular dilatation and congestion of central and portal veins were observed in low doses (2 and 4 mg mL⁻¹) given for 3 weeks. High doses given for 3 weeks showed peri-portal fibrosis and peri-vascular edema. Bile duct proliferation was a prominent feature in those specimens. Congestion and focal cellular changes were less in samples taken after 6 weeks however, some hepatocytes showed signs of apoptosis. Individual variation was observed in response to dose and duration of feeding. Insignificant differences were showed concerning the sex of animals. Further investigation is needed to clarify the cytological and biological effects of feeding on LS using enzyme immunoassay and electron microscopic study of hepatic tissue.

Key words: Garden cress seeds, Hab el Rashad, chemical analysis, minerals, fatty acids profile, liver functions, histology

INTRODUCTION

Lepidium sativum L. (LS) also named garden cress belongs to Brassicaceae (cabbage family) all cresses owe their aroma to isothiocyanates. Lepidium sativum called Hab el Rashad or Thula in Saudi Arabia (KSA) is an annual herbaceous plant (may sometimes be perennial) grown in many regions of KSA such Hijaz, AlQaseem and the Eastern Province (Ageel et al., 1978; Rahman et al., 2004). The vegetable form of garden cress is used as a food and the seeds used as herbal medication. In Europe and America, the leaves are used in salad (Maier et al., 1998). It is used in Kazakhst as food supplements, while in India the seeds are used for food purpose (Faecili, 1990; Gokavi et al., 2004).

The use of both herbal and herbal extracts in traditional medicine has recently increased. Although, history proved some beneficial effects, yet uncontrolled use may have serious impact on health especially vital organs such as liver and kidney. LS plant and seeds are considered one of the popular medicinal herbs used in the community of Saudi Arabia and some other Arabic countries as a good mediator for bone fracture healing in the human skeleton. Juma (2007) used an animal model of fracture to provide a scientific proof for this phenomenon noted publicly by traditional medicine practitioners and people in the community. He found that feeding LS seeds enhance callus formation on fractured femur of rabbit.

A number of recent studies point out the traditional uses of LS seeds extract in controlling many clinical problems. They were used as anti-asthmatic antiscorbutic, aperient, diuretic, galactagogue, poltice and stimulant. The leaves are antiscorbutic, diuretic and stimulant (Chopra et al., 1986; Eddouks et al., 2002). Maghrani et al. (2005) found that daily oral administration of the aqueous LS extract (20 mg kg⁻¹ for 3 weeks) exhibited a significant decrease in blood pressure (p<0.01) in Spontaneously Hypertensive Rats (SHR). Induced a significant increase of urinary elimination of sodium (p<0.01), potassium (p<0.001) and chlorides (p<0.001) in normal rat.

Aqueous LS extract was reported by Eddouks and Maghrani (2008) to have a potent inhibitor of renal glucose reabsorption in streptozotocin-induced diabetic rats which in turn reduces blood sugar. Studies concerning chemical analysis of L. sativum were few and mainly limited with isothiocyanates. These substances are
formed from inactive precursors called glucosinolates as a reaction to injuries (Kassie et al., 2002). An anticarcinogenic activity of organic isothiocyanate was reported by Zhang and Talley (1994). Kassie et al., (2002, 2003) reported that the chemoprotective effect of cruciferous vegetables including garden cress towards chemically induced colonic and hepatic preneoplastic lesions could be due to its constituents, Glucotropaeolin (GT) and Benzyliisothiocyanate (BITC), a breakdown product of GT.

Although, there are several literatures concerning uses of the plant (LS) as a traditional therapeutic agent or in experimental trial, there is no available toxicity study on the effects of the LS chemical constituents on liver. The possible side effects on liver if used without medical supervision in excess amount were not investigated. Adam (1999) reported some nephrotoxic effects and alteration of liver enzymes in rats. The main objective of present study was to determine the toxicity of the chemical components of LS in dried seed from 3 different region of Sudan Arabia, AL Ghassum (North), Alsuda and Ghamesh (South).

MATERIALS AND METHODS

Experiments were conducted at the Laboratory of Plant Physiology in the Department of Anatomy at king Fahd Medical Center for research (KFRMC) Lab., King Abdulaziz University to do chemical analysis and to study biological effect on rat liver.

Plant seeds collections: Experimental samples were seeds of L. sativum collected from three different regions of Saudi Arabia AL Ghassum (North), Alsuda and Ghamesh (South). The seeds were washed, then examined and photographed under stereomicroscope.

Chemical analysis of seed powder: Seeds of garden cress were washed twice by distilled water and ground in a coffee grinder powder then subjected to chemical analysis of the following:

Minerals: Calcium (Ca), Phosphate (P) Magnesium (Mg), Manganese (Mn), Iron (Fe) and Zinc (Zn) were determined by the method of AOAC (2002).

Vitamin C: HPLC was used to determine Vitamin C by using method in Food and Feed No. 1132 (1996).

Protein: Crude protein was determined in all samples according to methods used by Barneix et al. (1988).

Fatty acids: Fatty acids were extracted according to the method described by Whitaker (1986). Then, analysis of the fatty acid methyl esters was performed by Gas Chromatography (GC) (Varian 3400 GC).

Biological study

Preparation and dosage of LS seed for oral gavages: Seeds were washed thoroughly with distilled water, dried and powdered. The powder was insoluble in water. So a suspension was prepared in the following doses: low dose (2 g/100 mL), medium dose (4 g/100 mL) and high dose (8 g/100 mL). The present doses were chosen in view of previous research on L. sativum (Adam, 1999); Juma (2007).

Animals and experimental design: About 72 adult albino rats of both sexes (3 months old) with an average weight 150±5 g were used to test the biological effect of LS. (Animals were supplied by animal rearing unit, FMRC, King Abdulaziz University, Jeddah, Saudi Arabia). Rats of both sexes were divided into two groups: Control (n = 12) and experimental (n = 60). The animals were kept under controlled conditions (24±1°C, hum).

Experimental female and male rats was divided into 3 subgroups (n = 10 of each sex). Group 1 (n = 10 of each sex) giving 2 g/100mL (20 mg mL⁻¹) of L. sativum water suspension. Group 3 (n = 10 of each sex) giving 4 g/100 mL of (40 mg mL⁻¹) L sativum water suspension. G4 (n = 10 of each sex) giving 8 g/100 mL of (80 mg mL⁻¹) L sativum water suspension.

The suspension was shacked before use and gavaged daily to the animals using rat gastric tube (0.8 mL/animal/day). Blood samples were taken via retro orbital sinuses from all animals (under light ether anesthesia) at the beginning of the experiments for basal determination of some liver functions.

Biochemical study of some liver functions tests: Animals were sampled for blood after 3 and 6 weeks, respectively. Serum was sending to king Abdulaziz university, hospital laboratory for analysis of serum total protein, albumin, total bilirubin and liver enzymes (Aspartate Transaminase (AST) Gamma Glutamyl Transpeptidase (GGT) Alanine Transaminase (ALT) and Alkaline phosphatase (ALK).

Histological study of liver: Following blood withdrawal, the animals were decapitated. The abdomen and thorax were opened. Heart was perfused via left ventricle with normal saline followed by 10% neutral buffered formalin to ensure good fixation of the organs. Liver was removed, cut into small pieces (2×2 mm) and refixed in the same
fusative for 24 h. After that processed for light microscopic were studied. Haematoxylin and Eosin 5 μ paraffin sections from both control and experimental animals were examined and photographed.

**Statistical analysis**: The experimental design for data analysis was a completely randomized design with three replications. Data were recorded on standardized forms and were expressed as mean±S.E.M. for three determinations. ANOVA was applied to evaluate statistically significant.

**RESULTS AND DISCUSSION**

*Lepidium sativum* seeds morphology: The seed morphological characters of the *Lepidium sativum* as shown by Light Microscope (LM) are shown in (Fig. 1). Seeds are relatively large around 2.5 x 3 x 1 mm in dimension. Seed shape is obliquely ovate with smooth surfaces and ranged from brownish red to brown color. The seeds from different regions differ slightly in color and size.

**Chemical analysis of LS seeds**

**Total protein and minerals**: Table 1 shows that the average percentage of total protein in the 3 studied samples was 28.4% in AL Qusaim sample, 28.6% in Alsuda, however, lower protein content (22.8%) was found in Ghamesh Meshat. The seeds were found to contain different minerals such as calcium, phosphorus and magnesium. Alsuda seeds seemed to have the highest magnesium and phosphorus level (0.25-0.85%), respectively while Al Ghastum has the lowest percent of calcium. Zinc, manganese and iron were considered as trace elements and found in all samples in varying amounts. Alsuda seeds contain highest amount of Zinc (0.054%) and manganese (0.0050%). Iron was higher (0.036%) in seeds of Ghamesh Meshat.

**Vitamin C**: Vitamin C could not be detected in any of the analyzed samples.

**Fatty acids content of LS seeds**: Fatty acids analysis as shown in Table 2 indicates that the three samples of *L. sativum* seeds contain considerable amount of fatty acids. The major fatty acids in the total lipid fraction of LS included palmitic (16:0), oleic (18:1ω9), linoleic (18:2ω6), Linolenic (18:3ω3) and Gadoleic (20:1ω3). Essential fatty acids such as Oleic, Linoleic and Linolenic acid are present in considerable concentration. Differences among the three samples were detected in Oleic acid and Linolenic acid.

**Table 1**: Chemical contents of LS seeds of total proteins and some minerals (Means±S.E)

<table>
<thead>
<tr>
<th>Region of origin</th>
<th>AL Qusaim (%)</th>
<th>Ghamesh Meshat (%)</th>
<th>Alsuda (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>28.4±0.57</td>
<td>22.8±0.46</td>
<td>28.6±0.57</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.25±0.45</td>
<td>0.31±0.3</td>
<td>0.85±0.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.34±0.35</td>
<td>0.43±0.36</td>
<td>0.46±0.44</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.17±0.05</td>
<td>0.38±0.0</td>
<td>0.23±0.06</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.04±0.01</td>
<td>0.004±0.0</td>
<td>0.005±0.4</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.003±0.00</td>
<td>0.004±0.4</td>
<td>0.003±0.4</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.05±0.01</td>
<td>0.08±0.02</td>
<td>0.016±0.51</td>
</tr>
</tbody>
</table>

*Significant difference at 0.05 Levels (LSD) test; ** Significant difference at 0.01 Levels (LSD) test.

![Fig 1: Morphology of Lepidium sativum seeds as shown by LM from different region of Sudia Arabia: (a) AL-Qusaim (b) Ghamesh Meshat and (c) Alsuda](image-url)
determined by GC of Alsada sample. No significant differences among the three samples were detected in eucalyptus (22.1%) stearic (18.0%) and arachidic acids (20.0%).

**Biological effects of LS seeds in rats**

**Effect on animal body weight:** Body weight of the animals is shown in Table 3. Feeding animals on water suspension of LS seeds result in insignificant changes in body weight after 3 weeks, while significant statistical decrease in body weight of both sexes was observed after 6 weeks at doses 40 mg L⁻¹ and 80 g compared to control.

**Effect on liver function tests:** Table 4 shows the effect of different doses of LS seed suspension given orally for 3 and 6 weeks on some liver functions in male rats. Total blood albumin was significantly increased in males at 20, 40 and 80 mg L⁻¹ treated groups in comparison to control males. In female rats this was comparable among the groups. Alkaline phosphatase and AST were significantly increased at high dose in males only at 3 weeks after treatment.

**Histological changes in rat liver after 3 weeks of oral gavages of water suspension of LS seed powder** (Fig. 2-3): Compared to control (Fig. 2A), rat liver receiving (2 mg mL⁻¹) showed dilated Central Veins (CV) and focal endothelial degeneration of hepatic sinusoids however, hepatocytes nuclei still showed their vesicular appearance with increase in binucleated cells. At 4 mg mL⁻¹ dose, there was more congestion of Central Veins (CV) with extra vascular hemorrhage and endothelial separation. Lymphocyte aggregation was observed near necrotic hepatocytes which showed small or pyknotic dark nuclei. At higher dose (8 mg mL⁻¹) marked congestion and endothelial separation of Central Veins (CV), peri-vascular edema and extravasations of vessel contents were observed. Hepatocytes had dark acidophilic cytoplasm and small pyknotic nuclei (apoptosis). Bile duct proliferation and portal edema were also observed (Fig. 3).

**Histological examination of mice liver after 6 weeks of oral gavages of L. sativum seed powder water suspension:** Slight or no alterations in liver lobular architecture were observed upon prolonged administration of LS seeds.
Fig. 2: Liver sections at central vein region from control and experimental rats after 3 weeks of oral feeding with LS; a) control liver showing Central Vein (CV). Hepatocytes cell cords with central rounded vesicular nuclei, some are binucleated (N-arrows). Thin walled blood Sinusoids (3) separate cell cords. b) Section from liver (2 g) showing dilated Central Veins (CV) and sinusoids. Notice focal endothelial degeneration (arrow) hepatocytes nuclei still showed their vesicular appearance with increase in binucleated cells (white arrows); c) at 4 g dose, there is more congestion of Central Vein (CV) with extra vascular hemorrhage (star) and endothelial separation (arrow) lymphocyte aggregation (thick arrow). Most hepatocytes showed small or pyknotic dark nuclei (white arrow), d) at higher dose (3 g), marked congestion and endothelial separation of (CV), peri-vascular edema and extravasations of vessel contents were observed. Hepatocytes had dark acidicophilic cytoplasm and small (arrow) pyknotic nuclei (apoptosis) (Haematoxylin and Eosin stain photographed X400).

Fig. 3: (a) At a dose 2 mg (3 weeks) significant bile duct proliferation (arrows) and edema (star) was observed. Hepatocyte showed dark cytoplasm and nuclei (black arrows), bile duct proliferation was observed (white arrows); b) At higher dose (4 mg, 3 weeks) marked bile duct proliferation (arrows), edema (stars) and dilatation of Portal Vessels (PV). Hepatocytes looked disorganized with ill defined outlines, the nuclei are dark and pyknotic or deformed (Haematoxylin and Eosin stain photographed X400).

(Fig. 4) The changes seemed to be less than those observed after 3 weeks at all doses. No signs of necrosis were observed. However, focal regions showed shrunken hepatocytes with, dark stained cytoplasm but with intact undamaged cell membranes. The nuclei of cells become smaller, darker (apoptosis or programmed cell death). Portal areas showed dilatation of portal veins, bile duct proliferation but less than after 3 weeks. The ducts
Fig. 4: A) Part of rat liver near the Central Vein (CV) 6 weeks after oral gavages of LS water suspension (2 g) showed dark stained shrunken hepatocytes. The nuclei are small and pyknotic (arrows). No signs of necrosis (hepatocyte membranes are intact) or inflammation. B) At 14 g dose, similar changes were observed. Portal area showed dilated Portal Vein (PV) and Proliferation of Bile Ducts (BD). Hepatocytes were shrunken and separated from each other (arrows). C) Marked fibrosis of portal area (8 g) with lymphocyte and mast cell infiltration (arrows) and bile duct proliferation (stars). The duct have wide Lumina and low cubical lining cells (head arrow) (Haematoxylin and Eosin stain photographed X400).

appeared dilated with low height of lining cells. An increase in fibrous content and cellular infiltration were observed especially with high doses (3 mg mL⁻¹). The present research was designed to study the toxicological effects of chemical constituents of Lepidium sativum L. (LS) seeds collected from 3 region of Saudi Arabia kingdom, a plant that used in traditional medicine (Ageel et al., 1978; Czimber and Szabo, 1988; Ahman et al., 1989). Special interest in its use for enhancing bone healing (Juma, 2007). Chemical analysis of LS seeds showed that they contain high percentage of protein. The crude protein content observed for L. sativum seeds in this study are similar to those reported by Golapi et al. (2004). The high protein content explained the role of the seeds in healing process (Komesu et al., 2003). Diet rich in protein provide amino acid precursor for synthesis of different types of substances needed for cellular activities, repair or healing processes (Pollak et al., 1986; Delvin, 1992). Biochemical studies of rat serum in the present study showed that LS result in an increase in total serum protein with all doses and albumin in high dose. Both protein and albumin are also important to keep body volume and blood pressure homeostasis besides acting as body buffer (Mehler, 1992). Toxicological effect of LS seeds performed in the present study showed that feeding rat LS suspension produced decrease in body weight in both sexes after 3 and 6 weeks at the mid and high dose (4 and 8 mg mL⁻¹). Juma (2007) found no effect on body weight with LS dose of 6 g/kgBW in rabbits.

The decrease in body weight observed here may be due to linoleic fatty acids when LS seed were given in high dose Conjugated Linoleic Acid (CLA) supplementation helps improve the ratio of lean body mass to fat decreasing fat especially on the abdomen and enhancing muscle growth (DeLany et al., 1997).

The liver is one of the most important organs in the human body that performs a number of metabolic functions that are essential to human life. Several important proteins found in the blood are produced in the liver. One of these proteins, albumin helps retain calcium and other important substances in the bloodstream. Albumin also helps regulate the movement of water from the bloodstream into the body's tissue. Liver is the chief organ involved in detoxifying our body. When the liver is over-stressed all other organs start to dysfunction (Campbell et al., 2008). In the present study it was observed that LS seed had minimal effect on liver parenchyma if given in low doses (2 mg mL⁻¹).
chemical analysis done herein and also reported by other investigators (Gokavi et al., 2004) showed that LS seeds could be used as food additive or supplement. Mineral content especially calcium and phosphate and the presence of considerable levels of essential fatty acids make LS seeds of beneficial nutritional value for many body organs including the liver. In high dose (8 mg mL⁻¹).

LS showed some toxic effect on liver parenchyma represented by focal necrosis, hepatocytes apoptosis and inflammatory cell infiltrate in portal areas. These changes may be due to isothiocyanates reported to be one of LS constituents (Burow et al., 2007). From a human health perspective, isothiocyanates are quite important because they are major inducers of carcinogen-detoxifying enzymes with marked anti proliferative activity (O'Hare et al., 2007). Apoptosis seen in liver parenchymatous cells in the present study could be a part of anti-neoplastic effect of isothiocyanates constituent of LS taken in high dose.

Ökulicz et al. (2008) found that there was time dependent effect of gluconasturtiin and phenethyl isothiocyanate on metabolic and anti oxidative parameters in rats. Its administration caused a considerable increase in liver cholesterol and triglyceride content with a concomitant drop in the amount of glycogen. In view of these reports vaculation in hepatocytes may be explained by glycogen depletion or increase hepatocytes lipid content.

Ökulicz et al. (2008) found that short-term administration of gluconasturtiin and phenethyl isothiocyanate constituted a significant factor interfering with liver metabolism. This may explain bile duct proliferation, an observation reported in rat in association with metabolic changes in hepatocytes by Aktas et al. (2003).

The increase in acidophilic staining of cells 6 weeks after LS administration denote activation of detoxification process in hepatocytes with increase in smooth endoplasmic reticulum and mitochondria (Kumar et al., 2006), the organelles well known to be involved in detoxification process and tend to be stained intensely by acidic dyes. Future electron microscopic study of hepatocytes was needed to confirm such suggestion. Liver enzymes were not significantly altered by LS except of AST which indicate that damage in hepatocytes cellular membrane was mild. The increase in some liver enzymes like ALT and AST in groups receiving high doses (40 or 80 mg mL⁻¹) could be due to focal damage of some hepatocytes and bile duct proliferation. A toxicity study was performed on LS seeds used in Saudi traditional medicine for the treatment of various ailments (Adam, 1999). The researchers found that LS seed fed to Wistar albino rats at 2% (w/w) was non-toxic, Ten percent (w/w) was toxic but not fatal and 50% (w/w) of the diet for 6 weeks was lethal and caused depression in growth rate and entero-hepato-nephrotoxicity. He found also, that organ lesions accompanied by anemia and leukopenia were correlated with alterations in serum AST and ALT activities and concentrations of total protein, cholesterol, urea, and other serum constituents.

In the present study the preservation of hepatocytes membrane integrity, the minimal necrotic changes in liver parenchyma and the slight alteration of liver functions even in high doses may be due to protection offered by oleic acid and antioxidant effect of zinc constituents of collected LS seeds. Clandinin (1991) reported that dietary fat; determine cellular membrane structure. Schmidt-Erfurth found that LDL-R (+) mice fed a standard diet showed more diffuse focal alterations than control mice fed a high-fat diet.

Hart et al. (1990) reported that specific monounsaturated fatty acids (oleic and linoleic) are implicated in the protective effect against toxicity induced by paracetamol as these fatty acids prevent oxidant injury to be incorporated into the cell membranes. Oleic acid modulates lipid metabolism; it is integrated in the membrane structure and thus participates directly in the fatty acid profile of membrane phospholipids (McGarry, 1992). The apoptotic changes observed may be due to isothiocyanates (Zhang and Talalay, 1994) and this may explain the anti proliferative anticancer effect of LS seeds mentioned previously (Kassie et al., 2002).

CONCLUSION

It is concluded that the effects of LS on liver structure and function observed in the present study are considered preliminary. Further investigation is needed to clarify the cytological and biological effects of feeding on individual LS constituents for more prolonged periods.

ACKNOWLEDGEMENTS

This study was supported by grant from Deanship of Scientific Research, King Abdulaziz University, Jeddah, KSA, project no. 427/518. The researchers also thank Dr. Mansour AlHazmi Prof. of animal physiology Fac. of sciences, KAU for his helpful suggestions on biochemical and statistical parts of this study as well as Dr. Ali Faqih for his editing and revision of the manuscript.
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