THE EFFECT OF Lepidium Sativum SEEDS EXTRACT ON SOME OXIDATIVE STRESS, ANTIOXIDANTS AND HISTOLOGICAL CHANGES IN RAT TREATED WITH CCl4

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Article received 12.5.2019, Revised 18.6.2019, Accepted 25.6.2019

ABSTRACT:
The aim of the current study is to investigate the effect of hepatoprotective and antioxidant of seeds extracts of the Lepidium Sativum extract against liver damage induced by carbon tetrachloride. In our study, white rats were used. CCl4 (0.1 ml) /100 gram body weight was used to inject the rats intraperitoneal mixed in olive oil same amount, twice a week for twelve weeks and treated orally with (LSS) (200 mg/kg) daily for twelve weeks and compared with a group of rats injected intraperitoneal with CCl4 (0.1 ml, 100g b.w.) mixed in olive oil same amount, twice a week for twelve weeks as a control group. CCl4 administration is caused significant changes in serum enzymes (ALP GGT and GPT), oxidant substances (MDA, CAT, GSH and SOD) indicative of hepatic injury. The results revealed that the (LSS) extract significantly decreased AST, ALT and ALP levels. The antioxidant parameters GSH, GPx, SOD and catalase levels were increased considerably compared to their levels in groups not treated with (LSS). Histopathological findings revealed that liver of CCl4-treated rats showed Degeneration, thickness wall of the central vein; the inflammatory cells are infiltrating into portal areas and central vein. There are ameliorated significantly in the rat’s groups, that treated by LSS result in a decrease in degenerated hepatocytes and there are reduce in necrosis in the group that treated by LSS (200) mg/kg followed by CCl4 administration, and demonstrated less nuclear degeneration as compared to CCl4 group. Treatment of LSS (200) mg/kg and then followed by CCl4 injection results in a decrease in nuclear degeneration. It could conclude, LSS extract has a protective effect against CCl4 toxicity. Our results are very promising LSS extract could be used to protect liver tissues against CCl4.

Keywords: Lepidium Sativum, antioxidant, hepatoprotective, carbon tetrachloride

INTRODUCTION:
The environmental pollutants, drugs and toxic chemicals cause cellular damage by activation of reactive oxygen species (Sies, 2019). CCl4 is one of toxic substance that used in inducing hepatotoxicity in lab animal also, causes damage in heart, brain, testis and kidney (Fahmy et al., 2018). Depend on several studies, CCl4 showed that have great negative effect on many organs such as brain, liver, heart, kidneys, testis, lung, and blood. Also, CCl4 causes an acute and chronic lesion in several organs (Negi et al., 2018; Li et al., 2019b; Zamzami et al., 2019).

The medicine plants were used for the treatment of infections and diseases. Plant Treatment or cal-led Phyotherapy is the oldest methods used to cure the diseases. Pure chemicals that isolated from plants are used to treat diseases such as Jaundices, cardiovascular diseases, diabetes, heavy metal poisoning, scarlet fever and abdominal and pelvic diseases etc. (El-Missiry et al., 2015; Kumar et al., 2017; Shahid et al., 2017).

El-Rashad or called Lepidium sativum Linn. is an edible herb, a fast-growing with aroma and tangy flavor (Hussein et al., 2017). Several substances of the plant are used in the treatment of liver disease, jaundice, gastrointestinal diseases, spleen diseases, fracture, menstrual problems and arthritis. For example, L. sativum is used to treat the throat infection such as asthma and applied to treat the uterine tumor, headache, breast cancer and nasal polyps (El-Missiry et al., 2015; Fahmy et al., 2018; Kumar et al., 2017; Shahid et al., 2017; Zamzami et al., 2019). L. sativum contains essential fatty oils, vitamins, carbohydrate, protein, isothiocyanate glycoside and flavonoids. Many studies showed which L. sativum has an antioxidant effect, anti-inflammatory effect, antihypertensive effect, anti-asthmatic effect, diuretic effect and hypoglycemic effect (Emhofer et al., 2019; Hussein et al., 2017). Using LSS as protective substances are the aim of our study and staying some biochemical, physiological changes in rat that injected by CCl4.

2. MATERIALS AND METHODS
2.1. COLLECTION OF PLANT MATERIAL:
The local market is provided with our study with LSS in Ramadi, Iraq and was keeping at airtight closer containers.

2.2. EXTRACT PREPARATION: Soxhlet apparatus is used for plant extraction by taking seed 60 and ground to convert to powder. The powder was dissolved into 600ml ethanol for 6–8 hours. Filtration of the extract and heated the solution at (Bauchi company, Switzerland) at 60°C; and pressure 175 bar. The extract stored at 4°C (Al-Asmari et al., 2015).
2.3. THE CHEMICAL SUBSTANCES: Carbon tetrachloride was provided by Sigma company (USA).

2.4. ANIMALS: Thirty-six wistar rats have weight range 190–200 gram, it was provided by the University of Baghdad, Faculty of Veterinary Medicine, central animal house. It keeps at cages at 25 ±3°C with 12 hours dark and 12 hours light and provided standard food and water. The animals (Rats) are divided for 6 groups. The first group represents the control group and provided pellets and water for twelve weeks. The second group was provided with 1mL/kg olive oil intraperitoneal two times a week for twelve weeks. The third group was provided CCl4 0.1ml/100 gram body weight with 50% in olive oil intraperitoneal two times a week for twelve weeks. The fourth group was provided LSS extract 200 mg/kg b.w. Orally for twelve weeks. The fifth group was provided LSS extract 200mg/kg b.w. orally and then provided CCl4 0.1ml/100g b.w. (50% in olive oil) Intraperitoneal 2times a week for two–1ve weeks. The sixth group was provided CCl4 0.1ml/100g b.w. (50% in olive oil) intraperitoneal two times a week for four weeks, and then pro-vided LSS extract 200mg/kg b.w. orally for orally eight weeks. After anesthesia, the animals were sacrificed. (2) MI Blood sample was collected from the heart directly from all animals and left to drying for produce serum then the centrifuge uses separate 3000 rpm for 15 min. The serum was stored at -20°C.

2.5. SERUM BIOCHEMISTRY: Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) alkaline phosphatase (ALP) and bilirubin were determined using Reflation plus Analyzer and Roche kits (Roche Diagnostics GmbH, Mannheim, Germany). While urea and creatinine levels were estimated using standard test kits (Randox Laboratories, Crumlin in County Antrim, Northern Ireland, UK).

2.6. OXIDATIVE STRESS ANALYSIS: MDA amount will assess lipid peroxidation. MDA level is estimated depending on the manufacturer’s instructions (Sigma-Aldrich company, Germany). SOD, CAT and GSH level were assayed by using kits (Biodiagnostic-Sigma-Aldrich Company, Germany).

HISTOPATHOLOGICAL EXAMINATIONS: Liver tissues were fixed in formalin and the sample embedded in paraffin for cutting 5m in thickness. All samples are stained with eosin and hematoxylin (Sigma Company, Germany). The microscope is used to read the results.

2.7. STATISTICAL ANALYSIS: ANOVA is the test used in analysis of the results in Graph Prism Software V. 7.00.

3. RESULTS:

3.1. LSS AND LIVER ENZYMES: Figure 1, 2, 3 and 4 showed that CCl4 results in increased in liver enzymes level GOT, GPT, bilirubin and ALP, Group III as compared to the first group. Enzymes level was decreased at (P ≤ 0.05) due to LSS use (Groups IV, V and VI) as compared to Group III.

Figure 1: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of GOT enzyme in rats treated with CCl4

Each group represents mean± S.D. of six animals Mean values in each row having different superscript (A, B, C and D) are significant.

Figure 2: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of GPT enzyme in rats treated with CCl4

Each group represents mean± S.D. of six animals Mean values in each row having different superscript (A, B, C and D) are significant.
Figure 3: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of ALP enzyme in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript (A, B, C and D) are significant.

Figure 4: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of TSB enzyme in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript (A, C and D) are significant.

3.2 Oxidative state results: As compared to the first group, the CCl4 treated animals (third group) showed exhibited an increase in serum MDA level at (p < 0.05). While CCl4 group recorded significant decrease in GSH, CAT and SOD (%) levels, Treatment with LSS extract significant decrease in MDA level and significant increased SOD, CAT and GSH activities have been enhanced after treatment with LSS extract in (group IV, V and VI (Fig 5,6, 7, 8).

Figure 5: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of MDA in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript A, B, C and D are significant.

Figure 6: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of CAT enzyme in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript A, B and C are significant.

Figure 7: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of GSH enzyme in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript A and C are significant.

Figure 8: Effect of treatment with ethanolic extract of Lepidium sativum (L) seeds on serum level of SOD enzyme in rats treated with CCl4
Each group represents mean± S.D. of six animals Mean values in each row having different superscript A, B and C are significant.
3.3: Histopathological study: The control group showed that the liver is normal as Fig. 9. The liver tissues sections contain distinct hepatic cells with a prominent nucleus and normal cytoplasm; the liver cell is arranged around the central vein. But the sections of the CCL4 treated group showed cytoplasmic vacuolation necrosis, Degeneration, central vein thickness due to inflammatory cell infiltration and haemorrhage as shown in Fig. 10, 11, 12. All the histological changes are ameliorated in the groups treated by LSS included a decrease in necrosis and degenerated hepatocytes (Fig. 13). In treated LSS group with (200) mg/kg of LSS then followed by CCl4 injection, showed little degeneration in the nucleus (Fig. 14). Treatment by LSS 200mg/kg then followed by (CCl4) results in showed moderate nuclear degeneration, (Fig. 15). The current study showed that LSS reduce CCl4 toxicity.

Fig. 9: Histopathologic findings in the Liver of normal control rat (group1) showing no histopathological changes Central vein (CV), Hepatocyte (HC), Sinusoidal spaces (S), Kupffer cell (KC)

Fig. 10: Histopathologic findings in the Liver of normal control rat (group2) showing no histopathological changes Central vein (CV), Hepatocyte (HC), Sinusoidal spaces (S), (H & E X 400)

Fig. 11: Histopathologic findings in the Liver of CCl4 group (group3) showing Degeneration in hepatocytes (D), inflammatory cells infiltration (MNC), hemorrhage (H), (H & E X 400)

Fig. 12: Histopathologic findings in the Liver of CCl4 group (group3) showing thickness wall of Central vein (TW), inflammatory cells infiltration (MNC), increase in fibroblast (F), (H & E X 400)

Fig. 13: Histopathologic findings in the Liver of Ls group (group4) showing no histopathological changes Central vein (CV), Hepatocyte (HC), (H & E X 400)
Discussion

Liver diseases are a public health problem due to increasing obesity rates in all age groups. The liver disease most common in all world countries (Breda et al., 2019). The classical treatments of liver diseases have adverse effects (Doulberis et al., 2019; Maheshwari and Ranka, 2019). Medicinal plants contain natural substances without side effects (Ali, 2017; Doulberis et al., 2019).

Our results revealed the hepatoprotective effect of L. sativum that extracted by ethanolic. CCl4 is a toxic substance inside the cell wherever in the endoplasmic reticulum converted to C13COO and CCl3 by cytochrome P-450 (Li et al., 2019b). That will result in several changes included increase permeability in the plasma membrane of the calcium that leads to severe disorder of calcium and leads to cell death (Negi et al., 2018; Strehler and Thayer, 2018). CCl3 causes several negative changes included degeneration of liver fatty layer and necrosis of the centrilobular (Ferreira et al., 2018). Also, CCl4 is elevated of liver enzymes such as APT, ALT, AST and bilirubin. ALT and AST could easily leak into the blood vessels from damaged liver cells (Slama et al., 2018).

If the AST level becomes high, that indicates to the disorder of the liver, muscle injury and cardiac infraction. ALT is converted alanine to pyruvate and glutamate (Gupta, 2019a). Elevation of ALP is mean internal and external biliary obstruction due to infiltration. If bilirubin level increased that means because of hepatic obstruction. The elevated concentration of enzymes with increase concentration of the total bilirubin that mean to cellular leakage (Adekomi, 2019; Gupta, 2019b).

Our results founded treatment with LSS decrease liver damage, that agreement with (Chhipa and Sisodia, 2019) wherever, they founded LSS have a hepatoprotective effect. Also, we founded significant improvement in histological structure. But the explanation of LSS mechanism work is not known (L’hadj et al., 2018), there are several reports founded LSS have an anti-oxidative effect.

That may be attributed to phenolic compounds in LSS composition, and there are many studies founded a phenolic compound in LSS extract (L’hadj et al., 2018), and some reports suggested the phenolic compounds have a significant positive role in liver damage (Al-Asmari et al., 2015).

CCl4 is one such widely used environmental toxicant to induce animal models of acute nephrotoxicity and hepatic damages experimentally. The administration of CCl4 led to a statistically significant (p<0.05) increase in urea and creatinine level in the rats during the experimental period when compared to the control. On administration of extract LSS, a reduction in urea and creatinine levels were observed. The increased urea and creatinine level suggests the decline of glomerular filtration rate. But the protective and curative treatment of ethanolic extract of LSS significantly reduced the level of urea and creatinine that indicates increase glomerular filtration rate.

Hepatotoxicity is affected by oxidative stress. Trichloromethyl Production dines from CCl4 (Slama et al., 2018), the lipid peroxidation is caused changes in function and structure of cellular membranes and membrane damage (Abdollahi et al., 2019; Ali and Abdulhadi, 2017). MDA is produced through lipid peroxidation, MDA is enzyme used as indicator oxidative stress in liver toxicity by CCl4 (Hsouna et al., 2019; Lee et al., 2019).

GSH, CAT and SOD are endogenous antioxidants; its function is deactivation of free radicals (Jamore et al., 2019). GSH, CAT and SOD will deplete in CCl4 treated animals, that explained losing of antioxidant in oxidative stress (Li et al., 2019a). Free radicals will accumulate during the loss of antioxidant enzymes. CCl4 is a toxic substance, that stimuli for production of superoxide radicals and perox (Ikekuchwu et al., 2019). Malondialdehyde level becomes high after injection CCl4 (0.1ml/100 gram) body weight. Malondialdehyde will result in loss of antioxidant defence and tissue damage. All that lead to disorder in the ratio of polyunsaturated fatty acids and other fatty acids, thus, also, at the end that leads to cell death (Haryama and Riezman, 2018). LSS extract is oral administration at (200 mg/kg) to reduce MDA and increase in GSH, CAT and SOD levels for provided strong protection against CCl4. Many studies confirmed LSS extract efficacy against CCl4 (Al-Asmari et al., 2015; Sangekar et al., 2018; Shail et

Fig. 14: Histopathologic findings in the Liver of CCl4 & LS group (group 5) showing inflammatory cells infiltration (MNC 400) (H & E X 400).

Fig. 15: Histopathologic findings in the Liver of CCl4 & LS group (group 6) showing inflammatory cells infiltration (MNC) (H & E X 400).
al., 2016), but, the histological observation that treated with LSS showed marked protection of the liver against the toxicant materials, protection effect is including lesser fatty infiltration, the absence of necrosis and hepatic cords and that agree-ment with our results.

CCl4 is chemical substances used as toxicant material for induction hepatic damages and nephro-toxicity in the animal. The administration of CCl4 led to a statistically significant (p<0.05) increase in urea and creatinine level in the rats during the experimental period when compared to the control. On administration of extract LSS, a reduction in urea and creatinine levels were observed.

REFERENCES
Harayama, T. a


