Original article

Potential Hepato-healing effect of *Solenostemma argel* and *Origanium majorana* against Carbon Tetrachloride-induced Hepatic Damage in Rats

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Abstract

The use of medicinal plants as natural remedies is achieving popularity for cure liver disorders, supported by their safety, cost-effectiveness, and availability. This study is carried out on Wistar rats to evaluate the anti-hepatotoxic activity of aqueous *S. argel* and *O. majorana* extracts against CCL4 induced acute liver damages. Twenty-four rats were used and different concentrations of aqueous extracts were administrated orally for 30 days. Hepatic injury was achieved by injecting 3 mL/kg of CCL4 in day 7 subcutaneously. The study showed that there were no differences in mean body weight gain among the groups of rats treated with *S. argel*. There was significant decrease in the values of MCHC, and significant increase in MCV value, and elevated ALP and AST activities as well as liver and kidney damages. Necropsy finding and serum chemistry indicate that 500 mg/kg *S. argel* cause hepatic damage in rats. *O. majorana* extract given to the rats at 250 and 500 mg/kg caused a significant increase in body weight gain, mild alteration in haematological parameters and non-significant decrease in serum ALP and AST activities. The administration of the aqueous *O. majorana* extracts demonstrated a significant protective effect by lowering the level of hepatic marker enzymes (AST, ALT and ALP) and improving the histological architecture of the rat liver and kidneys, which were compared with Silymarin as standard hepatoprotective drug. In conclusion, the present study revealed that aqueous extracts of *O. majorana* treatment play protective role by improving the change in histopathological structure against CCl4 - induced liver damages and enhancing liver enzyme activity in rats. The study hypothesized that safety administration of *S. argel* depends on the accuracy of dose measurement.

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Introduction

Liver problems are still on aggravation (Saleem *et al*., 2010). Jaundice and hepatitis are two major hepatic disorders that account for a high death (Thnaian, 2013). These disorders have many etiologies, including chronic viral hepatitis, alcohol abuse. 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 disease, nutrient supply, energy provision and reproduction (Crispe, 2003). In spite of the scientific evolution in the field of hepatology, metabolic syndrome,

and autoimmune disorders although the cellular and pathological mechanisms leading to hepatic fibrosis and cirrhosis are relatively similar. Liver fibrosis is characterized by accumulation of extracellular matrix proteins, and activated hepatic stellate cells (HSC). (Scholmerich and Holstege, 1990 and Heymann *et al*., 2009). Currently, very few hepatoprotective drugs derived from natural sources, are used for the treatment of liver problems (Kumaresan and Vipin,2011).

According to Abere *et al*., (2010), herbal remedies have a therapeutic effect and are acceptable curative for diseases and symptoms. Many plants possess a hepatoprotective activity, which contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes (Bhawna and Kumar, 2009).

Solenstomma argel belongs to *Apocynaceae* family, *Solenoslemma* Genus and S. *argel* Species. Locally known as Hargel Ar. *(*Murwan and Murwa, 2010 and Elkamali,, 2001). The plant leaves are used for the treatment of gastrointestinal disturbances, liver and kidney diseases. They are also used as antispasmodic, carminative and diuretic agent (Kamel *et al*., 2000). Chemical constituents of *S. argel* are including acylated phenolic glycosides, namely argelin and argelosid, choline, flavonoids, monoterpenes, pregane glucoside, sitosterol, and a triterpenoid saponin. (Elkamali, 2001; Kebbab *et al*., 2017) reported that *S. argel* have potent radical scavenging activities.

Origanum majorana L.is a bushy shrub belonging to *lamiaceae* family, *Origanum* Genus and *Origanum majorana* L. Species. Locally known as Pardagosh (Adam and Ahmed, 2014). *O. majorana L*., is a tender perennial herb of 'Origanum' genus, popularly known as marjoram (Vagi *et al*., 2002). It is an aromatic plant native to Cyprus, Turkey and Mediterranean region. It has a tangy flavor and a bitter taste. Contains up to 5% volatile oil the thymol and carvacrol only occur in small amounts (Baratta *et al*., 1998; Novak *et al*., 2000). *O. majorana* oil possesses good antimicrobial activity. (Ibrahim, *et al*., 2017) and has been used in the form of herbal infusions in folk medicine for asthma, cold, coughs, cramps, depression, dizziness, gastrointestinal disorders, hay fever, headache, toothache, and sinus congestion and as a diuretic and to promote menstruation (El-Ashmawy *et al*., 2007 and Ramadan *et al*., 2012).

Considering the fact that herbal remedies have been used traditionally as Hepatoprotectors against liver damage, but they are usually self-prescribed by the consumers even with the slightest knowledge of efficacy, and minimum idea of toxicity, there is an increased need for experimental studies to assess a hepatoprotective plant potential effects and specific manner of administration.

This study has been designed to evaluate anti-hepatotoxic potentiality of two different concentrations of aqueous extracts of *S. argel* and *O. majorana* in CCL4 induced acute liver injury rats.

Material and methods

Plant material

The *Solenostemma argel* and *Origanum majorana* were purchased from a local market in Khartoum (April, 2017). The plants tissues were cleaned, shade-dried and ground with a mechanical grinder.

Animals

Forty-two both sexes Wister albino rats were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, weighting 130-160g, were housed in propylene cages and provided bedding with sawdust. The rats were clinically healthy and housed within the premises department of biochemistry and molecular biology, Faculty of Science and Technology, Al-Neelain University. Animals were acclimatized to the experimental conditions for a period of one week prior to the commencement of the experiment. Housing conditions including temperature (30°C ± 2°)**,** light (12-h light/dark), and relative humidity (60–70%) were set. The rats were supplied with standard diet (flour 55.6%, meat 35%, edible oil 7.5%, sodium chloride 1.2% and vitamins and minerals

0.7) and water *ad libitum*

Experimental design

Forty-two rats were weighed after the adaptation period and marked with serial numbers and divided randomly into 7 groups, 6 rats each, the rats were fasted for 12h prior to carbon tetrachloride treatment. Single dose of 3 ml/kg of carbon tetrachloride diluted with olive oil (1:1) was administrated subcutaneously for the rats.

Group 1 received distilled water represented as normal control; group 2 animal received distilled water as induction control. Group 3 was treated with Silymarin (100 mg/kg, orally) which served as standard treated group. Group 4 and 5 animals were treated with 250 and 500 mg/kg /d *O. majorana* aqueous extract Group 6 and 7 animals were treated with 250 and 500 mg/kg/d *S. argel* aqueous extract respectively. The doses were calculated according to individual body weights and administrated orally through cathedral tube for four weeks. Average body weight for each group of rats were measured weekly .Clinical signs and average body weight were reported for each group on day 0, 7, 14, 21 and 30 of the study.

At the end of the fourth week, overnight fasted animals were sacrificed by cervical decapitation under mild anesthesia, collection of blood for hematological analysis, blood for serum preparation and tissue samples of liver and kidney for histopathology were immediately occurred. The kidney samples were selected because renal dysfunction is a common and serious problem in patients with advanced liver disease.

Methods

Plant extract preparation

The crushed leaves of *S. argel* and aerial parts of *O. majorana* were sequentially extracted with petroleum ether (60-80 C), methanol (99.8%) and sterilized distilled water. (Enayde, 2005). 300 g of the fine powdered of plant was macerated in 900 ml petroleum ether for 72 hrs. After that the residue was extracted two times again with petroleum ether and the extract was separated from solvent using rotary evaporator, the plant residues were further dried, weighted and reused for methanol extraction and, subsequently, with distilled water and submitted to extraction process as described above. The petroleum ether and the methanolic extracts were kept a side and aqueous extracts was filtered, evaporated and freeze drier, the yields were calculated, the residue obtained was kept in dry clean bottles till used (Adam and Ahmed, 2014).

Biochemical methods:

Evaluation of activities of serum enzymes such as Aspartate transaminase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum albumin and total protein concentration were determined in serum using (BS-380 Chemistry Analyzer, China,2013).

Haematological methods:

Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total white blood cell (WBC) counts and differential white blood cell counts were measured by the Automated Haematology Analyzer. (Sysmex, XP 300, Japan)

Pathological method:

The fixed liver and kidney tissues were sectioned (5-micronthickness), embedded in paraffin and sections stained with Hematoxylin and Eosin (H&E). Tissue sections from each tissue were microscopically examined to identify gross lesion

Statistical analysis:

The results were analyzed for statistical significance by one-way ANOVA using the statistical package of social sciences (SPSS) version 21. All data were expressed as means (mean ± Standard error (M± S.E). P. Values ($P<0.05$) were considered statistically significant. (Snedecor and Cochran, 1989).

Results

Growth changes

The effects of treatment with aqueous extracts of the study

plants on body weight gain of the rats shown in Table 1. CCL4 given to the rats in Group 2 caused a significant decrease in body weight gains at week 2 and 4 when compared with control group. There were no significant differences in mean body weight gains among the rat treated with Silymarin. The rats on 250 mg/kg *S. argel* for 4 weeks (Group 4) showed no differences in mean body weight gain compared to control group. The average mean of rats on Group 5 showed a significant increased weight gain at week 4. *O. majorana* given to the rats at 250 mg/kg (Group6) and 500 mg/kg (Group7) showed significant increase weight gain ($P<0.05$) at the first 2 weeks, followed by a significant increase in weight gain for the next 2 weeks at Group7 only. No death among the rats wa recorded along the treatment

Haematological changes

The effects on haematological parameters of rats in compare with control group are shown in Table 2. In CCl4 group there were significant decrease in the values of RBC and MCHC. In Group 4 there were was significant decrease in the values of MCHC, lymphocyte and significant increase in MCV value. In Group 5 there were significant decreases in the value of lymphocyte and significant increase in neutrophil values. The statistical analysis showed that there was a significant increase in neutrophil and decrease in lymphocyte cell count. In the rat on the 500 mg/kg O. majorana (Group7), PCV value and lymphocyte cell count showed significant decrease, while monocyte count increased. No significant change in the control and Silymarin groups.

Serobiochemical changes

In comparison with the control group, there were significant decreases in total protein and albumin concentration in group treated with CCL4 only. Group 5 showed significant decrease in albumin concentration. The average mean of ALP activity, total protein and albumin concentration indicated a significant decrease in group of rats fed with 250 mg O. majorana (Group 6). Neither ALP, ALT, AST activity nor total protein, albumin, globulin concentration changed in Groups (2, 4, and 7).

Table 1. Body weight and body weight gain of experimental rats given different concentration of aqueous extracts of *S. argel* and *O. majorana* orally for 4 weeks

Groups	Body weight (g) 0 day	Weight gain (g) after 2 weeks	Weight gain (g) after 4 weeks
Control (no treatment)	155.6±5.7	15.2±0.4	12.4±0.6
CCL_4 control	156.4±1.6	4.8±0.6*	3.6±0.3*
Silymarin control	157.6±5.7	15.4±7.0	13.4±0.7
S. argel 250 mg/kg	159.2±2.8	16.4±0.1	13.4±0.3
S.argel 500 mg/kg	158.0±7.3	15.6±0.3	19.8±0.7*
O.majorana 250 mg/kg	158.0±7.5	20.0±0.8*	13.2±0.1
O.majorana 500 mg/kg	155.6±2.7	18.8±0.0*	17.4±0.8*

Table 2. Hematological changes of rats given different concentrations of aqueous extracts of *S. argel* and *O. majorana* orally for 4 weeks.

Groups	**Hb g\dl**	**PCV (%)**	**RBCs 10^6 mm^3**	**MCV (m^3)**	**MCH (%)**	**MCHC %**	**WBCs 10^3 mm^3**	**Lym (%)**	**monocyte (%)**	**Neut (%)**
1. Control	**14.1±.6**	**16.6±.3**	**8.4±.2**	**53.0±.6**	**45.4±1**	**31.4±.5**	**10.3±.1**	**69.1±1.7**	**19.0±1.3**	**11.8±1.2**
2. CCL_4 control	**13.9±.2**	**17.4±.7**	**7.2±.3***	**55.8±4**	**46.8±1**	**29.8±.7**	**8.9±1.0**	**58.2±7.0**	**17.5±2.4**	**24.1±6.6***
3. Silymarin control	**12.4±.8**	**16.3±.6**	**8.0± .4**	**55.5±2**	**40.1±1**	**29.4±.9**	**11.1±.3**	**53.1±17**	**24.6±8.8**	**21.9±9.1**
***4. S.argel 250* mg/kg**	**13.6±.7**	**17.1±.6**	**7.7±.4**	**60.2±2***	**47.8±2**	**28.6±.1***	**8.8±1.4**	**46.9±7.6***	**26.5±4.7***	**26.7±8.7***
***5. S.argel 500* mg/kg**	**14.4±.9**	**15.9±.3**	**8.3±.3**	**52.5±.9**	**43.9±2**	**30.3±1**	**8.3±.6***	**36.3±8.4***	**21.0±2.6**	**24.5±1.8***
***6. O.majorana* mg/kg**	**14.0±.4**	**16.6±.3**	**8.2±.1**	**55.8±1**	**46.8±1**	**30.2±.7**	**9.1±.9**	**54.7±4.1***	**22.7±2.1**	**22.5±2.3***
7. *O.majorana* mg/kg	**13.1±.3**	**15.6±.1***	**8.2±.4**	**51.9±.4**	**52.5±6***	**30.1±.4**	**10.7±2**	**49.8±5.9***	**24.8±1.9***	**15.8±4.3**

Values are expressed as mean ± S.E; *Significant = ($P< 0.05$) as compared to normal control

Serobiochemical changes:

In comparison with control group, there were significant elevation of ALP, ALT and AST activities and significant decreases in total protein and albumin concentration in group of rats treated with CCL_4 only (Table 3). In the rats treated with 250 mg/kg *S. argel* (Group 4) for 4 weeks, an increase in ALP activity and decrease concentration of albumin was significantly seen. Group 5 of the rats that had been treated with 500 mg/kg *S. argel* showed significant increase in ALP, ALT and AST activities decrease in albumin concentration in compere to control group, treatment with 500 mg/kg *S. argel* reduced the elevated average mean of ALP, ALT and AST activities recorded in group of rats treated with CCL_4 only. The average mean of ALP activity, total protein and albumin concentration indicated a significant decrease in group of rats fed with 250 mg *O. majorana* (Group 6). Neither ALP, ALT, AST activity nor total protein, albumin, globulin concentration changed in Groups (3 and 7). to CCL_4 group.

Table 3. Serobiochemical changes of rats given different concentrations of aqueous extracts of S. *argel* and *O. majorana* orally for 4 weeks

.Groups	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	T. Protein (g/l)	Albumin (g/l)	Globulin (g/l)
1.Control	89.4±4.8	15.2±2.9	27.8±3.4	7.6±.1	5.3±.1	2.0±.1
2.CCL_4	109.0±8.2*	23.0±3.3*	39.6±3.3*	6.1±.4*	4.3±.2*	1.8±.2
3.Silymarin	82.2±10.2	18.2±1.8	29.0±4.3	6.9±.3	4.4±.4	2.7±.3
4.S.Argel 250 mg/kg	111.6±9.1*	17.4±3.2	27.8±2.1	7.6±.6	4.3±.2*	2.0±.6
5.S.Argel 500 mg/kg	94.8±6.6*	19.4±1.8*	35.4±6.0*	7.3±.5	4.3±.2*	2.9±.3
6.O.majorana 250 mg/kg	76.2±6.7*	20.0±1.9*	26.6±3.3	6.0±.4*	3.8±.4*	2.1±.3
7.O.majorana 500 mg/kg	84.0±6.0	17.4±4.5	26.0±.6	6.7±.5	4.7±.6	2.0±.4

Values are expressed as mean ± S.E; *Significant = (P< 0.05) as compared to normal control.

Table 3. Serobiochemical changes of rats given different concentrations of aqueous extracts of S. *argel* and *O. majorana* orally for 4 weeks.

Groups	**ALP (IU/L)**	**ALT (IU/L)**	**AST (IU/L)**	**T. Protein (g/l)**	**Albumin (g/l)**	**Globulin (g/l)**
1.Control	89.4±4.8	15.2±2.9	27.8±3.4	7.6±.1	5.3±.1	2.0±.1
2.CCL_4	109.0±8.2*	23.0±3.3*	39.6±3.3*	6.1±.4*	4.3±.2*	1.8±.2
3.Silymarin	82.2±10.2	18.2±1.8	29.0±4.3	6.9±.3	4.4±.4	2.7±.3
4.S.Argel 250 mg/kg	111.6±9.1*	17.4±3.2	27.8±2.1	7.6±.6	4.3±.2*	2.0±.6
5.S.Argel 500 mg/kg	94.8±6.6*	19.4±1.8*	35.4±6.0*	7.3±.5	4.3±.2*	2.9±.3
6.O.majorana 250 mg/kg	76.2±6.7*	20.0±1.9*	26.6±3.3	6.0±.4*	3.8±.4*	2.1±.3
***7.O.majorana* 500 mg/kg**	84.0±6.0	17.4±4.5	26.0±.6	6.7±.5	4.7±.6	2.0±.4

Values are expressed as mean ± S.E; *Significant = (P< 0.05).

Histological changes:

After 30 days of treatment, Histological profile of the rats treated with *S. argel* at a dose of 250 and 500mg/kg showed sever degenerative changes in the liver as in Fig. 1 (IV and V) and kidneys as in Fig. 2 (I and II). There were, in addition, to hepatic degenerative changes, fatty cytoplasm vacuolation and massive haemorrhages. The cells of renal tubules were degenerated or necrotic and some of the glomerular tufts were disappeared.

The sections of the liver and kidneys taken from the rats treated with aqueous extract of *O. majorana* at doses 250 and 500 mg/kg showed conserved architecture (Fig.A[7]) and moderate tubular epithelial degeneration as in Fig. 2 (III and IV), illustrating protection against the CCL4 induced damage. In CCl4 dosed rats (Groups 2) there was heavy necrosis, congestion of sinusoids, hydropic degeneration and infiltration of inflammatory cells (Fig. 1(II)).

The kidney sections of the CCL4 treated group showed shrinkage of glomerular tuft, severe tubular necrosis and vacuolation. The histomorphology of liver and kidneys in rats treated with standard drug Silymarin showed the hepatic architecture, which was similar to that of control (Fig. 1(III)). Neither macroscopic nor microscopic lesions were detected in the liver and kidney of the rats which were treated with normal saline (control group) (Fig. 1 (I)).

Discussion

The finding of this study revealed that there were no significant differences in mean body weight gain between the group of rats administrated with 250 mg/kg *S. argel* for 4weeks' period, which indicate that administration of *S. argel* at dose 250 mg/kg is not toxic as evidenced by the absence of mortality and growth impairment. In the rats treated with 500 mg/kg *S. argel*, there were no differences in mean body weight gains at the first 2 weeks then increased throughout the rest of experimental period suggest the renal damage had led to excretory dysfunction and fluids accumulation. Macrocytic hypochromic anemia indicated by increased MCV and decreased MCHC values were observed in group of rats treated with low dose of *S. argel.* A clear relationship between *S. argel* alkaloids and blood parameters has been proved in previous experimental study conducted by Shyoub *et al* (2013).

However, administration of 250 and 500 mg\kg of oral dose of *S. argel* showed an increased monocyte count. Duffield, *et al* (2005); Heymann *et al*., (2009) reported that Monocytes and monocyte-derived cells such as macrophages and Kupffer cells have been shown to be essential mediators of fibrosis progression as well as fibrosis regression, because they possess the ability to induce as well as remove deposition of extracellular matrix material.

Mahgoub, (2016) record that treatment with100 mg/kg of *S. argel* against ethanol induced hepatotoxicity ameliorated the elevation of AST and ALT activity. This in reverse of the result of the current study that administration of different doses of *S. argel* in the rat causes elevated activity of serum AST, ALT and ALP activity and decrease albumin concentration.

Hepatonephrotoxicity was a feature of *S. argel* treatment in rats as evidenced by histological changes of the liver and kidney that include cytoplasmic changes, hydropic degeneration, sever fatty change, sinusoidal dilatation and necrotic glomerular tufts.it is necessary, however, to consider the possibility of injury to other organs such as the intestine and skeletal muscles as factors contributing to the changes in the activity of AST in serum of the experimental animals. Shyoub *et al* (2013) reported that histopathological examination of some of the vital organs of the young Nubian goats given a 5g/kg dose of *S. argel* syrup once daily for 45 days revealed some tissue abnormalities mainly including: congested heart; hyperemia of the intestinal tissues revealing catarrhal inflammation with lymphocyte infiltration; liver tissue necrosis of centrilobular hepatocytes, fatty cytoplasm vacuolation and slight congestion of the sinusoids; kidneys tissue necrosis of the renal tubules, pyknosis, karyolysis of tubular epithelial cells, and interstitial mononuclear cells infiltration.

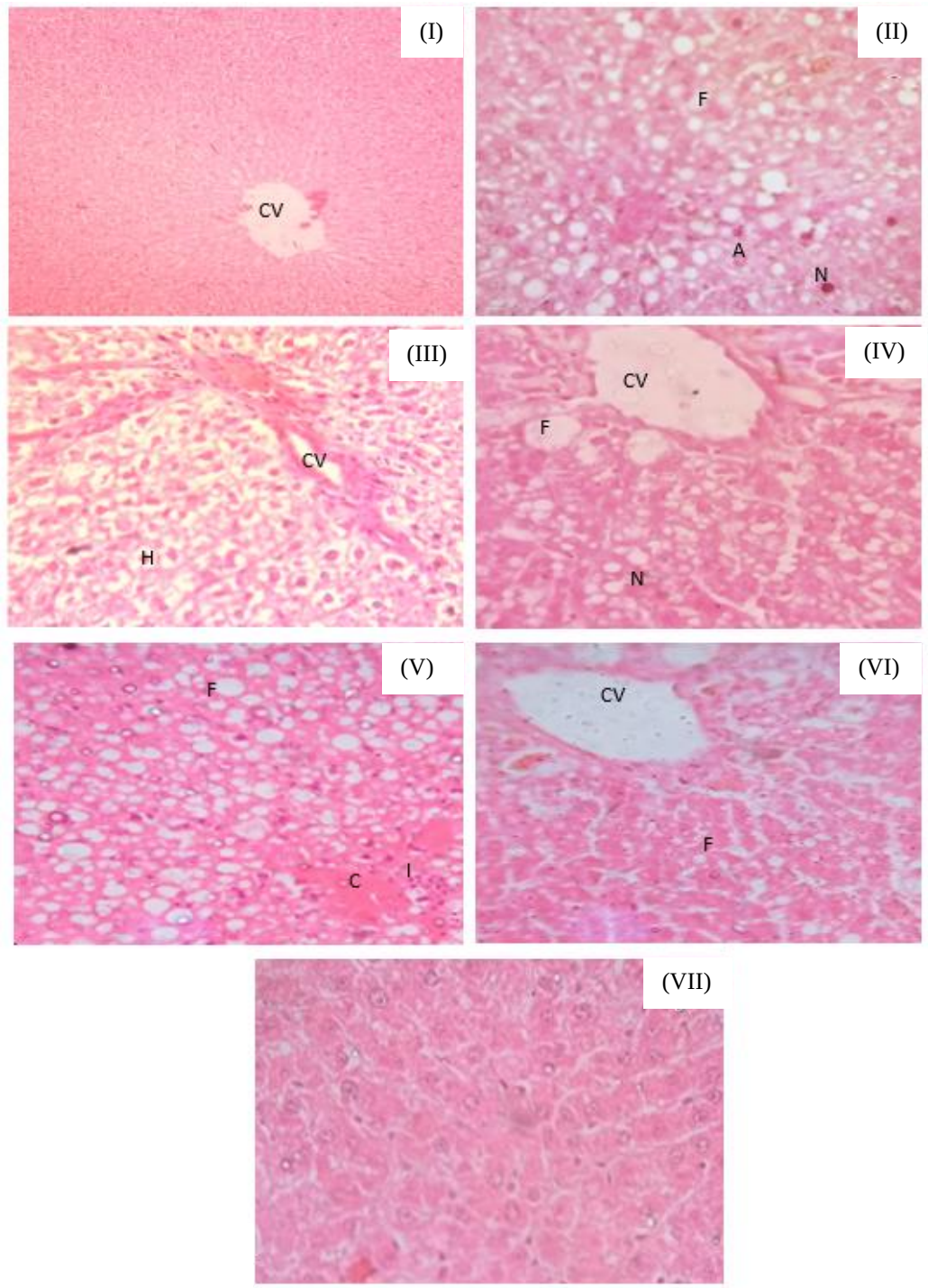

Figure 1: Photomicrograph of liver sections. I) Untreated control group. II) CCl4 Group: massive necrosis, areas of apoptosis. III) Group treated with Silymarin, conserved architecture centrilobular vein, no necrosis. IV) Group treated with 250mg/kg *S. argel*: sever fatty change V) Group treated with 500mg/kg *S. argel*: generalized fatty changes, lymphocytic infiltration, congestion in the central vein. VI) Group treated with 250 mg/kg *O. majorana*: centrilobular veins dilated, fatty cytoplasmic vacuolation VII) Group treated with500 mg/kg O. majorana: conserved architecture. **CV** = central vein, **F**= fatty changes, **A**= areas of apoptosis, **N**= necrosis, **H**=hydropic degeneration, **C**= congestion, **I**= infiltration of inflammatory cells.

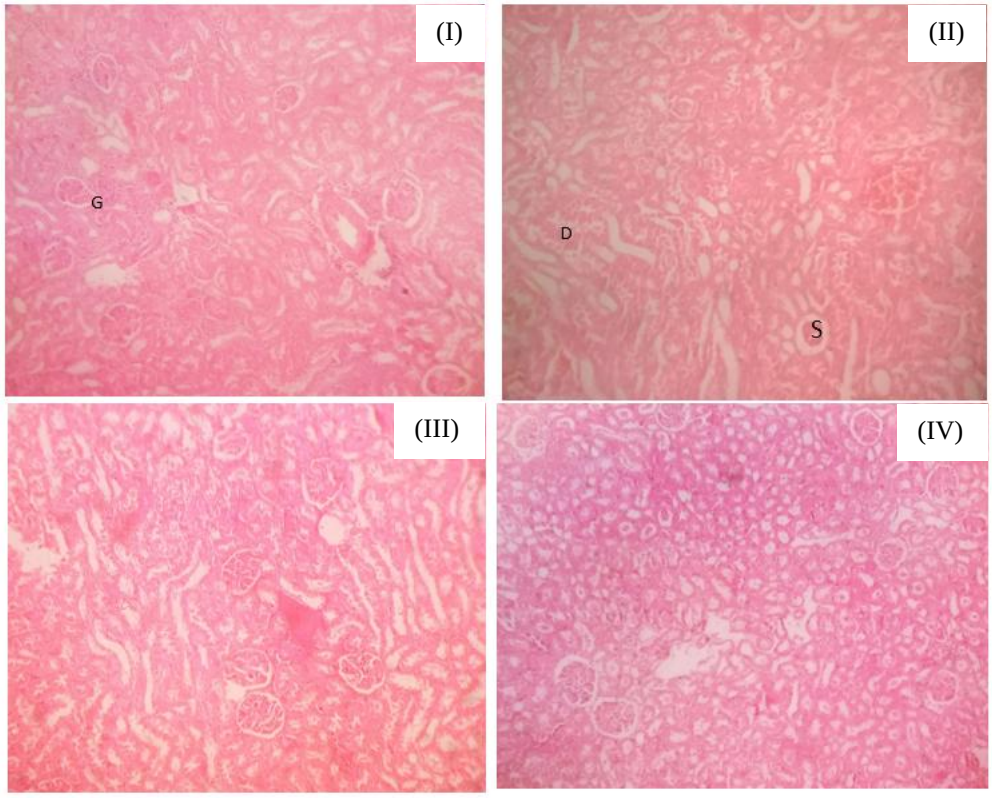

Figure 2: Photomicrograph of kidney sections. I) Group treated with250mg/kg *S. argel*: slight congestion. II) Group treated with 500mg/kg *S. argel*: necrosis of the cortical tubules and congestion of the glomerular tufts. III) Group treated with250mg/kg O. majorana: shrinkage of glomeruli IV) Group treated with500 mg/kg *O. majorana*: conserved architecture. **G=** glomerular tufts**, D=** dilatation of renal tubules**, S=** segmentation of the glomerulari.

Discussion

It seems, therefore, that the safety and efficacy of *S. argel* to improve liver damage depend, at least, upon the standardization of the dose of extract administrated. Previous study showed that *Argemone mexicana* L. at dose 100 mg/kg has a reasonable potential in healing liver parenchyma and regeneration of liver cells, while the high dose showed toxic effect in treated rats. (Adam *et al.*, 2011)

Pretreatment with aqueous extract of *O. majorana* for 4 weeks showed significant increase weight gain. This suggests the susceptibility of the plant to enhance weight gain. Effects on body weight and non-significant decrease elevated ALP and AST activity indicate potential hepatoprotection activity of *O. majorana* against CCI4 induced hepatotoxicity in rats evidenced by significant mitigation of the severity of the CCL4 toxicity, resulting in the preservation of the hepatic and renal tubular histology by significantly reducing the scores of histopathological damages. Evidence from earlier study showed that the methanolic extract of *O. majorana* possess free radical scavenging activity against CCL4 induced oxidative stress. (Kumar *et al.*, 2013). *O. majorana* leaves could be used as therapeutic option antagonize pesticide hepatotoxicity (Mossa *et al.*, 2015).

Conclusion

- From above observations of biochemical parameters and histopathological examination, *S. argel* at 500 mg/kg has

a reasonable potential in healing liver injury and regeneration of liver cells hence it may act as toxic and not safe to use for treatment of diseases and may disturb the haemopoietic process.

- In addition, this study demonstrated that aqueous *O. majorana* extract is effective agent in the treatment and protects rat liver from CCL4-induced and suggests a potential therapeutic use.

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