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Study of the antioxidant, immunomodulatory and antibacterial properties of *Origanum majorana* leaf acetone extract

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Abstract: *Origanum majorana* (OM) is known to have antioxidant properties. The present work was designed to evaluate, for the first time, the hepato/nephroprotective, immunomudulatory and antibacterial potentials of OM leaves acetone extract (OMLE). OML was collected from Al-Soudah, Aseer, Saudi Arabia, and OMLE was prepared. Active biomolecules were screened utilizing FT-IR spectroscopy, protein electrophoresis and HPLC. Reactive oxygen species (ROS) were measured using ELISA. Male rats were treated with OMLE and livers, kidneys and sera were collected. Liver enzymes, kidney function markers, antioxidants in liver and kidney tissues and tumor markers were quantitated. OMLE immunomodulatory potentials were tested using rat splenocytes. Antimicrobial power was tested against Gram negative/positive bacteria. The extract contained many functional biomolecules and ROS but no sugars and proteins. OMLE treatment did not affect liver and kidney functions or the tumor markers. There were some changes in measured antioxidant biomolecules. The extract is not harmful to hepatocytes as indicated by levels of AST and ALT. It is not carcinogenic as it did not make any changes in tumor marker levels. The extract could modulate the splenocytes. The use of OMLE is useful in protecting normal vital organs from oxidative stress. It can also be used as immunostimulant.

Keywords: *Origanum majorana,* oxidant, cancer marker, Aseer, hepato and nephroprotection.

INTRODUCTION

Kidney plays a fundamental role in health, sickness and overall development and growth. The basic job of the kidney is to eliminate waste products resulted from metabolic pathways and xenobiotic from the circulation. Also, kidney works to keep up all body liquid volume, its structure and acid base equilibrium. During these processes, the kidney may become vulnerable to toxicity. Living organisms are daily exposed to countless natural/synthetic chemicals. These may act as nephrotoxicants like heavy metals, hydrocarbons, halogenated herbicides, mycotoxins included as contaminants in water and food, these affect liver and kidney functions (George *et al*., 2017; Perazella, 2009). Oxygen free radicals were shown to participate in toxicity of large number of chemicals and induce pathogenesis of various sicknesses (Kalender *et al*., 2012; Mossa *et al*., 2012; Pizzino *et al*., 2017).

Reactive species, for example, H_2O_2, hydroxyl radicals (HO^-) and superoxide anions (O_2) enhance oxidative process and produce lipid peroxidative harm to the membrane of the cell. The hydroxyl radical is suggested as an abettor of lipid peroxidation by acting as an iron-catalysis through Fenton reaction (Jomova *et al.*, 2010). The utilization of natural antioxidants biomolecules for the curing of contaminants enhanced kidney toxicity or injury is being investigated extensively. Likewise, there are a few reports on oils showing that it brings about modifications of pharmacological reactions to drugs.

Liver, the largest organs in the body, is regarded as the chemical factory that is essential for survival. It receives the blood through two sources; the hepatic artery (20%) and the portal circulation (80%). All elements absorbed from the alimentary canal (nutrients, toxins and drugs) should first pass the liver. It likewise assumes a significant job in the metabolic processes and has other functions like storage of carbohydrate (glycogen), degradation of old red blood corpuscles, blood protein production and detoxification (Ueno and Komatsu, 2017). Hepatitis is an aggravation and corruption of liver cells. This might be because of substance and organic pollution of food and water or terrible ecological conditions, and lack of healthy sustenance are the fundamental elements for the rising liver brokenness which prompts jaundice (Serber and Blaney, 2015; Teschke, 2019). Further, the participation of free radicals as hydroxyl radicals and superoxide anions and other ROS like H2O2 in different ailments has been built up. Several factors, including resistible operators, prescriptions, poisons, and mediators that induce inflammation may cause a wide range of diseases that may end with the insufficiency of ordinary liver histological design, limit cell mass and a diminishing in the progression of blood prompting functional liver capacity loss. Many trials were made to find hepatoprotective materials. However, no effective

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therapies are available until now (Vasas, Orbán-Gyapai and Hohmann, 2015).

Nowadays, medicinal plants have a special interest to be used in treatment instead of synthetic drug consumption. *Origanum majorana* L. (*O. majorana*) is one of the mint family Lamiaceae and it is regional at North Africa, southern Europe and Asia Minor. *O. majorana* is known as Wazab in Saudi Arabia. Marjoram has numerous utilizations with various medical advantages and is used as a home remedy for cramps, nervous headaches, gastrointestinal disorders, depression, dizziness, migraine, paroxysmal coughs and as a diuretic (Vági *et al.*, 2005; Ouedrhiri *et al.*, 2016). *O. majorana* has an antimicrobial potential (Pepa *et al.*, 2019). Many studies showed that *O. majorana* essential oil and ethanolic/aqueous extracts could protect from lead acetate induced kidney and liver genotoxicity and damage (Leeja and Thoppil, 2007). It contains several metabolites like flavonoids (apigenin, diosmetin and luteolin), phenolic terpenoids (carvacrol and thymol), phenolic glycosides (methyl arbutin, vitexin, orientinthymonin and arbutin), tannins, hydroquinone, cis-sabinene hydrate, triacontaneacids (oleanolic acid), and sitosterol (Hajlaoui *et al.*, 2016; Bhardwaj and Dubey, 2018). The essential oil of *O. majorana* has some biological activities including hepatoprotective, antimutagenic and antioxidant activities (Bina and Rahimi, 2017). Its major ingredients are γ-terpinene (15.40%), 4-terpineol (29.97%), trans-sabinene hydrate (10.9 %), γ-terpinene (6.8 %) and 3-cycolohexene-1-1 methanal, a,a4-trimethyl-,(S)-(CAS) (6.5 %). As indicated by (Mossa and Nawwar, 2011; Mossa *et al.*, 2015), leaves extract of *O. majorana* has a suppressing action on lipid per oxidation, hydroxyl radical, $DPPH^{\cdot}$ and hydrogen peroxide, reducing power in a dose-dependent manner.

As of now, an almost no data is accessible in the literary works on the protective effects of *O. majorana* on healthy kidney and liver. Therefore, this study aimed to investigate the effects of *O. majorana* leaf extract on renal and liver function biomarkers, lipid peroxidation, and antioxidant enzyme activities and in rats.

MATERIALS AND METHODS

Plant collection and extract preparation

Leaves of *O. majorana* were gathered in March, 2019, from the Al Soda, Aseer, Saudi Arabia. Leaves of *O. majorana* (220g) were air-dried and ground by electrical grinder to get a coarse powder. The powder was soaked in acetone at room temperature for 30h with continuous stirring. The blend was separated two times through Whatman filter paper (No.1). The filtrate was dried using a rotary evaporator and obtained material extract (1.8g) was dissolved in acetone (180mL) to get 1% solution (stock solution). Stock solution was filter-sterilized (0.45 µm, Amicon) and stored at -30°C.

Functional group analysis

Functional group found in the *O. majorana* Leaves acetone extract (OMLExt) were explored using FT-IR spectroscopy (Perkin- Elmer Spectrum 2000, USA) within the range 600–4000 cm−1 at a rate of 16 times and the clarity of 4 cm−1(Ghramh, Khan and Ibrahim, 2019).

Extract's sugar and protein content

Sugars (fructose, glucose, maltose and sucrose) in OMLExt were estimated utilizing the high performance liquid chromatography (HPLC) the same way described by Ghramh *et al*. (Ghramh, Ibrahim and Kilnay, 2020) using Agilent 1260 infinity II and its supplied software (Open Lab CDS Chem Station Edition, Rev C. 01.10[201]). ROLAExt at 1% concentration was explored for the presence of proteins utilizing a protein standard (Precision Plus Protein™ Standards, Unstained, Bio-Rad), the ready-to-use SurePAGE (GenScript, USA) gradient (4-12%) polyacrylamide gel and electrophoresis cell (Mini-PROTEAN, Bio-Rad) (Ghramh, Ibrahim and Kilnay, 2020).

Reactive oxygen species (ROS) in the plant extract

Reactive oxygen species (ROS) were determined in OMLExt using an immunosorbant assay kit (EIAAB) following the included instructions. ROS in the tested samples is specified by paralleling the O.D. of the samples to a standard curve.

Antimicrobial susceptibility testing (AST)

The antimicrobial activity of OMLExt was assessed against Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Proteus mirabilis and Escherichia coli*) pathogenic bacteria. Further processing was carried out as per previous process (Kilany, 2017).

In vitro effects of OMLExt on splenic cell division

Splenocytes culture preparation

Splenocytes were prepared for *in vitro* culture, according to Ibrahim *et al*. (Ibrahim *et al.*, 2019). Cell density was set to $0.5X10^5$/mL in culture medium. The study was proceeded in conformity with the guidelines put by the Ethical Committee of King Khalid University.

Study of anti-proliferative effects of OMLExt

To prepare stimulated splenic cells, phytohaemagglutinin at final concentration of 6.5µg/mL was added to part of the cells suspended in culture medium. OMLExt was added at different final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15 and 7.8µg/mL to wells containing cells separately in triplicates to test its anti-proliferative potentials. Cells in media, as rapidly dividing cell, were included as a control. All cell cultures were incubated at 37 °C/5% CO_2 humid incubator (Memmert, Gmbh) for 72 hours in. The number of cells in each well was estimated using MTT assay according to Ghramh *et al*. (Ghramh *et al.*, 2019).

Study of cytotoxic/proliferative effects of OMLExt

Prepared untreated rat splenic cell suspension was used to test the cytotoxic/proliferative potentials that may be found in OMLExt. The extract was added at different final concentrations (1000, 500, 250, 125 and 62.5, 31.25, 15. and 7.8µg/mL) separately to wells having cells in triplicates. Cells in medium were used as untreated control cells. The increase/decrease in cell count was evaluated as indicated above.

In vivo studies

To test acute cytotoxicity, oxidant/antioxidant and carcinogenic effects which may be found in OMLExt, 10 adult healthy Sprague Dawley rats (200-250g) were injected with a single dose regimen of 500 µg (in 500µL OMLExt) (Oves *et al.*, 2013). Untreated (10 rats) and acetone treated (500 µL, 10 rats) were used as controls. Rats were left for 48 h at 22±2°C, 12 h dark/12 hlight, free access to food/water, and then sacrificed and sera, livers and kidneys (after perfusion with phosphate buffered saline to get rid of red cells) were collected. Rats were kindly supplied by animal house found at King Khalid University. The experiments were approved by the King Khalid Ethical Committee and used the guidelines and the NIH Guide for the Care and Use of Laboratory Animals. Livers and kidneys, separately, were homogenized in ice cold 0.1 M Tris-HCl buffer (pH 7.4, 10% w/v). Each homogenate was centrifuged at 20000 g for 15 min at 4°C (Ogunlana *et al.*, 2018). The supernatant of each sample was harvested and utilized in all experimental parameter measurements.

Functionality of livers was tested by measuring the level of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using colorimetric method (Randox Kit, UK) as described by Reitman and Frankel method (Reitman and Frankel, 1957). Serum creatinine and urea were estimated using Creatinine Abcam Assay kit and Urea Abcam Assay kit (both colorimetric) following the supplied directions in the kit (Medić *et al.*, 2019).

Lipid Peroxidation capacity in liver and kidney homogenate supernatants were measured using Lipid Peroxidation Assay Kit (Colorimetric) following the supplied directions in the kit. The kit is sensitive to detect malondialdehyde (MDA) formed as an end product of lipid peroxidation. The assay was acid treated to precipitate all proteins, so making the majority of the MDA found in the sample free enabling the detection of total MDA.

Total antioxidant capacity in liver and kidney homogenate supernatants were measured using Total Antioxidant Capacity Assay Kit (Colorimetric, Abcam) following the supplied directions in the kit. The experiment was run without masking the proteins (Buico *et al.*, 2009).

Following the supplied directions in the kit (Colorimetric, Abcam), activity of superoxide dismutase found the in liver and kidney homogenate supernatants were measured. Catalase Activity in liver and kidney homogenate supernatants was determined using Catalase Activity Assay Kit (Immunocapture, Abcam) to determine the relative specific activity (activity and quantity) of catalase following the supplied directions in the kit. Catalase enzyme is captured by the wells of the microteter plate. In this work only the quantity of the catalase was measured.

Glutathione (GSH) in liver and kidney homogenate supernatants were estimated utilizing the GSH Assay Kit (Colorimetric, Abcam) following the supplied directions in the kit. The Kit utilizes the enzymatic cycling way in the existence of GSH and a chromophore. The formation of reduced chromophore frames a steady item, which can be identified by kinetic method at 450 nm wavelength.

Arginase Activity in serum was measured using the Arginase Activity Assay Kit (Colorimetric, SIGMA-ALDRICH) following the supplied directions in the k. Sera were filtered through 10 KdDa cutoff filter (Amicon® Ultra-4) as remove urea found in the serum.

α-L-Fucosidase activity in rat sera was estimated using ready to use α-L-Fucosidase assay kit (Colorimetric, DIAZYME). In this experiment, the synthetic chloro p-nitrophenol derivative (R-pNP) is used by α-L-Fucosidase as a substrate to releases a chlorinated pNP derivative. This derivative can be kinetically evaluated under acidic conditions.

STATISTICAL ANALYSIS

Results were expressed as means 6 values ±SEM of the number of experiments. A Student's t-test (GraphPad Prism-Version 7.0 for windows) for values was performed and a p value of 0.05 was considered statistically significant.

RESULTS

Functional groups

Strong broad engendered at 3341.6cm-1 imputed to stretching O-H due to alcohols and phenols. Strong bands emerged at 2924.1 and 2853.3cm-1 is attributed to the stretching C-H bond corresponding to alkane. Weak bands arisen at 2118cm-1 may be consequent to stretching CΞC bond of alkyne. Weak band arisen at 1863.3 cm-1 may be due to bending C-H bond of aromatic compound. Weak bands generated at 1686- 1653.1 -1602.8 -1507.7-1457.4 cm-1 could be resultant to stretching C═C bond of disubstituted alkenes. Strong to weak peaks engendered at 1269.2- 1209.5- 1157.3cm-1 may assign to C-O stretching nascent of alkyl aryl ether. Strong broad peaks emanated

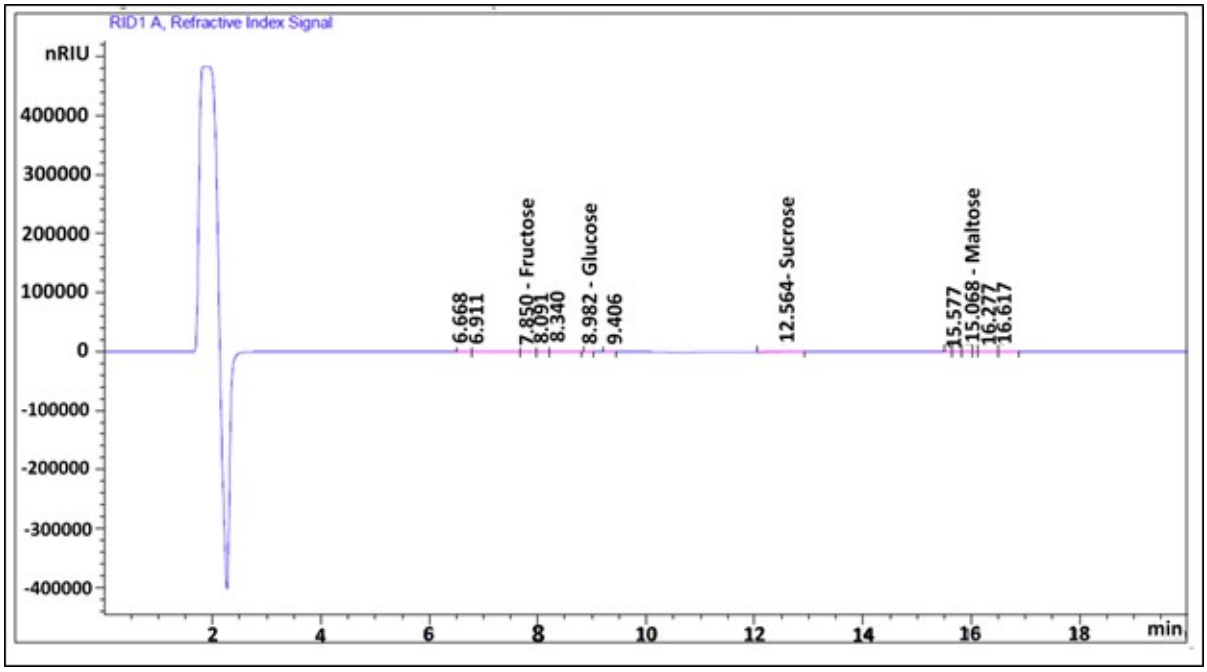

Fig. 1: Representative HPLC chromatograms of the sugars in OMLExt.

Table 1: Percent of splenic cells growth stimulation after treatment with OMLExt

OMLExt (µg/mL)	% of splenic cell growth stimulation	
	Normal	PHA-treated
1000	284±11.21[a]	516±41.38[a]
500	121±9.53[b]	441±17.11[b]
250	114±7.18[b]	354±19.55[c]
125	108±8.85[b]	351±14.14[c]
62.5	114±5.87[b]	333±14.43[c]
31.25	102±8.37[b]	333±16.92[c]
15	90±6.42[b]	318±15.66[c]
7.8	97±2.96[b]	300±13.44[c]

NB: % of splenic cell growth stimulations are expressed as the average of three replicates ± SD. Means with same superscript letters are not significantly different.

at 1067.9- 1032.5 cm-1 may ascribe to C-O imputed to primary alcohols. Strong peak at arisen at 777.1-713.8cm-1 may attributed to the stretching C-H bond of monosubstituted benzene. Strong peaks manifested at 661.6-520cm-1 ascribed to stretching C-Br halo compound.

Sugar and protein content in OMLExt

Results demonstrated that OMLExt contains a very minute amount of fructose, glucose, sucrose and maltose (fig. 1). There were no protein bands detected in the gel after staining.

Reactive oxygen species (ROS) in the plant extract

Quantification of ROS in OMLExt using ELISA indicated that the content of ROS is 134.5pg/mL.

Antimicrobial potential

OMLExt showed no antibacterial potential toward the tested Gram positive/negative pathogenic bacteria used in this study (fig. 2).

Effects on splenic cells proliferation

Anti-proliferative Potentials

OML Ext showed no inhibitory effects on stimulated splenic cells at all concentrations tested (fig. 3, table 1). In contrary, the extract at 500 and 1000 µg/mL significantly (0.0001) increased the cell proliferation of PHA-stimulated splenic cells. There was a slightly significant (0.005) or non-significant cell proliferation stimulatory effect of OMLExt on PHA-stimulated splenic cells at 7.8-250 µg/mL concentrations.

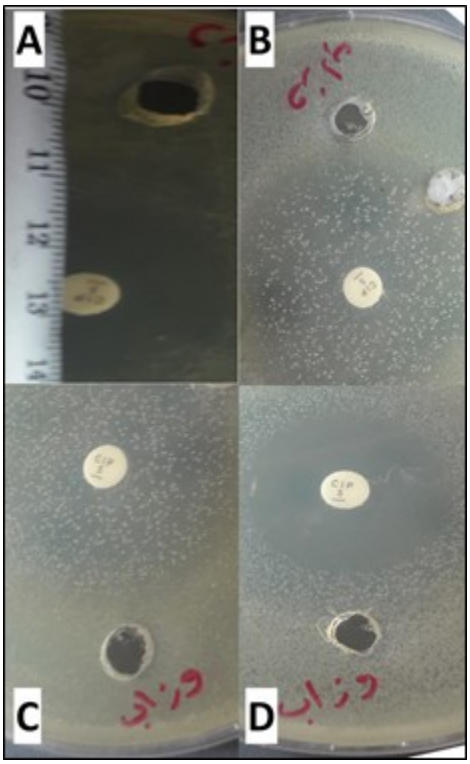

Fig. 2: Antimicrobial activity of OMLExt, where A: *Escherichia coli*; B: *Proteus mirabilis*; C: *Staphylococcus aureus*; D: *Bacillus subtilis* and CIP 5: 5 µg ciprofloxacin.

Study of cytotoxic/proliferative effects

The cytotoxic/stimulatory properties of OMLExt were examined. The outcomes revealed that the extract at 500 and 1000µg/mL significantly (0.0001) increased the cell proliferation of normal splenic cells. There was a non-significant cell proliferation stimulatory effect of OMLExt on normal splenic cells at 7.8-250µg/mL concentrations (fig. 3 table 1).

Liver and kidney functions

The administration of acetone and OMLExt into animals did not result in any significant changes in both serum ALT and AST levels over control untreated group (fig. 4A). Also, serum urea and creatinine levels did not show any significant changes over untreated control after administration acetone and plant extract into the animals (fig. 4B).

Antioxidants activities

The lipid Peroxidation end product, the malondialdehyde (MDA), showed a significant increase (<0.001) in liver tissues when treated with the plant extract treated group over the acetone treated and untreated groups (fig. 5A). In contrary, treatment using both acetone and the plant extract revealed no significant changes over control in kidney tissue (fig. 5A).

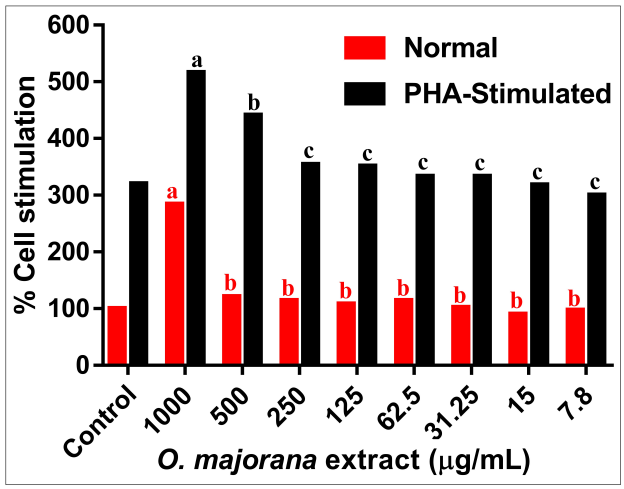

Fig. 3: Effects of OMLExt on normal and PHA-stimulated splenic cells at different concentrations.

NB: % of splenic cell growth stimulations are expressed as the average of three replicates ± SD. Means with same superscript letters are not significantly different.

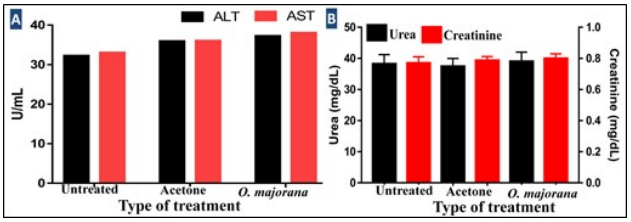

Fig. 4: Levels of AST, ALT, urea and creatinine in different groups.

The level of total antioxidant activity showed no changes among groups in both liver and kidney tissues except a slightly significant increase (<0.01) in liver tissue of OMLExt t treated group over the control untreated group (fig. 5B).

Level of SOD level showed no changes among groups in kidney tissues, but in case of liver tissue, there was a slightly significant increase (<0.01) in liver tissue of OMLExt treated group over the control untreated group (fig. 5C). There SOD content in liver of OMLExt treated group was significantly (<0.001) higher than that of the acetone treated group.

Acetone treatment did not make a change in CAT level in kidney tissue. The level of CAT in renal tissue was significantly (<0.0001) lower than that of untreated and acetone treated groups (fig. 5D). CAT level showed no significant changes in the livers of all groups. In all groups, there were no any significant differences in the levels of GSH in both liver and kidney tissues (fig. 5E).

Serum tumor markers

In all groups, there were no any significant differences in the serum levels of Arginase (fig. 6A). Treatment of

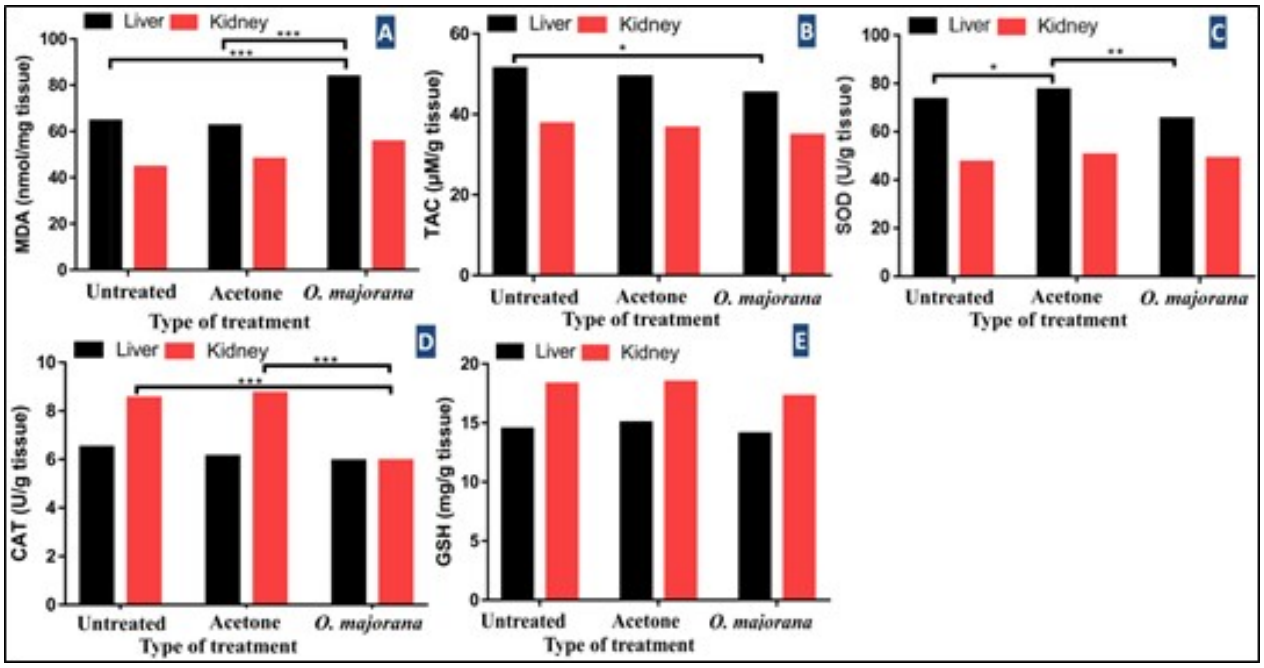

Fig. 5: Content of malondialdehyde (A), total antioxidant capacity (B), superoxide dismutase (C), catalase (D) and reduced glutathione (E) in liver and kidney tissues of different groups. *= p<0.05, **= p<0.01, ***= p<0.001

animal with both acetone and the plant extract did not make a significant change in α-L-Fucosidase serum level. But, there was a slight significant difference (<0.05) between extract and acetone treated groups (fig. 6B).

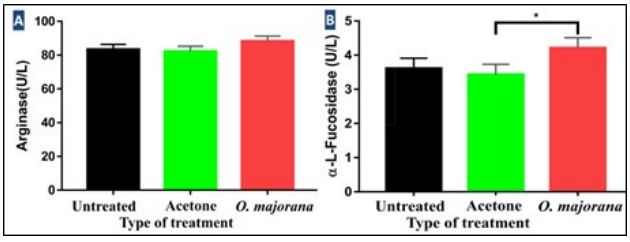

Fig. 6: Levels of serum arginase (A) and serum α-L-Fucosidase (B) in different treated groups. *= p<0.05

DISCUSSION

One of the fundamental sweeping strengths, which assist to preserve human life, is the biochemical reactions that occur within the body cells, specifically in the organelles. Free radicals can be clarified as receptive compound classification gaining a sole unpaired electron in an outside orbit (Riley, 1994). Majority of the free radicals that hurt biological organizations are oxygen-free radicals, and these are all the more ordinarily known as ROS. These are the essential results made in the cells out of oxygen consuming animal, , and can start autocatalytic responses, with the goal that particles to which they respond are themselves changed into free radicals to duplicate the chain of harm. It has been settled that ROS can be both terrible and great in biological systems relying upon the circumstance (Lopaczynski and Zeisel, 2001; Brieger *et al.*, 2012). The dominant ROS made in the aerobic creatures is super oxide that is a hugely receptive and cytotoxic factor. Super oxide is changed to H_2O_2 by a set of enzymes recognized as SOD. H_2O_2, thus, is transformed to H_2O and molecular oxygen by CAT or GP_X. The antioxidant safeguard framework of our body bank on large and various protection mechanisms to preserve ROS made from endogenous or exogenous origin at physiologically optimal levels.

OMLExt was previous shown to have many bioactive molecules (Dhull, Kaur and Purewal, 2016). The present study explored that OMLExt contains many biomolecules as indicated by FTIR analysis, but does not contain sugars or detectable proteins as indicated by HPLC analysis and PAGE. Absence of proteins in gel might be expected that the quantity is below the detectable limit or proteins are precipitated by absolute acetone.

In the present investigation, ROS was measured in OMLExt. A considerable quantity of ROS was obtained in the extract, so this ROS was taken as an exogenous source of oxidant material in the experiment. We did not need to any external materials to induce hepatic and renal injury. Most of the researchers used hepatic or renal toxic materials to induce liver and kidney injury (Mossa *et al.*, 2013, 2015; Refaie, Ramadan and Mossa, 2014; Soliman *et al.*, 2016), but here we did not.

The word “antioxidant” attributes to any substance able to preserve or deactivate free radicals prior they hit cells. Mammals have risen a profoundly entangled antioxidant agent framework (enzymatic and non-enzymatic), which

battle synergistically and in union with one another to safeguard the phones and organ frameworks of the body confronting free extreme harm. The oxidation prevention agents can be inside or gotten exogenously for instance, as a part of an eating regimen or as dietary added substances. Some nutritious exacerbates that don't kill free radicals, yet increase the inner activity may likewise be named cancer prevention agents.

A superb antioxidant ought to be handily consumed and extinguish free radicals, and chelate redox metals at physiologically appropriate levels. It ought to likewise battle in both fluid and/or membrane domains and influence gene expression in a positive method. Internal antioxidants assume an essential job in protecting ideal cell undertakings and henceforth foundational wellbeing and prosperity. Anyway, under conditions, which develop oxidative pressure, inner antioxidants may not be ample and nutrient antioxidants might be compulsory to safeguard ideal cell obligations. Regarding this statement, we examined the natural plant products, *O. majorana* leaves. Acetone extract of the leaves was not studied before, here; we examined the use of OMLExt *in vivo*.

The most effective enzymatic antioxidants include glutathione peroxidase, catalase and superoxide dismutase (Ighodaro and Akinloye, 2018). There are two states of glutathione peroxidase enzyme, one which is selenium-subordinate (GPx) and the other, which is selenium-autonomous (glutathione-S-transferase, GST). It likewise fights with a catalase for hydrogen peroxide as a substrate and is the essential wellspring of protection facing low degree of oxidative stress (Weydert and Cullen, 2010). Gene expression of GP_X is up-directed by H_2O_2 and different ROS. GSH levels in the tissue are chiefly reliant upon the level of biosynthesis and usage in oxidation/reduction responses (Rebrin *et al.*, 2005).

While the plant extract used in this study contains enough quantity of ROS to increase the rate of glutathione peroxidase expression, other antioxidants may help in the reduction of ROS content of the injected plant extract. This lead to the no change in glutathione peroxidase content in both liver and kidney of extract treated group over the untreated control group.

Catalase enzyme is a tetrameric peroxidase that is found in the peroxisome of aerobic cells and changes H_2O_2 to H_2O and atomic oxygen and whose gene expression is governed by H_2O_2. CAT plays a significant job in ROS metabolism and in adjustment to oxidant stress (Baud *et al.*, 2004). In the current study, there was a little reduction in kidney catalase content of extract treated group when compared to untreated control. Previous study (Yoshioka, Fogo and Beckman, 1992) showed that the renal antioxidant enzymes can be down-regulated by specific biological inducers. When antioxidant enzyme power in kidney cortex were thought about between intensely water denied and non-water denied rodents, water denied rodents were found to have catalase and superoxide dismutase power that were diminished to about half of gauge levels. In our study, injection of the plant extract may lead to a diminution in renal catalase.

Superoxide dismutase is one of the very powerful intracellular antioxidant enzymes and it catalyzes the adjustment of superoxide anions to dioxygen and hydrogen peroxide. The ethanol extract of the leaves of marjoram shows antioxidant and free radical scavenging activity where it exhibited a marked effect in DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay (Brand-Williams, Cuvelier and Berset, 1995; Vági *et al.*, 2005; Amarowicz *et al.*, 2009). FTIR results showed the presence of alcohol and phenol in the extract. Ethanol extracts (root and stem) of *O. majorana* exhibited a strong antioxidant action, reducing capability, free radical scavenging task and metal chelating power when correlated to standards such as ascorbic acid, which may be due to the existence of phenols, tannins, triterpenoids and flavonoids (Vági *et al.*, 2005). This may lead to the down-regulation of DOD production in the liver tissue.

Lipid peroxidation is developed as a result of ROS and causes noticeable alterations in the cell membrane. Lipid peroxidation makes an approved mechanism of cellular damage (Al-Rawi, 2011). It is brought into by the assault on a saturated fatty acid or fatty acyl side chain of any chemical species which has satisfactory reacted to prevent a hydrogen atom from a methylene carbon in the side chain. The more noteworthy the number of double bonds in a fatty acid side chain, accessible will be the elimination of the hydrogen atom which is why fatty acids are more sensible to peroxidation (Halliwell *et al.*, 1993; Rahman, 2007). ROS was found in the plant extract, this may be the cause of elevating the content of the lipid Peroxidation end product (MDA) in hepatic tissue.

In our examination, there was no cytotoxic impact of OMLExt on both normal and PHA-stimulated cells. Some researchers showed that *O. majorana* extract is not toxic to normal cell line (Hajlaoui *et al.*, 2016; Waller *et al.*, 2017). Others showed non-significant decrease in cell viability at high extract concentration (Rao, Timsina and Nadumane, 2014). Many reports demonstrated a cytotoxic impact of the *O. majorana* extract, this may be the effect of solvent used or the effect of the solvent itself on cell viability.

The extract showed very weak or no antibacterial activity against selected bacteria. Other publications showed *O. majorana* methanolic and oil extracts have antibacterial activities (Leeja and Thoppil, 2007; Omara, Abd El-Moez and Mohamed, 2014; Pepa *et al.*, 2019). These differences between our results and others results may be due solvent used in preparation.

CONCLUSION

The acetone extract of *O. majorana* leaves was used as a source of ROS and as antioxidant in the same time. OMLExt was found to be safe to both liver and kidney as indicated by levels of AST, ALT, urea and creatinine. The extract does not contain any carcinogenic substances as it did not make any changes in Arginase and α-L-Fucosidase levels. Acetone extract contained ROS, this and other components found in the extract made the liver and kidney to manage the oxidative stress to maintain the integrity of themselves. The extract has no cytotoxic activities. The use of OMLExt is useful in protecting normal vital organs from oxidative stress.

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