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Original Article

Antimicrobial and toxicological evaluation of *Origanum vulgare*: an *in vivo* study

Avaliação antimicrobiana e toxicológica de *Origanum vulgare*: um estudo in vivo

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Abstract

Origanum vulgare has been of great interest in academia and pharma industry due to its antioxidant, antifungal and antitumor properties. The present study aimed to find the anti-MRSA potential and *in vivo* toxicity assessments of *O. vulgare*. *O. vulgare* extract was used to monitor anti-MRSA activity in mice. Following MRSA established infection in mice (*Mus musculus*), treatment with *O. vulgare* was continued for 7 days. Autopsies were performed and re-isolation, gross lesion scoring and bacterial load in various organs were measured. Additionally, blood sample was analysed for hematological assays. Toxicity assessment of *O. vulgare* potential as medicine was done at 200 mg/kg and 400 mg/kg by evaluating liver and kidney functions. Bacterial load and gross lesion in lungs and heart were significantly low compared to positive control following *O. vulgare* treatment. Likewise, *O. vulgare* treated groups had hematological, neutrophil and TLC values similar to control groups. Increased AST, ALP and total bilirubin alongwith marked hepatocellular degeneration and distortion around the central vein, inflammatory cell infiltration, and cytoplasmic vacuolization of hepatic cells was observed at higher dose. It is concluded that crude extract of *O. vulgare* may contain beneficial secondary metabolites and in future may be explored for curing infectious diseases.

Keywords: *O. vulgare*, MRSA, crude extract, histology, *In vivo* study, liver, kidney.

Resumo

Origanum vulgare tem despertado grande interesse na academia e na indústria farmacêutica devido às suas propriedades antioxidantes, antifúngicas e antitumorais. O presente estudo teve como objetivo encontrar o potencial anti-MRSA e avaliações de toxicidade in vivo de *O. vulgare*. O extrato de *O. vulgare* foi usado para monitorar a atividade anti-MRSA em camundongos. Após infecção estabelecida por MRSA em camundongos (*Mus musculus*), o tratamento com *O. vulgare* foi continuado por 7 dias. As autópsias foram realizadas e o reisolamento, pontuação das lesões grosseiras e carga bacteriana em vários órgãos foram medidos. Além disso, a amostra de sangue foi analisada para ensaios hematológicos. A avaliação da toxicidade do potencial de *O. vulgare* como medicamento foi feita com 200 mg / kg e 400 mg / kg, avaliando as funções hepática e renal. A carga bacteriana e as lesões graves nos pulmões e no coração foram significativamente baixas em comparação com o controle positivo após o tratamento com *O. vulgare*. Da mesma forma, os grupos tratados com *O. vulgare* apresentaram valores hematológicos, de neutrófilos e de TLC semelhantes aos grupos de controle. Aumento de AST, ALP e bilirrubina total juntamente com degeneração hepatocelular marcada e distorção ao redor da veia central, infiltração de células inflamatórias e vacuolização citoplasmática de células hepáticas foram observados em doses mais altas. Conclui-se que o extrato bruto de *O. vulgare* pode conter metabólitos secundários benéficos e, no futuro, pode ser explorado para a cura de doenças infecciosas.

Palavras-chave: *O. vulgare*, MRSA, extrato bruto, histologia, estudo in vivo, fígado, rim.

1. Introduction

Nowadays, the vegetative parts and biochemical extracts of many plants including *Origanum vulgare* are in use in the food and spice industries. *O. vulgare* is an aromatic plant distributed throughout Asia including Pakistan for cure of respiratory diseases, hypoglycemic disease, and leukemia. It exhibits multiple biological activities such as insecticides, expectorant, antimicrobial, antifungal, anti-inflammatory and anti-oxidant. The majority of its components include rosmarinic acid, eriocitrin, luteolin-7-oglucoside, apigenin-7-*O*-glucoside, origanol A and B, and ursolic acid (Shokrzadeh et al., 2014). Rosmarinic acid and origanol A and B, found abundantly in aqueous extract of oregano,

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have antioxidative activities (Veenstra and Johnson, 2019). Previous studies have reported that components such as thymol, carvacrol, δ-terpinene, and *p*-cymene of *O. vulgare* have antioxidant capacity (Shokrzadeh et al., 2014). Also, there are other reports showing that components including ursolic acid and rosmarinic acid of the aqueous oregano extract exhibit potent antioxidant activities by scavenging free radicals (Baranauskaite et al., 2017).

Previously, oregano was reported to have many uses as food preservative of animal origin, controller of microorganisms and endoparasites and as a growth promoter in farm animals. In community, there is a common belief that herbs are safer natural products and have been used since long in ancient times. The leaves of oregano have been used as a condiment herb for flavoring fish, meat, vegetables, salad dressing, and wine since approximately the seventh century BC, however it is also true that herbs re not completely safe (González and Marioli, 2010) and have wide effects depending upon growth region. *In vitro* study has reported both synergistic and antagonistic effects of these substances found in oregano. Due to their allelopathic effects, they can be a potentially used as bio-herbicide rather than chemicals. Multipurpose use of these compounds has developed our interest in doing research on this important plant (Dhifi et al., 2016).

Recently, researchers have become more interested in potential antimicrobial effects of plants that are capable of minimizing the adverse effects of antibiotics and different medicines. Previously, we have described *in vitro* anti-MRSA potential of 29 herbs (Mehreen et al., 2016). Since *O. vulgare* was found to be very effective in controlling MRSA at low MIC, focus of this study was to validate the *in vivo* efficacy of the anti-MRSA potential of this particular plant. Furthermore, to confirm *O. vulgare* safety and rule out its toxic potential in *in vivo* system, experiments related to evaluation of toxic potential of the methanolic extract of *O. vulgare* in mice at two different concentrations (200 mg/kg and 400 mg/kg) equivalent to 1x and 2x of recommended human dose were performed to evaluate their effect on liver and kidney functions.

2. Materials and methods

2.1. Plant extract and MIC determination against S. aureus strains

O. vulgare extract preparation and MIC determination against *S. aureus* strains (KY698020) were performed by method described by Mehreen et al. (2016). Briefly, Plant extract were prepared in absolute methanol (10 g/100 ml), followed by sonication, drying and storage at 4°C. Final suspension was made in ethanol before administering to *Mus musculus* (200 mg/ml per kg of body weight).

Staphylococcus aureus strain was isolated from sore throat patients, characterized and kept at −80°C. App $10^{7\text{-}11}$ cfu/ml of inoculum was used to inoculate 03 mice (*Mus musculus*) groups. MIC of the alkaloids and flavonoids ranged from 3.25 to 12.5 (µg/mL) and zone of inhibition ranged from 13 - 18 mm.

2.2. Animals

Experiments were performed in compliance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and approved by the Ethical Committee for Research on Animals (ECRA) of the University of the Punjab, Lahore, Pakistan. Hundred *S. aureus* free mice (*Mus musculus;* 8 weeks old; weighing 25-30 g) were obtained from Department of Zoology, animal house and kept in isolated common metallic cages of size (14"× 10"× 7") under controlled conditions with temperature at 30° C with 12 hours variation of light and dark period. Animals used were from both genders and acclimatized for one week prior to the experiment. Water and feed were provided *ad libitum*. Three mice were randomly selected, sacrificed and using impression technique, presence of *S. aureus* in throat, lungs, heart, joints and blood was monitored on Mannitol Salt Agar (MSA) supplemented with 2.08mg/ml vancomycin.

2.3. Animal grouping, MRSA infection and O. vulgare treatment

In main study, animals were divided into four groups based on positive, negative and antibiotic control, and *O. vulgare* treated respectively. All groups except negative control were inoculated using MRSA strain through intra-tracheal route with the most effective selected dose. Treatment with a dose of 200mg/kg of *O. vulgare* extract was started on 3rd dpi after confirmation of infection. On 8th day animals were sacrificed and blood sample was analysed for hematological assays. Autopsies were performed and gross lesion score were recorded on lung and heart. In addition, bacterial load in throat, lungs, heart, blood and joints was also determined.

2.4. Toxicity evaluation of O. vulgare extract

Next part of this study was to work on experiments related to evaluation of toxic potential of the methanolic extract of *O. vulgare*. Two different concentrations (200 mg/kg and 400 mg/kg) equivalent to 1x and 2x of recommended human dose were used to evaluate their effect on liver and kidney functions. Extracts were forcibly fed to each group up to 7 days consecutively and on 8th day animals were sacrificed and blood serum were analyzed for LFT and RFT. In addition, a part of liver and kidney were processed for histological examinations.

2.5. Statistical analysis

The data were analyzed using one-way ANOVA followed by Duncan's multiple range tests. All analyses were performed in SPSS software for window version 16.0 (SPSS). Probability values ≤ 0.05 were considered significant.

3. Results

The dose 10^7 CFU/ml was considered for the experimental study due to observation of less mortality and re-isolation from all the organs. The effect of treatment with plants extract in mice is presented in Table 1. Body weight of the

Table 1. Effects of *O. vulgaris* extract treatment on clinical outcome of infected animals at 8 days post infection.

Parameters	Infected group (n=6)	Negative control	Antibiotic-treated	*O. vulgaris*
Mortality	1/6	0/6	0/6	0/6
Body weight	23.36±0.65	26.83±0.29	25.18±1.05	25.93±0.95
Re-isolation at day 8 post infection				
Throat	6/6	0/6	3/6	6/6
Lung	6/6	0/6	3/6	5/6
Heart	6/6	0/6	0/6	0/6
Joint	6/6	0/6	0/6	0/6
Blood	6/6	0/6	0/6	0/6

animals at the end of the experiment was significantly less in infection control group however, in the treated groups there was no difference in the final body weight of the animals. In positive control group mortality was 16% (n=1/6) and 0% in *O. vulgare* extract treated group only. Re-isolation frequency from the throat, heart and lungs from plant treated group was similar to that of positive control. However, in antibiotic treated group it was recovered only from 3/6 animals. Inoculated strain could not be isolated from heart of any treated group while re-isolation from joints remained negative in all groups treated with plant extracts, as far as reisolation from blood was concerned, the inocculated strain could not be recovered from any treated group except for positive control only

3.1. Gross lesion scoring

Gross lesion scoring of the heart and lungs of treated groups was significantly lower in *O. vulgare* extract treated group. The comparison of lesion score among different treatments is given in following Table 2.

3.2. Estimation bacterial load from different organs

Bacterial load from different organs were count in term of CFU (log_{10})/g of tissue or CFU (log_{10})/ml of blood. In the throat of the positive control it was 4.88 ± 0.20 CFU (log_{10})/g while significantly reduced [3.18±0.65 (log_{10})/g] bacterial load was observed in *O. vulgare* treated group, almost similar to vancomycin-treated group [2.50±1.02 (log_{10})/g] (Table 3) .

In the lung of the positive control, bacterial load was 5.36 ± 0.58 CFU (log_{10})/g with significantly low value [3.19±0.60(log_{10})/g] in *O. vulgare* almost similar to that of vancomycin-treated group [2.50± 1.02 (log_{10})/g] (Table 3).

The heart, joint and blood of untreated groups were found to contain 4.52 ± 0.09, 3.89 ± 0.23 (CFU (log_{10})/g) and 4.11 ± 0.11 CFU (log_{10})/ml respectively. While vancomycin and *O. vulgare* treatments succeeded in removing strain completely in given tissues (Table 3).

3.3. Toxicity assessment of O. vulgare

All treatment groups (A-D) had apparently healthy animals without any sign of toxicity, in terms of their behavior, feed consumption and activity during the treatment period. Analysis of serum sample for liver and kidney, revealed the slightly different picture. AST level in group treated with high dose of *O. vulgare* was significantly higher from the control group (Table 4).

Table 2. Comparison of Gross lesion scores in lungs and heart following treatment of infection with *O. vulgaris* extract.

Treated groups	Positive control	Antibiotic-treated	Negative control
Antibiotic treated	0.034, 0.43		
Negative control	0.034, 0.034	1.00, 0.317	
O. vulgaris	0.005, 0.005	1.00, 1.57	1.00, 1.00

ALT and ALP levels remained unaltered in animals which were treated with *O. vulgare*, while reduction in creatinine and urea was observed. Bilirubin level was significantly high in *O. vulgare* treated group compared to control (Table 5).

3.4. Haematological assessment

Comparison of erythrocytes sedimentation rate (ESR), hemoglobin level (Hb), red blood cells count (RBC), platelets and packed cell volume (PCV) of all experimental groups is presented in Table 5. ESR value of positive control differ significantly *O. vulgare* and vancomycin-treated group. *O. vulgare* and vancomycin-treated group had significantly lower ESR value from positive control and could not become closer to then negative control group (Table 5).

Hb value for positive control was significantly ($P < 0.05$) lower from the *O. vulgare* treated group which were similar to negative control and vancomycin-treated group. Erythrocyte count value for positive control was significantly lower ($P < 0.05$) from the all treated groups. *O. vulgare* treated group had erythrocyte count similar to that of negative control group. The difference in treated and positive control was significant (Table 5). Platelet count of positive control group were lower significantly ($P<0.05$) from all treated group. Vancomycin and *O. vulgare* platelet count were comparable to negative control. Packed cell volume (PCV) for positive control was significantly lower ($P < 0.05$) from all other group, While variation among treated and negative control were not significant.

Comparison of total and differential leukocyte count of group I-IV is given in Table 5. Total leucocyte count

(TLC) of positive control was higher than all other groups. *O. vulgare* treated group showed TLC similar to that of the negative control. Neutrophil count of vancomycin and *O. vulgare* treated group was similar but significantly lower than positive control (Table 5). Vancomycin and *O. vulgare* treated group show lymphocyte count equivalent to negative control. Monocytes were higher in positive control followed by almost equal in negative control and vancomycin treated group, however significantly lower count was observed in *O. vulgare* treated group. Eosinophil was higher in positive control and almost similar vancomycin and *O. vulgare* treated groups.

3.5. Histological examination of liver and kidney

3.5.1. Control group

Histology of liver tissue of the control mice showed normal portal triad, interlobular bile duct, radiating hepatic cells and sinusoids lined by endothelial cells (Figure 1A). Light microscopic examination of kidney of control mice display the Bowman's capsule, lined by outer squamous capsular cells and inner podocyte cellular layer. Bowman's space, vascular pole, proximal and distal convoluted tubules all was normal (Figure 1D).

3.5.2. Origanum vulgare (200mg/kg)

The animals treated with crude methanolic extract *O. vulgare* a dose of 200 mg/kg, the hepatic parenchyma showed normal appearance. Hepatocytes were aligned in cords with normal sinusoidal spaces while nuclei of hepatocytes are normal having nucleolus and chromatin material. It was similar to that of control group (Figure 1B).

The renal parenchyma of this group was also normal in appearances *i.e.,* Bowman's capsule was lined by outer squamous capsular cells and inner podocyte cellular layer. Bowman's space, vascular pole, proximal and distal convoluted tubules all was normal (Figure 1E).

Table 3. Effect of treatment with *O. vulgaris* on bacterial load of different tissues.

Plants treated	Organs				
	Throat	Lungs	Heart	Joints	Blood
Positive control	4.88± 0.20^{a}	5.36±0.58^{a}	4.52±0.09 ^{a}	3.89±0.23 ^{a}	4.11±0.11 ^{a}
Vancomycin	2.50±1.02^{b}	2.50± 1.02^{b}	0.00±0.00 ^{b}	0.00±0.00 ^{b}	0.00±0.00^{b}
O. vulgaris	3.18±0.65^{ab}	3.19±0.60^{ab}	0.00±0.00 ^{b}	0.00±0.00 ^{b}	0.00±0.00 ^{b}

Analysis was performed using one-way ANOVA followed by DMRT. Values in a column having no common letter in superscript are significantly different from each other. Bacterial load is represented as Mean ± SE of CFU (log_{10})/g of tissue or CFU(log_{10})/ml of the blood.

Table 4. Effect of different doses of *O. vulgaris* on liver and renal function markers after 7 days treatment.

	AST	ALT	ALP	Bilirubin	Urea	Creatinine
Control	51.65 ± 2.34^{b}	45.83 ± 2.23^{b}	96.8 ±5.25	0.43 ± 0.03^{b}	44.36 ± 3.30^{a}	1.2 ± 0.23^{a}
O. vulgaris (200mg/kg)	49.4 ± 3.24^{b}	29.08 ± 7.13^{c}	111.14 ± 20.23	1.52 ± 0.23^{a}	36.88 ± 1.21^{b}	0.64 ± 0.17^{b}
O. vulgaris (400mg/kg)	62.82 ± 5.30^{a}	38 ± 3.21^{a}	103.5 ± 2.27	1.9 ± 0.15^{a}	38.15 ± 1.30^{b}	0.9 ± 0.15^{ab}

Table 5. Effect of *O. vulgaris* extract on hematological parameters of mice at 8th -day post infection.

Parameter	Positive control	Negative control	Vancomycin	*O. vulgare*
Hb (g/dL)	10.10 ± 1.25 ^{b}	14.33 ± 0.33 ^{a}	14.67 ± 0.33 ^{a}	14.17 ± 0.40 ^{a}
RBC(x 10^{6}/mm^{3})	6.19 ± 0.80^{c}	7.34 ± 0.33^{b}	8.79 ± 0.30^{a}	8.67 ± 0.00^{a}
Platelets(x 1000)	129.33 ± 3.48^{b}	380.00 ± 22.91^{a}	385 ± 7.63^{a}	372.50 ± 12.69^{a}
PCV (%)	33.53 ± 4.61^{b}	40.00^{a}±0.00^{a}	41^{a}±0.00^{a}	40.00 ± 0.25^{a}
ESR (mm/hr)	32.00 ± 1.73^{a}	6.50 ± 0.33^{b}	3.67 ± 0.33^{c}	6.67 ± 1.28^{b}
TLC (x 1000)	9.0 ± 0.23^{a}	5.63 ± 0.02^{c}	6.10 ± 0.02^{b}	5.83 ± 0.06^{bc}
Neutrophil (%)	21.00 ± 2.08^{a}	10.67 ± 0.42^{b}	12.33 ± 1.45^{b}	12.33 ± 1.45^{b}
Lymphocyte (%)	86.33 ± 5.20^{a}	71.17 ± 2.55^{b}	72.22 ± 1.20^{b}	72.39 ± 2.33^{b}
Monocyte (%)	2.00 ± 0.00	1.33 ± 0.33	1.33 ± 0.33	1.00 ± 0.00
Eosinophil (%)	2.33 ± 0.33	1.2 ± 0.2	1.33 ± 0.33	1.17 ± 0.16

The analysis was performed using one-way ANOVA followed by DMRT. Values in a column having no common letter in superscript are significantly different from each other. Data were presented in term of mean ± SE, the value of $P < 0.05$ consider significant.

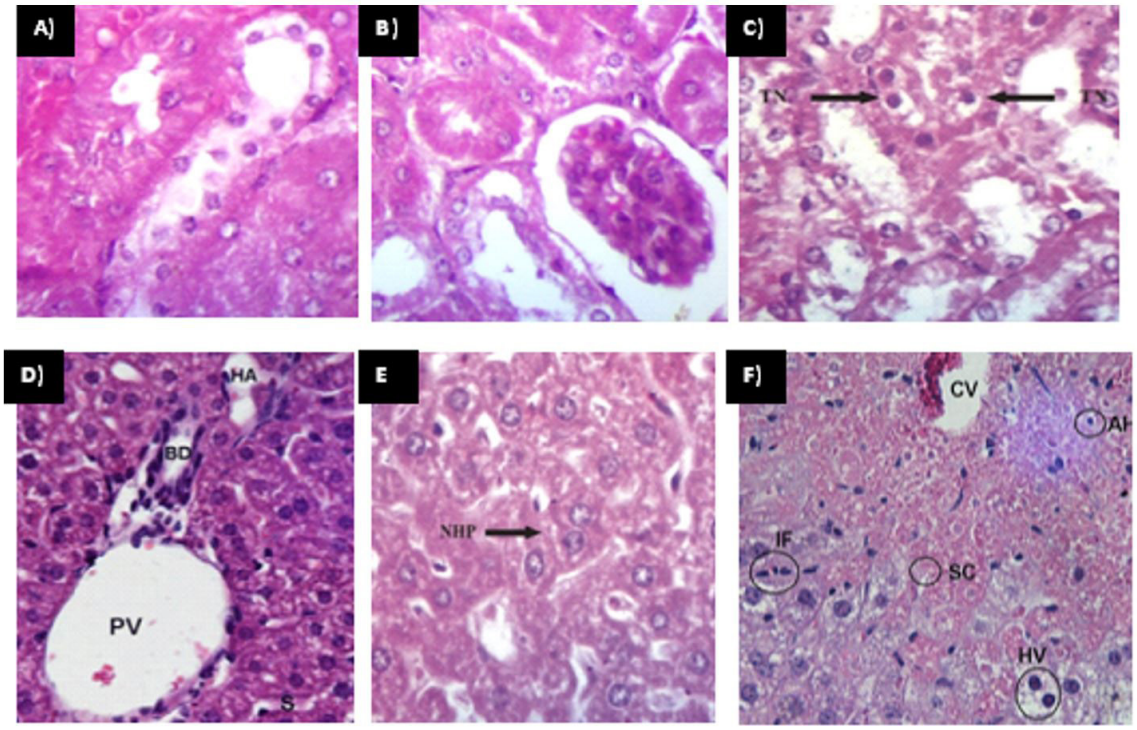

Figure 1. Histological study of kidney and Liver (A) Photomicrograph of kidneys from control group showing normal renal parenchyma (NRP) (H&E Staining 400X); (B) Photomicrograph of kidneys from *O. vulgare* 200mg/ml treated group showing normal renal parenchyma (H&E Staining 400X); (C) Photomicrograph of kidneys from *O. Vulgare* 400mg/ml treated group showing tubular necrosis (TN) (H&E Staining 400X); (D) Photomicrograph of Liver from control group showing normal hepatic parenchyma, consisting of cells that are large and polyhedral, with round nuclei and abundant heterochromatin and nucleoli. A typical portal triad displays a portal vein (PV), bile duct (BD), and hepatic artery (HA). Capillary sinusoids (S) are of normal caliber and there is no evidence of congestion (H&E Staining 400X); (E) Photomicrograph of Liver from O. *vulgare* (200mg/ml) showing almost normal hepatic parenchyma (NHP) (H&E Staining 400X); (F) Photomicrograph of Liver from O. *vulgare* (400mg/ml) showing marked hepatocellular degeneration and distortion around the central vein (CV), Inflammatory cell infiltration (IF), and cytoplasmic vacuolization (HV) of hepatic cells. Sinusoidal congestion (SC) was also apparent. Apoptotic hepatocytes (AH) were also observed in areas of centrilobular necrosisvacuolar (H&E Staining 400X).

3.5.3. Origanum vulgare (400mg/kg)

Animals treated with high dose of *O. vulgare* (400 mg/kg) showing marked hepatocellular degeneration and distortion around the central vein (CV), Inflammatory cell infiltration (IF), and cytoplasmic vacuolization (HV) of hepatic cells. Sinusoidal congestion (SC) was also notice. Apoptotic hepatocytes (AH) were also observed (Figure 1C).

The renal parenchyma of this group indicated lower degree of pycnotic changes indicated by mild tubular epithelial cell necrosis and even at some places urinary spaces of the glomeruli slightly increased (Figure 1F).

4. Discussion

O. vulgare is a popular herb having leaves that can enhance the flavor of food and widely spread in Europe and Asia. The species is also used in traditional and modern medicine and in pharmaceutical industry. Very little data exists on cytototoxicity and antimicrobial activities of this plant (Grbović et al., 2013). Previously we reported the *in vitro* antimicrobial activity of 29 herbs including *O. vulgare* (Mehreen et al., 2016). The current study was planned to investigate the *in vivo* activity of *in vitro* finding of methanolic extract of *O. vulgare* (in a concentration of 200mg/kg), followed by toxicity assessments.

Regarding first objective, *in vivo* study cannot be achieved without having an infection model. For this purpose, mice were exposed to freshly cultured MRSA for 7 days, morbidity and mortality was recorded. All animals were sacrificed and gross lesion on lungs and heart were noted on 8th day. Additionally, bacterial re-isolation from different organs such as throat, heart, lungs, and joints was recorded. Animals treated with *O. vulgare* extracts showed reduction in gross lesion scores. Gross lesion develops in tissues due to bacterial virulence. MRSA release toxins which lead to hemolysis in RBC, its adhesion in walls of tissue and resistance to phagocytosis (Shumba et al., 2019). Reduction in gross lesion score of lungs indicated some role of this extract in controlling MRSA infection in i*n vivo* system.

Re-isolation from throat, lungs, heart, blood and joint was performed as an indicator of persistency of infection. Bacteria could not be re-isolated from the heart, joint and blood samples of antibiotic and *O. vulgare* treated group indicating that these treatments help in controlling spread

of infection to other organs. The reduction in bacterial load may be due to bactericidal/bacteriostatic effects of phytochemicals present in crude extracts. In a study by Coccimiglio et al. (2016), gas chromatography/mass spectroscopy analysis of *O. vulgare* extract showed presence of monoterpene hydrocarbons and phenolic compounds (carvacrol and thymol abundantly) and less commonly 1-octacosanol, p-cymene, creosol and phytol. Possible antibacterial activity of *O. vulgare* extract might be due to active constituents (carvacrol and thymol) that interference with the pH gradient and membrane permeability of bacteria as suggested by Lambert et al. (2001). Burt et al. (2007) also suggested that carvacrol may also be involved in inhibiting flagellin of *E. coli*. Similarly, antibacterial activities of the main chemical constituents of oregano and other spices (For example thymol, cinnamaldehyde, eugenol, and carvacrol) were studied in separate and combined form. Authors reported that components contain antibacterial activities individually and in synergism (Lambert et al., 2001; Pei et al., 2009). However, the exact mechanism by which oregano extract and its components exhibit antimicrobial effect against various bacteria need further investigation All these data are in consistent with *in vitro* findings. *O. vulgare* has variable efficacy and seems to be good herbal medicine. Our data support findings of Chang et al. (2014) who reported a reduction in bacterial load in vital organs of mice after treatment with *Coptidis rhizome*. Likewise, Nath and Joshi, 2017 demonstrated the decrease in microbial load after application of inhibitory effect of endophytic fungi from tropical ethnoveterinary plants.

Among haematalogical parameters, ESR value in vancomycin-treated and *O. vulgare* treated group were lowered than the positive control group indicating their positive role in fighting the infection. Increased ESR is an indication of inflammation, anemia, autoimmune disorders, infections and kidney diseases. As the body return to normal condition value of ESR drops to normal range. Other findings include reduced Hb, RBC, platelets and PCV as outcome of *S. aureus* infection. *S. aureus* uses the Hb as the sole iron and its adherence with RBC. *O. vulgare* treated groups had hematological indicators value similar to that of the vancomycin treated and negative control group. These finding further confirms their effectiveness in controlling the infection.

The WBCs and neutrophil count are used as an indicator of infection while rise in basophil is usually associated with allergic response (Chapel et al., 2013). Neutrophil and TLC value rise during bacterial infections and drops to normal after recovery. In this study, during bacterial infection the values of neutrophils increased compared with control. Comparison of these values among all plant extract treated groups revealed that *O. vulgare* treatment positively influenced all indicators of infections. It is quite evident from our data that *O. vulgare* is helpful in controlling infections and is very best in controlling MRSA infections.

Considering the *O. vulgare* as an alternative medicine having potential therapeutic value, its effectiveness in *in vivo* system require to validate its safety and rule out any toxicity in animals. Appropriate animal models are mostly used to estimate presumptive health risks of any herbal medicine in humans. This is performed by questioning the probability of exposure to that particular treatment at recommended concentrations or doses.

Next aim of study was therefore to evaluate the toxic potential of the methanolic extract of *O. vulgare*. Two different concentrations (200 mg/kg and 400 mg/kg) equivalent to 1x and 2x of recommended human dose were used to monitor its efficacy on liver and kidney functions. Extracts were forcibly fed to each group up to 7 days consecutively and on 8th day animals were sacrificed and blood serum were analyzed for LFT and RFT. Additionally, a part of liver and kidney were examined histologically.

Total bilirubin level in *O. vulgare* treated group was higher than the control. AST level was higher while ALT level was lower in animals exposed to 400 mg/kg of *O. vulgare*. Raised level of total bilirubin indicate destruction of RBC some metabolite in crude extracts may be responsible for this outcome. AST and ALT are considered two of the most important tests to detect liver injury. Total bilirubin, ALT, ALP and AST are found within the hepatocytes and even in different tissues and organs. These are released into the blood due to cellular necrosis and variation in cell membrane permeability (Contreras-Zentella and Hernández-Muñoz, 2016). The rise in the level of AST was of clinical and toxicological importance as it indicates that plant extracts at higher dose have adverse effect on liver function. An increase in these enzyme levels reflects active liver damage. These observations were further supported by the histological examination of the liver where marked hepatocellular degeneration and distortion around the central vein, inflammatory cell infiltration, cytoplasmic vacuolization of hepatic cells, sinusoidal congestion and apoptotic hepatocytes in areas of centrilobular necrosisvacuolar were observed receiving a high dose of *O. vulgare*. Methanolic extract of *O. vulgare* did not influence renal functions. Urea and creatinine level did not cross above the control value and remained in low range (Sadrefozalayi and Farokhi, 2014).

From all the above finding it can be said that the high dose of *O. vulgare* adversely affects the liver function and low dose had no adverse effect. Increased level of total bilirubin both in low and high dose may be due to several phytochemical presents with in the extract. Saponins presence has already been reported to cause the hemolysis of RBC. As this study was conducted on crude extract we may reach to the conclusion that it may contain some harmful secondary metabolites along with beneficial and in future only pure compound having antibacterial activity must be used. To best of our knowledge and effort work done on *in vitro* and *in vivo* setting on *O. vulgare* have been summarized in Table 6.

There is no sufficient scientific evidence to state that *O. vulgare* can be harmful to human. *O. vulgare* also prove to be non-toxic at low level and at this high dose only a mild toxicity was observed. However, use of e bioactive compounds other than crude extract may reduce its toxic effect.

Table 6. *In vitro* and *In vivo* work carried out on the extract of *O. vulgare.*

Plant name	*In vitro*	References	*In vivo*	References
Origanum vulgare	Anti-oxidant	Barros et al. (2010)	Anti diabetic	Vujicic et al. (2015)
	Anti-bacterial	Chaudhry et al. (2007)	Anti inflammatory	Vujicic et al. (2015)
	Anti cancer	Paur et al. (2010)	Analgesic effect	Afarineshe Khaki et al. (2013)
			Antinociceptive	Rukh et al. (2016)

5. Conclusion

This study demonstrates the *in vivo* efficacy of methanolic extract of *O. vulgare*. This perennial herbaceous plant is quite bioactive having good antibacterial potential against the sore throat isolated MRSA with mild toxicity. Gas chromatography/mass spectroscopy analysis of *O. vulgare* extract showed presence of monoterpene hydrocarbons and phenolic compounds (carvacrol and thymol abundantly) and less commonly 1-octacosanol, p-cymene, creosol and phytol (Coccimiglio et al., 2016). Possible antibacterial activity of *O. vulgare* extract might be due to active constituents (carvacrol and thymol) that interference with the pH gradient and membrane permeability of bacteria as suggested by Lambert et al. (2001). Burt et al. (2007) also suggested that carvacrol may also be involved in inhibiting flagellin of *E. coli*. Similarly, antibacterial activities of the main chemical constituents of oregano and other spices (For example thymol, cinnamaldehyde, eugenol, and carvacrol) were studied in separate and combined form. Authors reported that components contain antibacterial activities individually and in synergism (Lambert et al., 2001; Pei et al., 2009). Our previous study also proved that it contains phenolic, alkaloids and flavonoid compounds (Mehreen et al., 2016), which may contribute to its antibacterial potential. However, the exact mechanism by which oregano extract and its individual components exhibit antimicrobial effect against various bacteria needs further investigation, since these are complex and inhibit microorganisms through various targets. This study proves that its use is not safe at all doses. In particular, crude extraction may not be safe for the continuous use because such extract may contain undesirable components causing liver and renal function impairment as observed in this study. Our study is a preliminary study, Further research is needed to validate the novel antibacterial bioactive molecules and effectiveness of effective pharmacological agents of *O. vulgare* extract studied here, through additional toxicity for safe use in humans.

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