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The Impact of *Origanum Vulgare* Leaves Aqueous Extract on The Physiological and Antioxidant Factors in Adult Albino Female Mice

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Abstract:

This research determined the biochemical impact of aqueous leaf extract of *Origanum vulgare* on the female albino mice. There were twelve mice who were randomly assigned control and treated groups (n=6). The extract was given orally at 100 mg/kg to the treated group in the course of four weeks. Serum samples were examined on the antioxidant status, metabolic markers, and liver functioning. A significant change toward reduced glutathione (GSH) levels (7.76 ± 0.49 mmol/L) and a decrease in malondialdehyde (MDA) levels (0.93 ± 0.06 mmol/L) was also found relative to the controls, which suggested an improved antioxidant defense and a decrease in lipid peroxidation. The level of vitamin C declined considerably, whereas the amount of vitamin E did not change significantly. Metabolic profiles showed that serum urea (53.66 ± 2.45 mg/dL) and blood glucose (80.33 ± 2.15 mg/dL) were significantly lower, which was an indication that there was better metabolism of nitrogen and possible hypoglycemia. There was no significant difference in creatinine levels which implies intact renal functioning. The liver function tests showed no improvement in the ALP, ALT, total protein, albumin or globulin. But there was a significant increase in the levels of aspartate aminotransferase (AST) in the treated group. These results indicate that *O. vulgare* extract has got strong antioxidant and hypoglycemic effects. The high levels of AST, however, should be investigated further in terms of hepatic metabolic activity. In general, the extract has therapeutic potential in spite of the slight enzymatic changes.

Keywords: *Origanum vulgare* leaves, *Antioxidants* parameters, Liver Enzymes, Kidney Functions.

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1. Introduction

Origanum vulgare is a common fragrant and medicinal herb, which is a useful plant in the culinary and medical industries and is a member of a family known as the Laminaceae. It is widespread in Africa, Asia, and Europe and may occur naturally in many different ecological contexts on several continents (U.S. National Plant Germplasm System Origanum vulgare). It is naturally found in the Mediterranean region and has been used traditionally mostly in its dried form. On the basis of phytogeographical and ethnobotanical evidence, Southern Eurasia is probably the place where *Origanum vulgare* was first introduced, where it was first domesticated and grown. This enabled the plant to propagate all over Europe and its surrounding nations. The genus *Origanum L* has approximately 39 species with a high center of diversity in the Mediterranean basin, which is characterized by high endemism and ecological diversity [1]. The 10 sections that form the genus Origanum (Lamiaceae) consist of 43 species, 6 subspecies, 3 cultivars, and 18 natural hybrids based on various taxonomic studies. Along with the range of secondary metabolites and the high concentration of natural compounds with beneficial nutritional and therapeutic properties, the study of oregano is important to the maintenance of biodiversity and economy. Oregano is recognized to have a commercial and scientific value that is of great importance in the world because it is one of the most widely cultivated fragrant and treatment species. Due to its increasing significance in the pharmaceutical sector, the cosmetic sector, and biotechnological sector, its value goes beyond the standard usage in the food sector as the flavoring ingredient and herb in the culinary sector. The main factor behind the increasing popularity of the plant is its abundance of phytochemicals, especially its essential oils, polyphenolic arsenal which has very good antibacterial and antioxidant effects [2]. These bioactive elements prove its potential as a natural alternative to artificial additives as well as justify its use in the production of both drugs and bioproducts. Oregano and other medicinal plants are applicable in industrial and agricultural production to enhance the health of animals, reduce production costs, increase overall production and reduce reliance on chemical preservatives [3].

The objective of the proposed study was to assess the biochemical and antioxidant properties of the Origanum vulgare aqueous leaf extract on adult female albino mice. The reasoning was based on the traditional medicinal properties of oregano and its high phenolic content, especially carvacrol and thymol that have been shown to have antioxidant effects. The main aim was to examine the effects of the extract on oxidative stress indicators (GSH, MDA, vitamins C and E), metabolic indicators (urea, creatinine, glucose), and hepatic active enzymes. Through a study of these parameters, the research aimed at scientifically confirming the therapeutic potential of O. vulgare and finding out its safety profile in future use in the treatment of oxidative stress related disorders and metabolic disorders.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract

Origanum vulgare dried leaves were taken and ground to a fine powder using a laboratory grinder. An aqueous extract was made by mixing the powdered plant material with distilled water. A fresh preparation of the extract was used and applied to the experimental animals in a dosage of 100 mg/kg body weight as it is already reported [4]. This treatment was carried through a period of four weeks.

2.2. Experimental Animals

The current research was performed in the animal house of the Department of Biology, College of Science for Women, University of Baghdad. The experiment used twelve adult female albino mice with a weight of between 25-30 g. Before the experiment, the animals were two-week-old acclimatized to the laboratory environment. The mice were put into clean cages and subjected to controlled environmental conditions with controlled temperature and humidity during the period of the experiment. The animals were kept on a 12-hour light:12-hour dark cycle and were fed on a normal laboratory diet and ad libitum water.

2.3. Experimental Design

The experimental animals were randomly allocated into two groups, and each group had 6 mice (n = 6). The control group acted as the first group and was given a regular laboratory diet and given orally distilled water throughout four weeks. This group was also kept in identical environmental and experimental conditions as the treated animals to provide the appropriate comparison.

The second group was the treated group that was given an aqueous leaf extract of *Origanum vulgare* at the dosage of 100 mg/kg body weight. The extract was administered orally at a single dose per day in a time span of four weeks. The animals were observed during the treatment period under the same housing, feeding, and environmental conditions with the control group to determine biochemical effects of the plant extract.

2.4. Blood Collection and Serum Preparation

By the termination of the experimental period, the animals were starved overnight and the blood was collected. The samples of blood were collected through cardiac puncture and without the application of anticoagulant with gel-activated tubes. Collected blood was left to clot and serum was separated by centrifugation at 5000 rpm at 10 minutes. The serum samples were separated and then stored at -20°C until subsequent biochemical tests.

2.5. Biochemical Analysis

Several biochemical parameters were examined in serum samples. The concentrations of glutathione (GSH), malondialdehyde (MDA), vitamin C, and vitamin E were determined by the standard biochemical methods and commercially available assay kits [5]. The serum urea, blood glucose, and creatinine levels were determined by a Diamond diagnostic enzyme kit as per the instructions given by the manufacturer [6][7].

A commercial diagnostic kit was used to measure serum total protein, and serum albumin concentration was measured with the help of a conventional colorimetric method [8]. The calculation of the serum globulin concentration was done indirectly using the equation as shown below:

$$Globulin = Total\ Protein - Albumin$$

Enzymatic assays kits were used to measure the activities of liver enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in accordance to standard procedures [9][10].

2.6. Statistical Analysis

Data obtained were tested with the help of Statistical Analysis System (SAS) software. The results were given in the form of mean ± standard error (SE) of each group. The statistical difference between the groups was tested with the help of the relevant statistical tests, and the statistical significance was taken as $P < 0.05$ [11].

3. Results and Discussion

The findings demonstrate that *O. vulgare* extract treatment considerably affected a number of antioxidant indicators relative to the control group. Reduced glutathione (GSH) concentration rose by an average of 5.10 ± 0.22 mmol/L in the control group to 7.76 ± 0.49 mmol/L in the treatment group, which is considered a great improvement in antioxidant defense (LSD = 1.763, $P < 0.05$) in Table 1 and Figure 1. Equally, malondialdehyde (MDA) which is a measure of lipid peroxidation declined in the control group (1.26 ± 0.08 mmol/L) to 0.93 ± 0.06 mmol/L in the treated mice, revealing significant reduction in oxidative stress (LSD = 0.288, $P < 0.05$). Conversely, the concentration of vitamin C decreased in the control group (0.34 ± 0.05mg/dL) to 0.19 ± 0.08mg/dL in the treated group, and this was statistically significant (LSD = 0.093, $P < 0.05$). Nevertheless, the difference in vitamin E levels between the control (3.33 ± 0.12 mg/dL) and treated groups (2.70 ± 0.08 mg/dL) was not statistically significant (LSD = 0.607, NS). Generally, these results indicate that an *O. vulgare* aqueous leaf extract improves antioxidant effects by elevating GSH level and decreasing lipid peroxidation in test animals, though its impact on vitamin E is not significant.

Table 1. Effect of *Origanum vulgare* aqueous leaf extract (100 mg/kg body weight) on antioxidant parameters in adult female albino mice.

Group	GSH (mmol/L)	MDA (mmol/L)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Control	5.10 ± 0.22	1.26 ± 0.08	0.34 ± 0.05	3.33 ± 0.12
Treated (*O. vulgare*)	7.76 ± 0.49	0.93 ± 0.06	0.19 ± 0.08	2.70 ± 0.08
LSD	1.763*	0.288*	0.093*	0.607 NS

Values are presented as mean ± standard error (SE), n = 6 animals per group.
*Significant at $P < 0.05$.
NS: Non-significant difference.

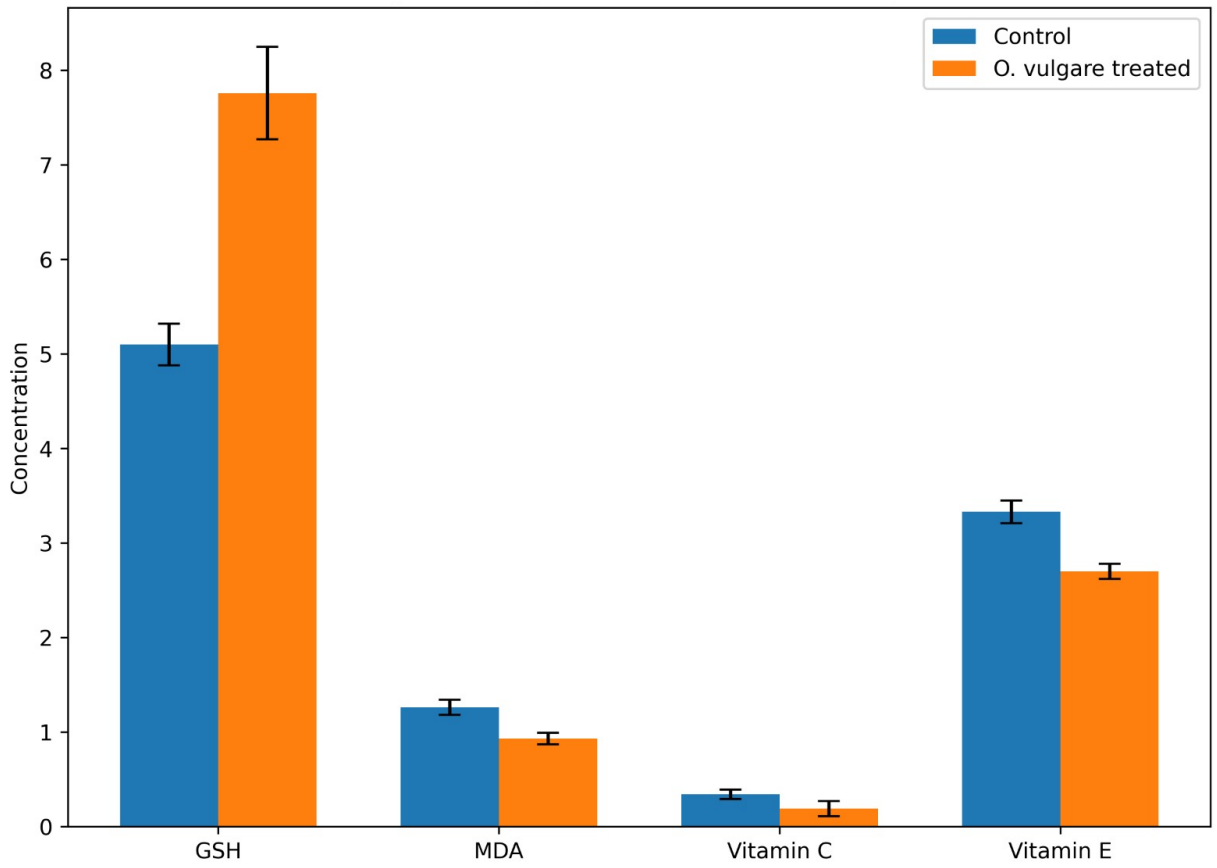

Figure 1. Effect of *Origanum vulgare* extract on antioxidant parameters

The normal female mice administered the *Origanum vulgare* showed significant increase in glutathione (GSH) levels relative to the control group as indicated in Table 1. One of the most prolific tripeptides that occur naturally is glutathione that serves as a major non-enzymatic antioxidant in the biological system. It is essential in the detoxification mechanisms and neutralization of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide (H_2O_2) and alkoxy radicals which are produced during oxidative stress and drug metabolism. Moreover, GSH helps in membrane protein thiols protection and cell defense against foreign substances [12]. The GSH levels that were observed to increase can be explained by the antioxidant effects of *O. vulgare*. It is also known that bioactive compounds in oregano (especially the phenolic compounds, including carvacrol and thymol) help maintain intracellular glutathione levels and inhibit their depletion. The results are in accordance with the earlier reports that have indicated the aspect of Origanum species in boosting the antioxidant defense system [13]. The ability of *O. vulgare* to scavenge free radicals is believed to be one of the important mechanisms in the prevention of lipid peroxidation chain reactions, which implies that the plant can be a good source of protection against the ROS-induced oxidative stress and related tissue damage.

Moreover, the *O. vulgare* administration led to a great decrease in the level of malondialdehyde (MDA) as opposed to the control group. Malondialdehyde is a well-known lipid peroxidation and oxidative stress biomarker; this means that a reduction in its levels means protective effect against oxidative damage. The decrease in the MDA content of the treated mice could be linked to the antioxidant effect of phenolic compounds existing in *O. vulgare*. These compounds are capable of suppressing the processes of lipid peroxidation and also minimizing the oxidative stress of

biological systems. The results hence indicate the possibility of Origanum to serve as a natural source of antioxidant molecules that can be useful in counteracting oxidative stress in vivo [14].

The leaves and flowers of *O. vulgare* have been known to have a number of aromatic and phenolic compounds with high antioxidant and free radical scavenging activities. Phenolic phytochemicals are also involved in protecting cells by inhibiting the damage of biomolecules by free radicals. Origanum is able to scavenge free radicals, and hence it may disrupt the chain reaction of lipid peroxidation and thus prevent the build-up of MDA which eventually helps in the protection of other tissues like the liver [15]. The first water-soluble antioxidant in the body is vitamin C, which is a well-established natural antioxidant. It has a vital role in the counteraction of free radicals in plasma and cytosolic baggage (Sies and Wihelm, 1995). Nevertheless, the decrease in the concentrations of vitamin C in the treated group can also be attributed to the fact that the level of using this antioxidant is likely to be higher during the process of scavenging ROS. Phenolic constituents of oregano extract, such as thymol and carvacrol, can change the state of the oxidative conditions or increase the level of the free radical interactions under particular conditions, which increases vitamin C intake to maintain redox homeostasis. As a result, vitamin C is quickly consumed as the antioxidant defense mechanism to counteract ROS and sustain the cellular oxidative homeostasis [16].

Vitamin E is a lipid-soluble and chain-breaking antioxidant that inhibits the escalation of free radical reactions in cell membranes. The changes in the levels of vitamin E observed in the current research were not statistically significant which indicates that the *O. vulgare* application did not significantly affect vitamin E levels in the conditions of the experiment [17].

Table 2. Effect of *Origanum vulgare* aqueous leaf extract (100 mg/kg body weight) on serum urea, creatinine, and blood glucose levels in adult female albino mice.

Group	Urea (mg/dL)	Creatinine (mg/dL)	Blood Glucose (mg/dL)
Control	79.33 ± 3.62	0.86 ± 0.07	105.33 ± 4.95
Treated (*O. vulgare*)	53.66 ± 2.45	0.78 ± 0.03	80.33 ± 2.15
LSD	7.912*	0.193 NS	17.47*

Values are presented as mean ± SE (n = 6).
* $P < 0.05$ indicates a significant difference.
NS: Non-significant.

Table 3. Effect of *Origanum vulgare* aqueous leaf extract (100 mg/kg body weight) on liver function parameters in adult female albino mice.

Group	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	6.96 ± 0.27	4.00 ± 0.09	2.94 ± 0.05	12.00 ± 0.13	24.00 ± 0.33	71.00 ± 1.64

Treated (*O. vulgare*)	7.60 ± 0.44	4.50 ± 0.08	3.16 ± 0.11	29.00 ± 0.36	28.00 ± 0.74	64.00 ± 1.53
LSD	0.893 NS	0.926 NS	0.561 NS	4.279*	6.118 NS	9.205*

Values are expressed as mean ± SE (n = 6).
* P < 0.05.
NS: Non-significant.

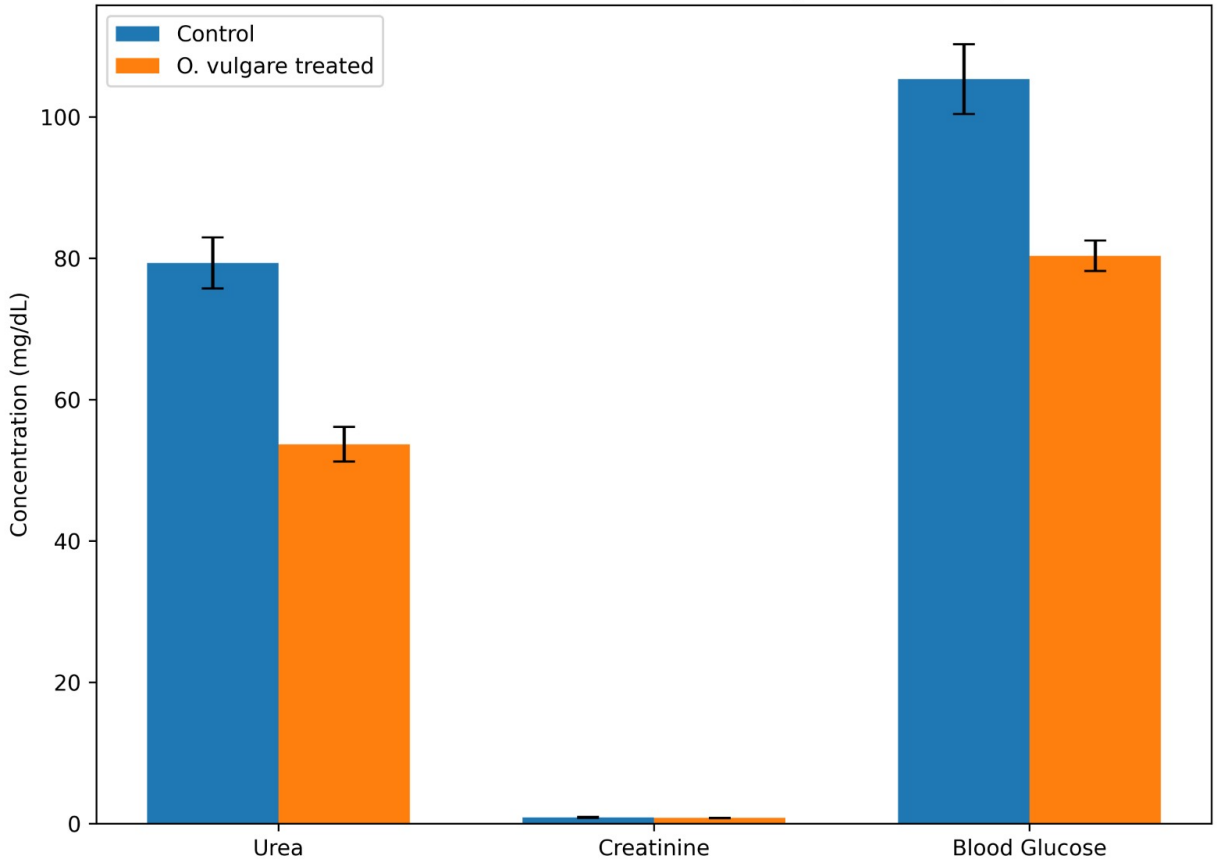

Figure 2. Effect of *Origanum vulgare* extract on kidney function and glucose

The serum urea levels were also significantly decreased in the treated group (53.66 ± 2.45 mg/dL) than the control group (79.33 ± 3.62 mg/dL) with a statistically significant level of decrease of (LSD = 7.912, P < 0.05). Likewise, the level of blood glucose in the treated animals (80.33 ± 2.15 mg/dL) was significantly lower than the control group (105.33 ± 4.95 mg/dL) (LSD = 17.47, P < 0.05) in Table 2 and Figure 2. Conversely, the serum creatinine levels declined insignificantly between 0.86 ± 0.07 mg/dL in the control group and 0.78 ± 0.03 mg/dL in the treatment group, but this was not statistically significant (LSD = 0.193, NS). Such findings indicate that *O. vulgare* extract probably controls the metabolism of nitrogen and glucose homeostasis without significantly influencing the renal filtration indicators under experimental conditions.

The decrease in serum urea can be explained by biological actions of phenolic compounds that are found in *O. vulgare*, especially carvacrol and thymol. These substances have antioxidant and anti-inflammatory effects which would lower protein metabolism and ammonia production hence lowering the urea production in the liver. It has been previously indicated that Origanum extracts

have the potential to impact the protein metabolism and lower the nitrogenous waste products, which is why a reduction in serum urea levels was observed [17]. Despite the slight variation in creatinine levels, the lack of any significant difference indicates that the renal functioning was not significantly affected by the provided dose of the extract.

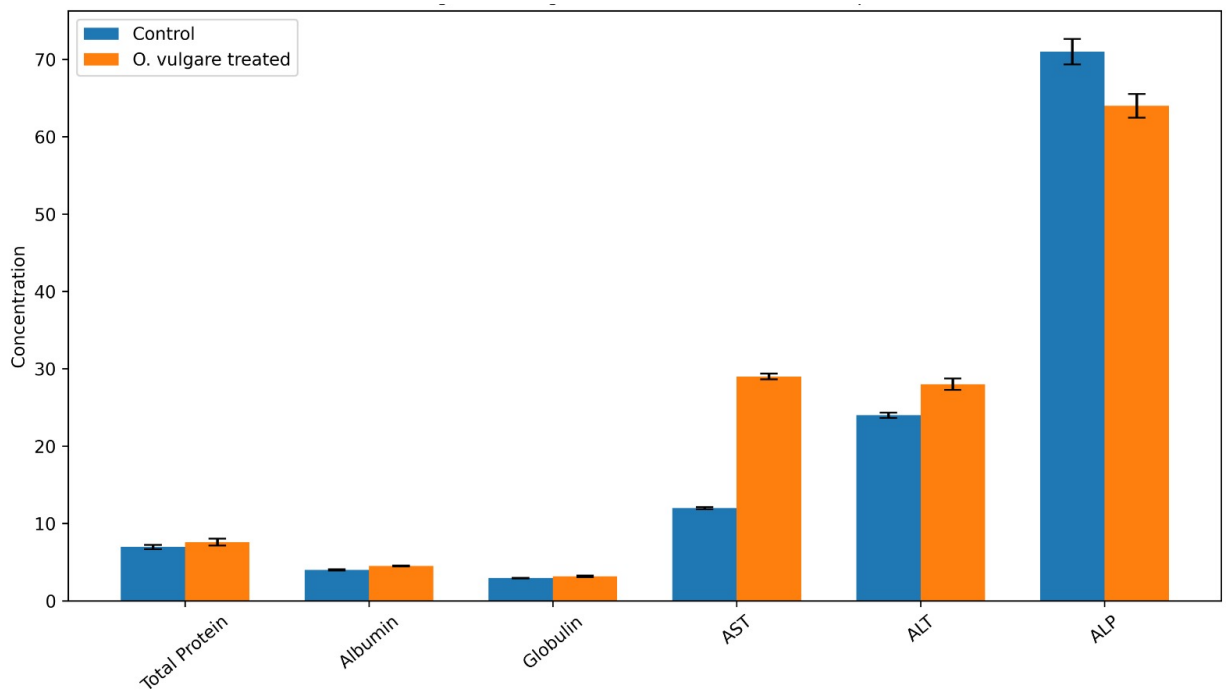

Figure 3. Effect of *Origanum vulgare* extract on liver function parameters

The substantial decrease of the level of blood glucose in the treatment group also suggests that *O. vulgare* may have a hypoglycemic effect. It is possible that this effect can be linked to the presence of bioactive phenolic compounds, including apigenin, luteolin, and rosmarinic acid. Such substances are known to prevent the major carbohydrate-digesting enzymes, especially a-glucosidase and a-amylase, and so, delay carbohydrate digestion in the intestines and slow down glucose absorption. As a result, this mechanism can help to achieve better glycemic control. Experimental research also proved *O. vulgare* extract to possess antidiabetic effects; it is capable of lowering the glycosylation processes and blocking the activity of a-glucosidase [18].

The bio-chemical analysis of other liver-related parameters revealed that serum total protein, albumin, globulin, alkaline phosphatase (ALP), and alanine aminotransferase (ALT) showed no significant differences ($P > 0.05$) between the control and treated groups. Nonetheless, the treated group (29 ± 0.36 mg/dL) and the control group (12 ± 0.13 mg/dL) showed higher and lower levels of serum aspartate aminotransferase levels respectively as shown in Table 3 and Figure 3. This increment can be linked to a higher hepatic metabolism evoked by some bioactive components of *O. vulgare*. The carvacrol and thymol compounds can be stimulating the hepatic detoxification processes and enzymes involved in drug metabolism, which can result in mild cellular stress or high membrane permeability in the hepatocytes. Consequently, there may be partial leakage of hepatic enzymes including AST into the blood. Moreover, an excess of the doses of phenolic compounds might cause moderate oxidative stress or inflammatory events in liver tissues, which might lead to the observed increase in the AST activity [19].

The important biological potential of *Origanum vulgare* as a natural therapeutic agent for enhancing antioxidant status and metabolic status. The observed elevation of glutathione levels and decrease of malondialdehyde signify higher protection against oxidative stress and suggest the involvement in the protection of oxidative stress-induced cellular damage. Additionally, fall in serum urea and blood glucose level shows its potential role in regulation of nitrogen metabolism and glycemic control. Although little effects were found on vitamin E and renal markers, a general stability of the extract was shown on biochemical levels. These findings highlight the usefulness of *O. vulgare* in the development of plant-based anti-oxidant and anti-diabetic drugs, towards safer and more sustainable approaches for the control of disease and functional nutrition.

4. Conclusion

The four-week administration of aqueous leaf extract of *Origanum vulgare* at the dose of 100 mg/kg body weight showed significant biochemical effects and antioxidant effects on adult female albino mice. The therapy provided a significant improvement in antioxidant defense as demonstrated by the increase in glutathione (GSH) and significant decrease in malondialdehyde (MDA), which represent a reduction in lipid peroxidation and oxidative stress. These results demonstrate the high antioxidant capacity of *O. vulgare*, presumably because of its phenolic components, including carvacrol and thymol. The extract too generated considerable decrease in serum urea and blood glucose, indicating positive effects on nitrogen metabolism and glucose control. Conversely, serum creatinine, total protein, albumin, globulin, ALT, and ALP did not have any significant changes and the fact that renal functions and most of the hepatic functions were not significantly affected. But, the rise in AST levels could be taken as indicative of mild hepatic metabolic stimulation or hepatic cellular stress. Altogether, the findings indicate that *O. vulgare* have good antioxidant and metabolic regulatory potentials, which contributes to its possible application as a natural therapeutic agent.

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