Indian Journal of Pharmacy and Pharmacology 2021;8(2):161–167

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Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

# Anti stress activity of *olea europaea*

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ARTICLE INFO

*Article history:*
Received 27-03-2021
Accepted 05-05-2021
Available online 17-06-2021

*Keywords:*
Swimming
Cold stress
Olea europaea
WBC
BUN
TG

ABSTRACT

Stress and stressor-related disorders are the global illnesses. The immune system is thought to be strengthened by apoptogenic substances due to their ability of restoring normal body functions, which were imbalanced due to the presence of stressful conditions. The aim of study is to evaluate anti stress potential in ethanolic extract of *Olea europaea* (*O. europaea*) leaves on swimming endurance test in mice, cold and immobilization stress in rats. The effect was assessed by swimming survival time and estimation of biochemical parameter, weight of gland, organs, blood count in cold and immobilization stress at a dose 400 and 600 mg/kg per oral(p.o). The results demonstrated on comparison with stress models with *O. europaea* leaf extract and Gerifort Syrup showing significant increase ($p<0.001$) in swimming time, significant decrease ($p<0.001$) in blood glucose, cholesterol, triglyceride (TG), plasma cortisol and Blood urea nitrogen (BUN) levels and also decrease in the weight of adrenal gland and organs weights. Blood cell count such as WBC, polymorphs and neutrophils significant decrease ($p<0.01$) under physiological limits. It was experimentally compared to control and gerifort group. Thus, obtained results revealed that the leaf extract of *O. europaea* have potential Anti-stress activity due to the existence of phenolic compounds, especially secoiridoids and iridoids and antioxidant molecules, such as oleuropein, hydroxytyrosol, tyrosol, caffeic acid, and ligstroside proving its significant anti stress activity.



## 1. Introduction

Stress and stressor-related disorders are the global illnesses.[1] The term Adaptogen was introduced into scientific literature by Russian toxicologist Nikolay Lazarev in 1957 to refer substances that increase the "state of non-specific resistance" in stress. Hans Selye's theory of stress and general adaptation syndrome, which has 3 phases: alarm phase, phase of resistance, and phase of exhaustion.[2]

The sequence of responses can be summarized as an initial alarming stage corresponding to fight or flight followed by an adaptation stage stressor resistant response and finally an exhaustion stage that may lead to the death of the organism.[3] However, the concept of stress could be altered with the replacement of a non-specific response with a specific response state. Rise in blood pressure (hypertension), immune suppression, peptic ulcer, and endocrine disorders like diabetes could be stimulated by any stressor while suggesting the HPA axis as the primary regulator of the stress. The adrenal cortex is considered as

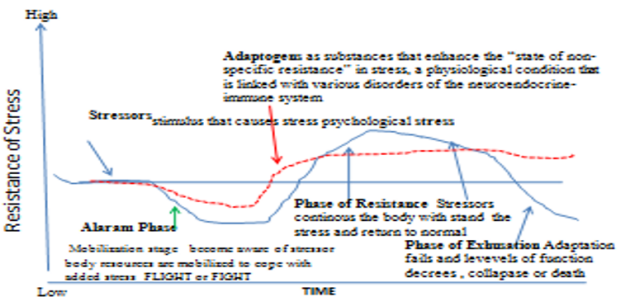


**Fig. 1:** General adaptation syndrome (GAS) Hans selye

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https://doi.org/10.18231/j.ijpp.2021.026

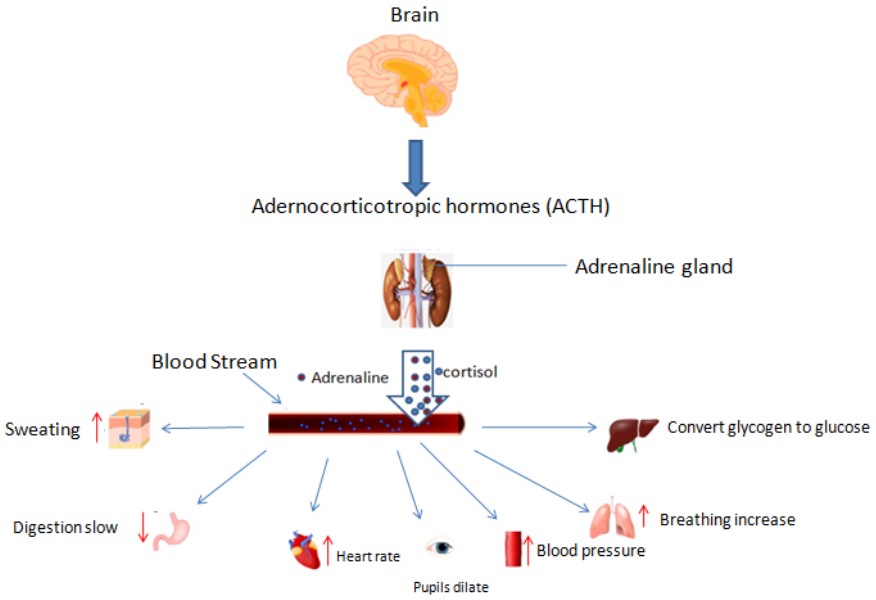


**Fig. 2:** Stress response to system

an organ of integration in a stressful condition.[4]

The immune system is thought to be strengthened by adaptogenic substances due to their ability to restore normal body functions, which were otherwise imbalanced due to the stressful conditions. Thus, they are considered to be protective agents against numerous emotional and environmental stress.[3]

*O.europaea* L, a Mediterranean plant, belongs to family Oleaceae, is one of the oldest cultivated plants known to humanity. Commonly it is called as Olive, and in Arabic, it is known as "zaitoon".[5] In traditional practices, Olive has been used to treat various diseases, including asthma, inflammation, rheumatism, diarrhea, urinary tract infections, gastrointestinal disorders, hemorrhoids, and hypertension. It has also been used as a laxative and vasodilator. The olive plant has been reported to contain phenolic compounds, primarily iridoids, and secoiridoids.[6–8]

Though the olive plant has many active phytoconstituent, several studies have revealed that oleuropein, a component of the secoiridoid group, is responsible for its significant therapeutic effects. Oleuropein is mostly present in the leaves of the plant and is considered to be effective in the treatment of arrhythmias, spasms, immunity booster, lowering the blood pressure, and is also used as cardioprotective, antithrombic, antioxidant, and anti-inflammatory agent.[9–13] The decoction of leaf *O. europaea* is used as an immunomodulator and rejuvenating in ethnomedicinal folk and traditional system. Pharmacological studies on anti-stress or adaptogenic activity have not been reported. Hence an effort has been made to establish the anti-stress or adaptogenic activity by investigating scientifically to validate the potential of O. europaea leaf extract.

## 2. Materials and Methods

The gift sample of *O. europaea* leaf extract obtained from Herbal plant, Spain and Gerifort syrup obtained from Himalaya Drug company, Bangalore batch No-25900531.

### 2.1. Photochemical evaluation

The Leaf Extracts of *O. europaea* were subjected to various chemical tests to detect the phytoconstituents present.[14]

### 2.2. Experimental animals

Albino mice (Swiss) 20-25gm and Adult rats (Wistar) of both sex weighing 160-180 g were purchased from Sanzyme lab Pvt. Animal feed was procured from National institute of animal nutrition and physiology, Hyderabad. Animals were accommodated in recommended laboratory environment at 25°C under 12 hr light-dark cycle. All the experimental animals had free access to chow and water ad libitum. The research protocol was approved by (IAEC/1657/CMRCP/T2/PhD-16/84-date 14-11-2016)

### 2.3. Swimming endurance test

Swiss albino mice of either sex weighing between 20 ± 5g were randomly categorized into four groups of six mice in each group.

1. Group I- swimming endurance test [ST] control,
2. Group II –ST + *O.europaea* 400 mg/kg (p.o) for 3 weeks.
3. Group III- ST + *O. europaea* 600 mg/kg (p.o) for 3 weeks,
4. Group IV - ST + Gerifort syrup 2ml/kg (p.o) for 3 weeks.

Swimming endurance test methods was modified in our laboratory.[15]

Above treatment was given to mice for 3 weeks. On $1^{st}$ week, $2^{nd}$ week and $3^{rd}$ week 1 hr after treatment, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 25 ± 1°C temperature. The mice were allowed to swim till exhausted and moment when animal drowned is considered as the endpoint. The mean swimming time for each group was calculated.[15,16]

### 2.4. Cold stress

Albino rats 160-180gms, male and female were categorized into five groups of six animals each.

1. Group-I: Normal
2. Group-II: Cold stress [CS],
3. Group-III CS + *Olea europaea* 400 mg/kg p.o for 10 days,
4. Group-IV: CS + *Olea europaea* 600 mg/kg p.o for 10 days and
5. Group-V: CS + Gerifort 2ml/kg p.o respectively for 10 days.

Cold stress was induced in $2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$ groups on animals, by exposing to 4 ±1°C temperature every day for 1 hr for 10 days. On $10^{th}$ day animals were sacrified, blood, organs and gland were collected. In blood, Cholesterol, Glucose, TG, Plasma cortisol, BUN, WBC and DL cell count were estimated. Organ such as Liver, spleen, testes and adrenal gland weight noted.[15,16]

### 2.5. Immobilization stress

Albino rats 160-180gms, either sex categorized into 5 groups of 6 animals each. Group-I: Normal,

1. Group-II: Immobilization stress[IMS],
2. Group-III: IMS +*O. europaea* 400 mg/kg for 10 days
3. Group-IV: IMS + *O. europaea* 600mg/kg for 10 days and
4. Group-V: IMS + Gerifort syrup at the dose 2ml/kg(p.o) for 10 days

Immobilization stress methods was modified in our laboratory. Immobilization stress induced on $2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$ groups, animals were immobilized by head downward in supine position fixing a card board inclined position at an angle of 60° daily for 1hr for 10 days. On $10^{th}$ day animals were sacrificed, blood, organs and gland were collected. In blood, Cholesterol, Glucose, TG, Plasma cortisol, BUN, WBC and DL cell count were estimated. Organ such as Liver, spleen, testes and adrenal gland weight noted.[16,17]

### 2.6. Statistical analysis

The results obtained from the pharmacological experiments were statistically analyzed using SPSS Version 19.0. The data is presented as mean of ± SEM. To compare the values and to establish the significance. ANOVA was used and P value are reported as mean ± SEM. *a p<0.001, *b p<0.01 and *c p<0.05.

## 3. Results

Preliminary phytochemical study shows O. europaea leaves extract presence of Alkaloids, Glycoside, Phytosteroids, Flavonoids, Terpinoids, Vitamins and Tannins.

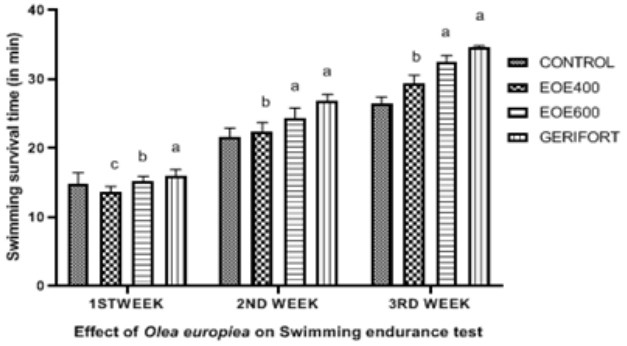


**Fig. 3:** Effect of *O. europaea* on swimming endurance test

In swimming endurance test, control group animals swim in normal time, treatment group of *O. europaea* 400 and 600 has shown significantly (p<0.01, p<0.001) increase in swimming time in 2nd and 3rd week compared to control as well as in geriforte group (Figure 3). In cold and immobilization stress-induced animals, there was a significant (p<0.001) increase in Cholesterol, BUN, glucose, TG, and serum plasma cortisol compared with control group. *O. europaea* in a dose dependent manner significantly (p<0.01, p<0.001) reduced the elevated biochemical parameters in both cold and immobilization stress models compared to Geriforte treated group (Figures 4, 5 and 6). Cold and Immobilization stress group showed increase in liver, spleen and adrenal gland weight, while, *O.europaea* leaf extract in dose-dependent manner, significantly (p<0.01) reduced spleen, liver and adrenal gland weight. (Figure 5). In cold and immobilization

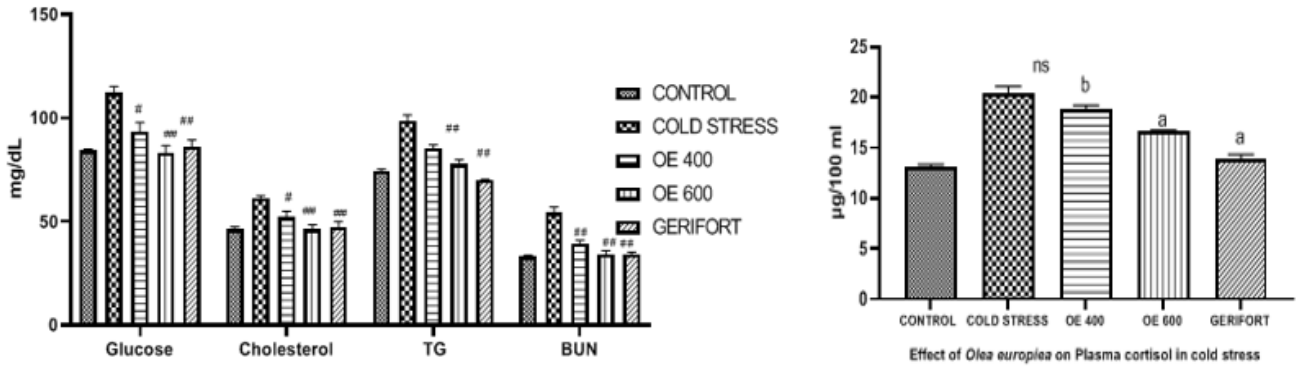


**Fig. 4:** Effect of *Olea europaea* on biochemical parameter in cold stress

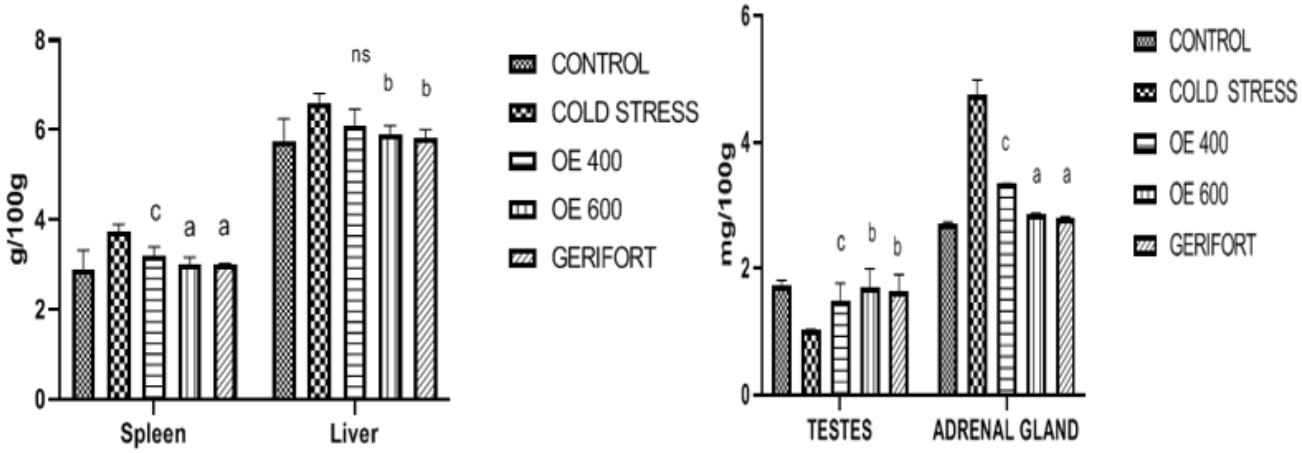


**Fig. 5:** Effect of *O. europaea* on organ and gland weight in cold stress

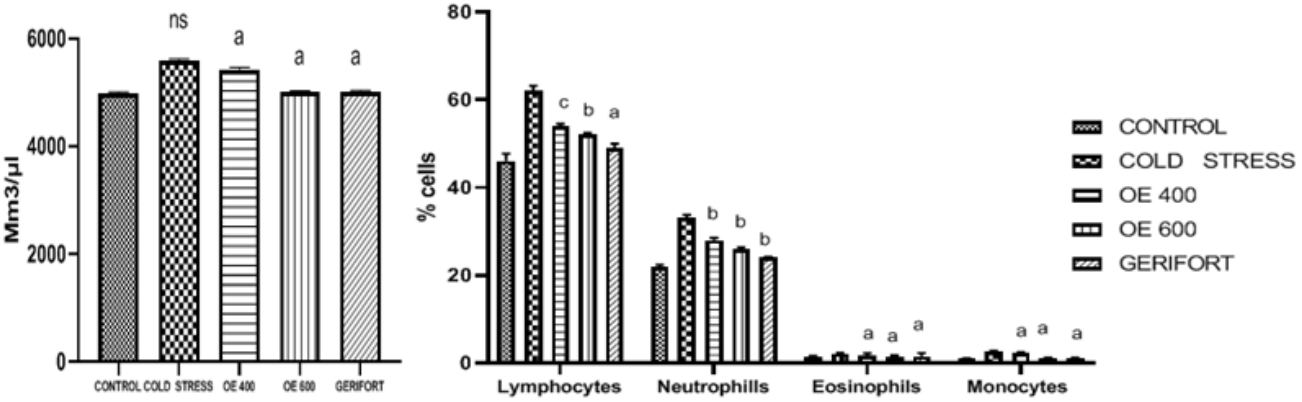


**Fig. 6:** Effect of *Olea europaea* on blood cell count in cold stress

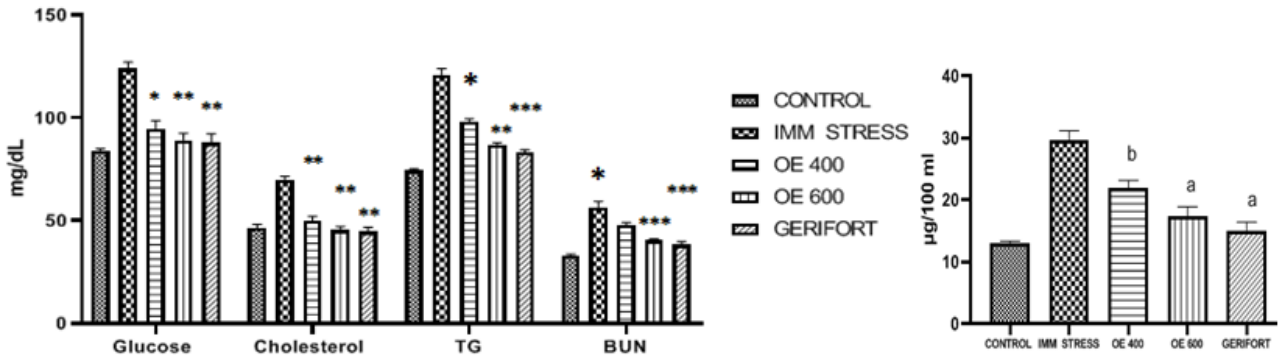


**Fig. 7:** Effect of *Olea europaea* on biochemical parameter in immobilization stress

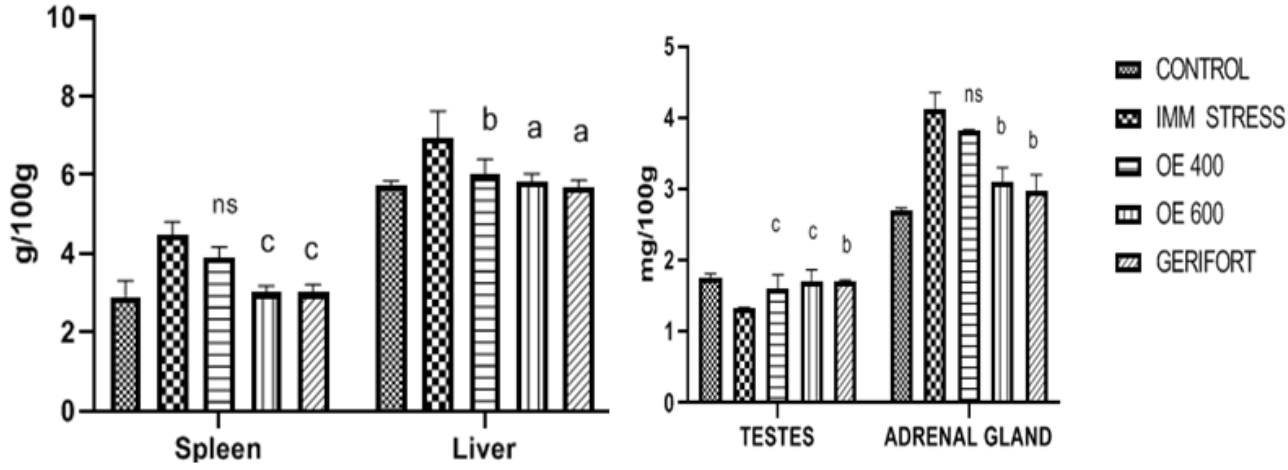


**Fig. 8:** Effect of *O. europaea* on weight of organ and gland in immobilization stress

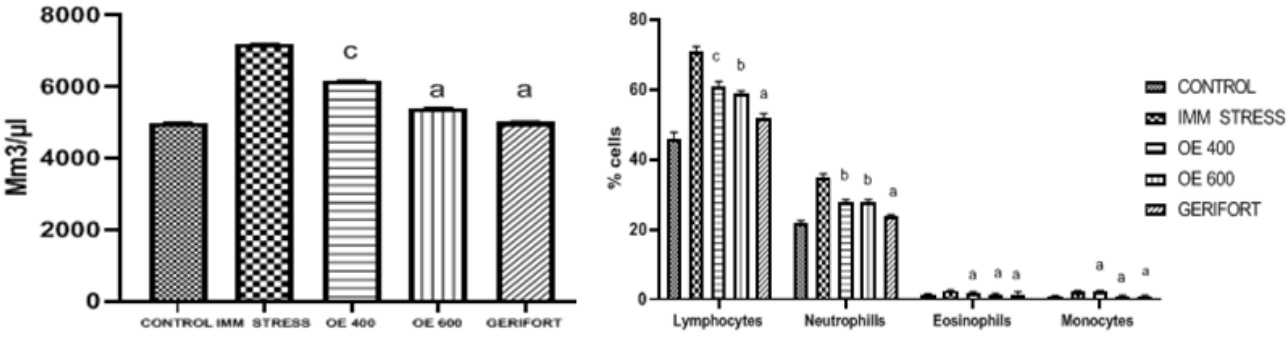


**Fig. 9:** Effect of *O. europaea* on blood cellcount in immobilization stress

stress-induced animals, with an exception to eosinophils ($p<0.05$), all the other hematological parameter including the WBC's and differential blood cell count (DLC) were significantly ($p<0.01$) increased, compared to the control group. However, treatment with *O. europaea* reduced the differential blood cell count and WBC's significantly ($p<0.01$) compared to stress control group (Figures 7, 8 and 9).

## 4. Discussion

The most common stressors could be physical (extreme environmental conditions, external force), emotional (tragedies, sadness, academic, etc.), and chemical stress (due to medicine, solvents, and exposure to the laboratory or industrial chemicals). Exposure to any of these stressors could readily imbalance the neuroendocrine systems of the body.[18] The hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system are the two major systems involved in stress response. The sympathetic nervous system secretes catecholamines, triggered mainly by a region in the brain stem (the lowest part of the brain) called the locus coeruleus. The hypothalamus is a major integrative center for receiving and converting messages from divergent centers to hormonal signals, via the regulation of pituitary gland and neural gland pathways.[19]

The activation of this HPA mechanism results in corticotrophin hormone, adrenocorticotropin hormone (ACTH), β-endorphin, and glucocorticoids being secreted into the bloodstream. In a stressful condition ACTH is released and triggers the adrenals to increase the secretion of corticosteroids, epinephrine, and norepinephrine.[20] Mice upon forcing to swim in a confined space from which they cannot escape, they will be immobile right after an initial vigorous activity. Indicating the effects of stress.[21] The immobility in the animals under stress is a distinct sign of fatigue, tiredness, as a result of reduced stamina, with the endpoint being the moment when the mice could not swim anymore and start drowning. However, an increased swimming time was observed in mice, pre-treated with *Olea europaea* with significantly enhanced physical performance compared to untreated (control) and Gerifort treated group.

Anorexia is a critical stressor as it blocks the oxygen supply and halts the vital functions of the body primarily being the respiration though affects all the other vital functions.[22] *O. europaea* leaf extract significantly prolonged the meantime to convulsion as compared to the positive control group.

Release of ACTH under the cold and immobilization stress leads to synthesis and release of cortisol through the stimulation of adrenal cortex.[23] An increased cortisol levels in the plasma results in mobilization of fats and stored carbohydrates reserves thereby increasing the blood glucose levels, cholesterol, and triglyceride. Anti-stress agents could reverse the increased cortisol levels.[24] *O. europaea* substantially decreased the elevated cortisol and blood glucose levels caused by stress. The standard drug Geriforte also provided similar results in this research. In stress-induced animals, the marked rise in serum cholesterol, triglycerides, and BUN levels is attributable to activation of the hypothalamic-pituitary axis (HPA) and sympathetic system, resulting in the release of catecholamines and glucocorticoids that suppress the immune system at multiple locations, such as the liver and kidneys.[25] The serum cholesterol, BUN, and triglycerides decreased with Olea europaea and the standard drug, which may be due to inhibition of the sympathetic nervous system. The increase in adrenal weight in stressed animals could be due to the stress-induced adrenomedullary response, which contributes to increased corticotropic hormone production, leading to an increase in adrenal weight.[26]

*O.europaea* and Geriforte reduced the liver, adrenal gland weight. This decrease in the liver and adrenal gland weight may occur due to the reversal of stress-induced adrenomedullary response and decreased production of the corticotropic hormone.

As a result of the draining of blood from the spleen to the lymphocytes, the spleen's size and weight will reduce.[27] Pretreatment with *O. europaea* and Geriforte increased the spleen weight. This may be due to the inhibition of recruitment of lymphocytes to blood from the spleen.

In stress spleen contracts and releases more amounts of lymphocytes, neutrophils, and eosinophils into circulations.[28] The increase in total WBC count is indicating a stressful state. Plant adaptogen is smooth stressors that reduce the reactivity of the host defense system. The mode of action of adaptogens is associated with the stress system. Adaptogen increase the capacity of stress to respond to the external signals of activating and subsequently deactivating mediators of the stress response.[24]

The phytochemical screening of *O. europaea* revealed the presence of alkaloids, steroids, saponins, flavonoid glycosides with tannins, protein, and carbohydrate.[8] The anti-stress activity could be due to the presence of these constituents, whereas standard Geriforte is an established anti stress/adaptogenic drug.

## 5. Conclusion

The obtained results from this study suggest that the Ethanolic extract of *O. europaea* leaves has potential Anti-stress activity due to the presence of phenolic compounds, especially secoiridoids and iridoids, having antioxidant molecules, such as oleuropein, hydroxytyrosol, and tyrosol; caffeic acid; and ligstroside.[6,7,9,11] The main active component in olive leaf is oleuropein, a natural product of the secoiridoid group. Several studies have shown that oleuropein possesses a wide range of pharmacological activity.

## 6. Source of Funding

None.

## 7. Conflict of Interest

We have no conflict of interest to declare.

## 8. Acknowledgement

Authors would like to acknowledge and thanks to Herbal Plant, Spain for Providing *O. europaea* gift sample, we also thank and appreciate MEDICULE for scientific solution Hyderabad, providing chemicals. We heartly gratitude to Assoc. Prof Dr. Abdullah Khan KPJ Healthcare University college, Nilai, Malaysia for his valuable suggestion in research work.

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**Cite this article:** Roshan S, Hussain SM. Anti stress activity of *olea europaea*. *Indian J Pharm Pharmacol* 2021;8(2):161-167.