World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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In vivo assessment of pharmacological potentials of different fractions of *Fagonia olivieri* DC

Umbreen Rashid [1, 2, 3, *], Muhammad Rashid Khan [2], Jasia Bokhari [2], Shumaila Jan [2], Hammad Ismail [4] and Bushra Mirza [5]

[1] *Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.*
[2] *Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.*
[3] *Department of Life Sciences, Abasyn University, Islamabad Campus, Pakistan.*
[4] *Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Pakistan.*
[5] *Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.*

World Journal of Advanced Research and Reviews, 2022, 16(02), 761–770

Publication history: Received on 07 October 2022; revised on 14 November 2022; accepted on 17 November 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.16.2.1243

Abstract

Introduction: Hemostasis mechanism involves blood coagulation as one of the step which is responsible for blood clot formation that restricts blood flow at site of injury. By inhibiting coagulation cascade and reducing thrombus formation, several cardiovascular diseases (CVD) and atherosclerosis can be prevented. Traditionally, *Fagonia olivieri* (zygophyllaceae) is reported in the treatment of diabetes, coughs, blood purifier, cooling agent, blood vascular diseases, digestive system disorders and cancer in initial stages.

Objectives: Different fractions of *F. olivieri* methanolic extract were evaluated in this study for their anticoagulatory, anti-inflammatory and antidepressant potentials.

Methodology: Anticoagulatory, anti-inflammatory and antidepressant potentials of ethyl acetate, butanol and aqueous extracts of *F. olivieri* were assessed using capillary tube experiment, carrageenan induced paw edema test and forced swim test respectively.

Results: The results of this investigation have revealed that ethyl acetate and butanol fractions possess significant anticoagulatory potentials as compared to control (P=0.0002). Butanol extract showed highest anti-inflammatory activity. Maximum anti-depressant activity was shown by ethyl acetate followed by n-butanol and aqueous extracts.

Conclusion: Hence, it can be concluded that *F. olivieri* exhibits anticoagulatory, anti-inflammatory and antidepressant potentials which justifies its use for the treatment of various disorders particularly blood vascular diseases.

Keywords: *Fagonia olivieri*; Anticoagulatory; Cardiovascular diseases; Anti-depressant; Anti-inflammatory

1. Introduction

Currently the world is exposed to various kinds of diseases. The most common among them are the diseases related to blood or heart. In middle to low-income countries, these kinds of diseases cause high mortality rate [1]. During periods of injury, hemostasis keeps the blood within the injured vascular system by the process of interaction between

* Corresponding author: Umbreen Rashid
Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

coagulants and anticoagulants. It is a complex process that consists of three main steps including vasoconstriction, temporary blockage of a break by a platelet plug and blood coagulation or fibrin clot formation [2].

Anticoagulants are the chemical agents that prevent and treat abnormal blood clots such as Pulmonary embolism (PE) and Deep vein thrombosis (DVT) which are types of Atrial fibrillation and Venous thromboembolism (VTE), by interacting with body's natural blood coagulation system. Anticoagulant drugs are most commonly employed to control blood coagulation in healthy as well as diseased conditions including cancer, diabetes mellitus and cardiovascular disorders. Despite the development of many drugs over the last few years, many are accompanied with side-effects for instance bleeding which can be in and around the brain, or their activity is affected by drug and food interaction. Hence, the research interest to discover natural anticoagulant drugs with fewer side effects and less toxicity is increased [3].

Carrageenan-induced paw edema assay is frequently employed to determine the acute phase of inflammation. Inflammation is a term derived from a Latin word – "inflammare", which mean burn. Inflammation is a normal protective response to tissue injury that is caused by microbiological agents, noxious chemicals or physical trauma. Numerous proliferative, chemotactic and vasoactive factors at different stages participate in inflammation and there are several targets for anti-inflammatory action [4].

A series of chemical changes are induced in the injured area of human body by any form of injury. Inflammation is a single disorder which results by body fluid disturbances as believed earlier. The latest view considered inflammation as a healthy process in response to some disturbance or disease. Redness, pain, swelling, heat and loss of function are the basic signs of inflammation [5]. Nonsteroidal and steroidal anti-inflammatory drugs are used at present to treat inflammatory diseases [6]. The use of these drugs is reduced in some parts of the population because unfortunately both of these extensively prescribed drugs have major negative side effects [7, 8]. Therefore, there is a requirement of novel drug development with least side effects and new modes of action. Anti-inflammatory agents derived from natural products with lower risk of side effects, good efficiency and transcriptional form of action prevents inflammation-related conditions and offer promising treatment. In the hunt for new anti-inflammatory drugs, a logical and fruitful research strategy is to research the plants which are used as anti-inflammatory agents in folk medicine [9].

Depression and anxiety are the most frequent stress related mood disorders [10]. Clinical depression is a condition of deep sadness, despair or melancholia that reaches to the point of being troublesome to an individual's social functioning or daily life activities. On at least one occasion of life, depression affects about 7-18% of the population, before the age of 40 [11]. According to monoamine theory, deprivation of serotonin, norepinephrine and/or dopamine in the central nervous system (CNS) is the main cause of depression. Drugs with antidepressant activity increase the level of these neurotransmitters in the central nervous system [12]. Recent theories suggest that monoamines act only as regulator to other more important brain neurobiological systems [13]. The traditional therapeutic strategy is the only current treatment that affects serotonin and norepinephrine system, but approximately half of affected people are insufficiently cured by psychotherapeutic approaches and existing medication [14, 15]. There is no success in developing fundamentally new antidepressants with distinct mechanisms of action despite remarkable efforts [16]. Synthetic drugs available for the cure of depression and anxiety have several side effects such as drowsiness, insomnia, libido with selective serotonin reuptake inhibitors and ataxia with benzodiazepines [17]. Drugs obtained from natural sources have the ability to cure diseases in exactly the same manner as their synthetic counterparts with less side effects. In order to look for novel therapeutic products for the cure of neurological diseases, there is a constant worldwide progress in medicinal plant research which demonstrates the pharmacological efficacy of plant species in different animal models [18].

2. Material and methods

2.1. Plant material

Fagonia olivieri was collected (as a whole) from Dhamyal Rawalpindi, Pakistan in April-May 2010. Voucher specimen was identified by Dr. Saleem Ahmad and deposited at Herbarium of Pakistan Museum of Natural History, Islamabad (Voucher No. 058608). The plant was dried in shade at temperatures range of 21-30 °C. It was stored at room temperature after grinded by blender into powder form. Following procedure was used to carry out extraction.

2.2. Preparation of extract

2.2.1. Methanol extract

The dried samples of plant were soaked in 95% methanol for one week with random stirring and shaking at room temperature. Whatman filter paper No. 42 (125 mm) was used to carry out filtration. This process was repeated thrice. The resulting filtrates were combined and concentrated in rotary vacuum evaporator at 40 °C to get a solid gummy mass. The extract was stored in airtight vials at -4 °C till further use.

2.3. Preparation of fractions

Liquid–liquid extraction technique was used to carry out fractionation of methanol extract. The resulting fractions i.e. ethyl acetate, n-butanol and aqueous were concentrated using vacuum evaporator and stored at -4 °C in airtight vials till further analysis.

2.4. Pharmacological tests

2.4.1. Preparation of test samples

The extracts were given in 400 mg/kg dosage that was made by suspending in a mixture of 0.75% sodium carboxymethyl cellulose (CMC) and distilled water. The animals in the control group were given the similar experimental handling like that of the treated groups unless of course the drug administration was swapped with proper amount of dosing vehicle. Diclofenac potassium (10 mg/kg) in 0.75% CMC was employed as the standard.

2.4.2. Animals

Adult Sprague-Dawley rats weighing between 160-210 g of either sex were used for experiment. These were kept under standard environmental conditions like, 12-h light/dark cycle, ambient temperature (22 ± 1°C) and relative humidity (55 ± 5 %). The rats received free accessibility to a standard pellet diet and water *ad libitum* and were kept in groups of three. The food was withdrawn 18-24 h before the experiment but allowed fresh water before administration of the plant extracts. The ethics for use of experimental animals were followed carefully. The doses of the control materials and test samples were given according to the weights of the rats before any treatment.

2.4.3. Anti-coagulatory Activity

Anticoagulatory potentials of ethyl acetate, butanol and aqueous extracts of *F. olivieri* were assessed using capillary tube experiment.

2.4.4. Capillary Tube Method

The anticoagulatory activity of extracts was measured using capillary tube method. The rats were orally administered with acute dose of extracts and saline in negative control. The blood was taken after one hour by piercing the tail of each animal using sterile lancet. The capillary tube was filled with the blood. Then the small portion of capillary tube was broken and the process was repeated till the appearance of fibrin thread. Time interval between the tail pricking and the formation of fibrin was recorded [19].

2.5. Evaluation of anti-inflammatory activity

2.5.1. Carrageenan induced hind paw edema method

In order to determine anti-inflammatory potential, carrageenan induced hind paw edema model was utilized [20]. Rats were split (randomly) into five groups having three rats in each group. Oral dose of vehicle (0.75% CMC suspension) was provided to Group-I, while ethyl acetate, *n*-butanol and aqueous fractions (400 mg/Kg) of *F. olivieri* were given to Group- III, IV and V. Group-II received Diclofenac potassium (10 mg/kg bw) and served as standard treatment group [21]. To induce edema, 0.1 ml of 1% suspension of carrageenan (prepared in normal saline) was injected into the subplanter region of left hind paw after 1 h of the treatment. The quantification of inflammation was measured by the volume displaced by the paw, employing a plethysmometer (Ugo Basile) at time 0, 1st, 2nd, 3rd and 4th h after the carrageenan injection. The value of actual edema volume was obtained from the difference between the initial and subsequent values which was then compared with control. Following formula was used to estimate the inhibition of inflammation:

$$\% = \left[\frac{\text{Volume of control} - \text{Volume of the treated}}{\text{Volume of control}}\right] \times 100$$

2.6. Evaluation of anti-depressant activity

2.6.1. Forced swim test

Forced swim test (FST) is considered the most frequently employed pharmacological *in vivo* model for evaluating antidepressant potential [22]. The apparatus includes a transparent plexiglass cylinder (40 cm high × 18 cm diameter) filled with 25 cm of water (25 °C) hence the rat would not be able to touch bottom with its hind paws. All the rats of either sex were split into six different groups (n=3). The first group allocated as control merely received vehicle (0.75 % CMC suspension). The other three groups received acute doses of ethyl acetate, n-butanol and aqueous fraction (400 mg/kg bw) of *F. olivieri*. In the pre-test session, each rat was kept separately into the cylinder for fifteen minutes, 24 h before experiencing the 10 min swimming test, wherein the time span of immobility was noted for the last 6 min. Oral administration of the doses was performed 1 h before final swimming test session. The rat was judged to be immobile when it stopped struggling and did no more attempts to escape (except for the movements needed to keep its head above the water) was recorded as the immobility time. A reduction in the time period of immobility is an indicator of an antidepressant like effect.

2.7. Statistical analysis

The values were expressed as mean±standard error. The results were evaluated by one way analysis of variance (ANOVA) using computer software, Statistics 8.1. Multiple comparisons among different treatments were made by Tukey HSD method at p-value≤0.05.

3. Results

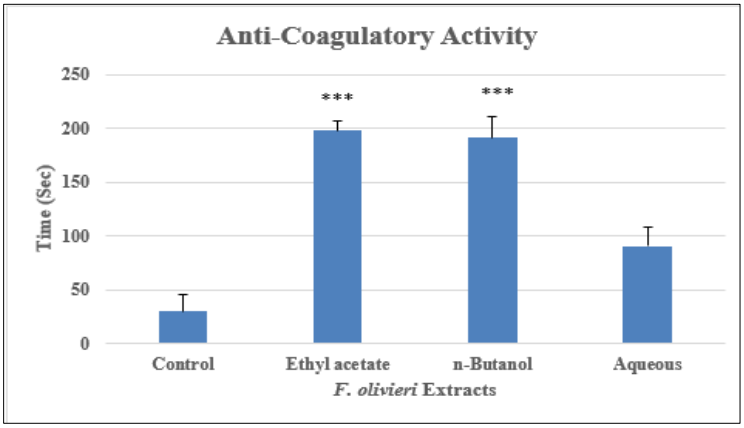
3.1. Anti-coagulatory activity

The effect of different fractions of *F. olivieri* on clotting time is given in Table 1 and Fig 1. The highest increase in clotting time is shown by ethyl acetate 197.67 ± 9.06 and n-butanol fractions 191.00 ± 20.42 as compared to control ($P < 0.001$). The aqueous fraction increases the clotting time non-significantly (90.33 ± 17.32).

Table 1 Effect of different fractions of *F. olivieri* on clotting time in rats

Extract	Dose (mg/kg)	Body Weight (g)	Clotting Time (seconds)
Control	-	160.33 ± 10.33	29.67 ± 15.38
Ethyl acetate	400	149 ± 9.71	197.67 ± 9.06***
n-Butanol	400	142.33 ± 8.08	191.00 ± 20.42***
Aqueous	400	105.66 ± 3.28	90.33 ± 17.32

Values are expressed as mean ± SEM (n=03). Statistically significant when compared to control; * p < 0.05; ** p < 0.01;*** p < 0.001



* p < 0.05; ** p < 0.01;*** p < 0.001

Figure 1 Changes in clotting time in control and treatment groups of rats

3.2. Anti-inflammatory activity

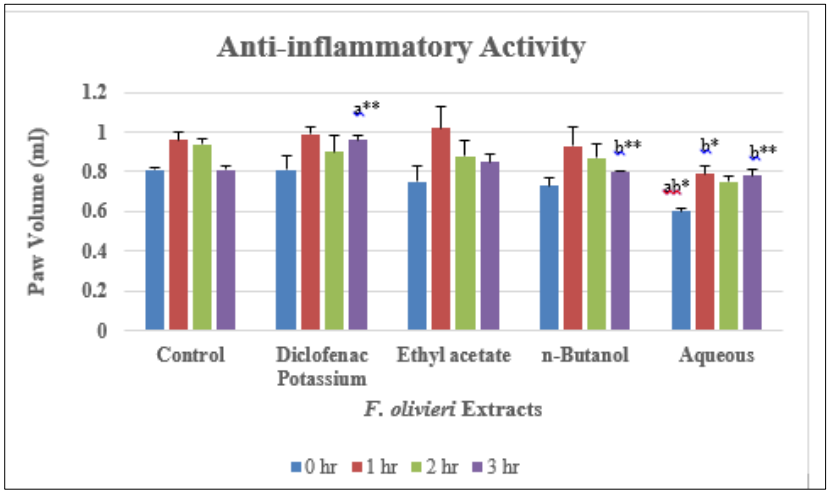
The effect of different extract/fractions of *F. olivieri* on carrageenan induced paw edema is depicted in Tables 2 and 3. The n-butanol extract showed highest inhibition (11.38 %) among all the extracts comparatively to the normal rats. The aqueous and ethyl acetate fractions produced 4.23 and -2.35 % inhibition respectively (Fig. 2 and 3).

Table 2 Effect of different fractions of *F. olivieri* on mean increase in paw volume (ml) in rats

Extract	Dose (mg/kg)	0 hr	1 hr	2 hr	3 hr
Control	-	0.81 ± 0.02	0.96 ± 0.04	0.94 ± 0.03	0.81 ± 0.02
Diclofenac Potassium	10	0.81 ± 0.07	0.99 ± 0.04	0.9 ± 0.08	0.96 ± 0.02^{a**}
Ethyl acetate	400	0.75 ± 0.08	1.02 ± 0.11	0.88 ± 0.08	0.85 ± 0.03
n-Butanol	400	0.73 ± 0.04	0.93 ± 0.1	0.87 ± 0.07	0.80 ± 0.008^{b**}
Aqueous	400	0.60 ± 0.02^{ab*}	0.79 ± 0.04^{b*}	0.75 ± 0.03	0.78 ± 0.03^{b**}

Values are expressed as mean ± SEM (n=03). Statistically significant when compared to control.

a Significantly different from Control; b Significantly different from Diclofenac Potassium; * p < 0.05; ** p < 0.01;*** p < 0.001



a Significantly different from Control; b Significantly different from Diclofenac Potassium

* p < 0.05; ** p < 0.01;*** p < 0.001

Figure 2 Effect of different fractions of *F. olivieri* on mean increase in paw volume (ml) in rats

Table 3 Effect of different fractions of *F. olivieri* on % inhibition in rats

Extract	Dose (mg/kg)	1 hr	2 hr	3 hr	Mean
Control	-	0.95 ± 0.04	0.94 ± 0.03	0.81 ± 0.02	0.9
Diclofenac Potassium	10	-4.23 ± 4.95	-5.13 ± 15.5	-14.35 ± 2.72	-7.90
Ethyl acetate	400	-12.2 ± 19.91	-2.42 ± 17.72	7.56 ± 6.17	-2.35
n-Butanol	400	17.19 ± 8.54	11.34 ± 8.23	5.63 ± 2.63	11.38
Aqueous	400	6.60 ± 1.29	5.89 ± 1.92	0.21 ± 6.02	4.23

Values are expressed as mean ± SEM (n=03). Statistically significant when compared to control.

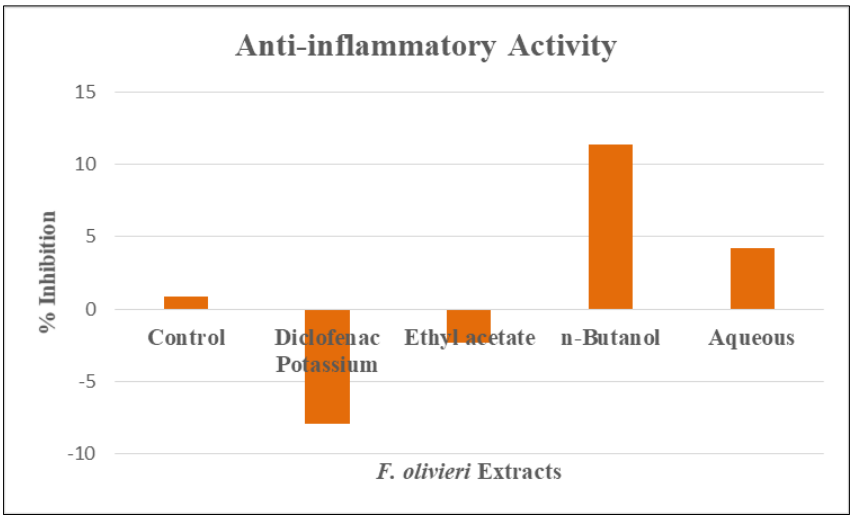


Figure 3 Effect of different fractions of *F. olivieri* on % inhibition in rats

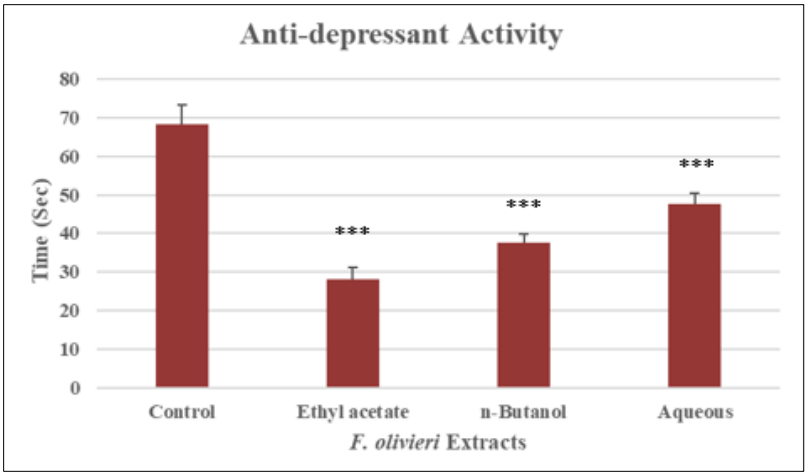
3.3. Anti-depressant activity (Forced swim test)

The effect of various fractions of crude methanolic extract of *F. olivieri* (400 mg/kg) in forced swim test in rats is shown in Table 4. The present findings suggests that ethyl acetate, n-butanol and aqueous extracts reduced the immobility time by 28.00 ± 3.05, 37.66 ± 2.02 and 47.66 ± 2.72 seconds respectively when administered at an acute dose of 400 mg/kg (P = 0.0002) in comparison to the immobility time of control i.e. 68.33 ± 4.91 sec. The ethyl acetate extract of the plant has shown the best results when it was compared with control (Fig. 4).

Table 4 Effect of different fractions of *F. olivieri* on immobility time in rats

Extract	Dose (mg/kg)	Body Weight (g)	Immobility Time (Seconds)
Control	-	160.33 ± 10.33	68.33 ± 4.91
Ethyl acetate	400	149 ± 9.71	28.00 ± 3.05***
n-Butanol	400	142.33 ± 8.08	37.66 ± 2.02***
Aqueous	400	105.66 ± 3.28	47.66 ± 2.72***

Values are expressed as mean ± SEM (n=03). Statistically significant when compared to control.; * p < 0.05; ** p < 0.01;*** p < 0.001



* p < 0.05; ** p < 0.01;*** p < 0.001

Figure 4 Changes in immobility time in control and treatment groups of rats

4. Discussion

Coronary disease is the most frequent and common ailment in the world that is prominent because everyone (especially adults) can accumulate it easily. This might lead to a heart attack, if left untreated. The formation of a clot in the heart or blood vessels causes heart attack. Hence anticoagulant drugs are recommended to reduce the formation of a blood clot. In the United States, a heart attack occurs every 40 sec according to the Centers for Disease Control and Prevention (2020) [1].

Plasma proteases activates proenzymes by sequential proteolysis, which results in thrombin formation, breaking the fibrinogen molecule into fibrin monomers, which polymerize to form the clot. This entire process is called blood coagulation that is divided into intrinsic and extrinsic pathways, which responds to vascular injury and tissue factor release respectively [23].

Different fractions of *F. olivieri* are studied for their effect on clotting time (CT) which is one of the screening test for the coagulation profile. All the fractions prolonged the clotting time in this test demonstrating anticoagulant potential. This might be credited to the presence of phytochemicals including flavonoids [24]. There is a potential interest in flavonoids for producing blood inhibitors and vessel wall interactions as they can affect a wide variety of enzymes due to their antioxidant properties, free-radical scavenging ability and inhibitory effects on leukocytes and platelets. They have also been recently shown to inhibit platelet secretion, aggregation and adhesion [1].

The organisms respond to several factors like infections caused by injury, microbes and immunological mechanisms in the form of inflammatory processes. Carrageenan induced edema is extensively employed as a working model of inflammation in the hunt for novel anti-inflammatory agents [25] and emerged to be the basis for the finding of Indomethacin, an anti-inflammatory drug [26]. Carrageenan induced inflammation is associated with infiltration of neutrophils along with free radicals and mediators produced and released from neutrophils.

Carrageenin-induced paw edema was taken as a prototype of exudative phase of inflammation. This edema depends on the involvement of polymorphonuclear leukocytes, kinins and their pro-inflammatory factors like prostaglandins [27]. The edema which is developed in rat paw after injecting carrageenan is illustrated as a biphasic event [28]. Serotonin and histamine are liberated in the first phase of inflammation which initiates instantly after injection and reduces within an hour [29]. Prostaglandin and leukotrienes are released in the second phase of swelling which starts at 1 hr and stays up to 3 hr [28]. The 2nd phase is sensitive to steroidal and nonsteroidal anti-inflammatory drugs. Usually, non-steroidal drugs strongly restrain the second phase of edema whereas several other drugs hinder both phases. The paw edema is sensitive to cyclooxygenase inhibitors. Hence this model is employed to assess the effect of non-steroidal anti-inflammatory drugs which mostly inhibits the cyclooxygenase involved in prostaglandin synthesis [30]. The significant anti-inflammatory effect shown by the extracts of *F. olivieri* might be due to inhibition of prostaglandin-like substances. Anti-inflammatory activity of the n-butanol and aqueous extracts of the plant could be due to chemical compounds such as flavonoids, saponins, tannins, sterols and triterpenes. Flavonoids and tannins are known to inhibit prostaglandin synthesis [31].

Infection, trauma or injury naturally produces inflammation in the body. The aim of inflammation is to remove the causative agent, damaged tissues, dead cells and start repairing them. Several mechanisms are known to relate inflammation with blood clotting. This inflammation/coagulation relationship is traditionally connected via platelets: production of TXA2 by the activation of arachidonic acid pathway which stimulates platelet aggregation that in turn leads to blood clotting.

Hence, it can be expected that medicinal plants that act on inflammation may also affect the process of blood clotting [23].

Recent lifestyle directs to various stress conditions, amongst which depression and anxiety are general and commonly prevailing senile neurological disorders. The generally used animal model for evaluating antidepressant activity in small animals is forced swimming test following acute or short-term treatment [22, 32]. It is anticipated that immobility happen in this test will be a sign of behavioral desolation or incapable to adapt the stress as seen in human [33, 34]. In central concept of forced swimming test, animal will get immobile stance when subjected to the short-term or unavoidable stress. This test is sensitive and quite specific to all major classes of antidepressants including MAO inhibitors, serotonin selective reuptake inhibitors and tricyclics [33, 35].

Immobility is the sign of depression. Antidepressants that selectively inhibit norepinephrine uptake decrease immobility and selectively augment climbing without upsetting swimming [36]. In assessing antidepressant activity by

the forced swim test, when rats are compelled to swim in a restricted space, they rapidly abandon swimming and stand immobile. Many antidepressant drugs lessen this despair behavior of rats which showed that this immobility behavior might be a measure of lowered mood in the rats. Drugs that potentiate alpha-adrenergic systems and central dopaminergic reduce immobility behavior of rats in this test [37]. Fluoxetine is selective serotonin reuptake inhibitor which facilitates serotonergic neurotransmission. In our tests, *F. olivieri* ethyl acetate, n-butanol and aqueous extracts showed antidepressant-like effects with decline in the immobility time which goes along with the boost in swimming time. It has been reported that swimming behavior is sensitive to serotoninergic agents, for instance fluoxetine [38, 32, 35], the selective serotonin reuptake inhibitor (SSRI). Based on these findings, it can be recommended that the *F. olivieri* extracts which are capable of decreasing the immobility time and increases swimming behavior in the rats exposed to these paradigms can put forth its effect through a mechanism comparable to that of the fluoxetine via the serotonin system. Several neural pathways are implicated in the pathophysiology of anxiety states and depression. Therefore, a large number of neurotransmitters are thought to engross in underlying mechanisms of these diseases, as apparent by the antidepressant and anxiolytic drugs. The anti-coagulatory, antidepressant and anti-inflammatory potentials of *F. olivieri* fractions might be due to a combination of diverse biologically active constituents relatively than any single compound, being the most remarkable are alkaloids, flavonoids and the triterpenoids. Latest reports have also pointed out that various flavonoids have anti-inflammatory activity [31, 39].

5. Conclusion

Hence, it can be concluded that *F. olivieri* exhibits anti-coagulation, anti-inflammatory and anti-depressant potentials which validates its use for the treatment of various disorders particularly blood vascular diseases.

Compliance with ethical standards

Acknowledgments

We would like to acknowledge Dr. Ihsan-ul-Haq, Department of Pharmacy, Quaid-i-Azam University, Islamabad for his support in this study. We are also grateful to Higher Education Commission of Pakistan for the provision of funds to carry out this research work (Indigenous Scholarship for MPhil leading to PhD, Batch IV).

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All animal handling were done according to the guidelines provided by the ethics committee of the Department of Biochemistry, Quaid-i-Azam University, Islamabad.

Funding

This research work is funded by Higher Education Commission of Pakistan (Indigenous Scholarship MPhil leading to PhD, Batch IV).

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