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Phytochemical and Pharmacological Evaluation of Antidepressant Activity of *Quercus infectoria* Plant

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Abstract
Depression is the second leading psychiatric disorder where 21 % of the world population suffers from this disease. The age range is markedly decreasing from 40–50 years age range to 25–35 years age range which observed worldwide. In last few decades, several drugs have been discovered to treat depression such as tricyclic antidepressants, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors (SSRI). But unfortunately, all of the drugs have serious side effects including insomnia, anxiety, weight gain etc. It is well known that nature is the best and safe source for all medicine. So it becomes worth to search for a new antidepressant drug from natural source with less side effects (It is assume that a drug from natural source could have less side effects) and complications. However, the neuropharmacological activity of *Quercus infectoria* plant is not investigated extensively which influenced us to design our study.

The aim of study is to screen the effect of selected plant on these transmitters in stress induced depression in mice. This herb is well established for other activity but the effect of that herb on CNS transmitters is not known, so the study will identify the effect of plant leaves on these parameters due to a pharmacologically active flavonoid compound.The present study investigated the antidepressant-like effect of MEBM in different behavioral model of depression in mice.
Key-words: Phytochemical, *Quercus infectoria,* Pharmacology

Introduction

Depression is disorders of mood or affect have been described since the 4th century BC. Despite this early acknowledgment, their aetiology is still a source of debate. Depression is one of several disorders affecting mood, along with mania, hypomania, and bipolar disorders. The primary symptoms of depression (depressed mood, low self-esteem, guilt, difficulty in concentration, suicidal ideation, thoughts of death) are by their nature difficult to model in animals. This problem is further confounded by their unknown etiology. Several theories have been proposed [1]. The clinical diagnosis of depression requires the presence of several "core" symptoms (depressed mood, decreased pleasure) often accompanied by more variable symptoms such as irritability, changes in weight, sleep disturbance, feelings of

guilt, poor concentration, thoughts of death, suicidal ideation, etc. It is clearly not possible to reproduce in animals all symptoms observed clinically. The principal symptoms observed in depressed patients and suggest analogous signs that can be observed in animals. These signs can be used as dependent variables (end point measures) allowing behavioral assessment in different animal models of depressive states. Several of the behavioral signs are, however, amenable to preclinical testing.

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Measures thought to be related to resignation (often termed "behavioral despair" or "learned helplessness") are used as the main behavioral parameter in screening tests for antidepressant activity (forced swim and tail suspension tests), as well as in the learned helplessness model [2].Depression means selective diminution of activity of specialized cells, e.g. barbiturates depress CNS, quinidine depresses heart, omeprazole depresses gastric acid secretion. Certain drugs stimulate one type of cells but depress the other, e.g. acetylcholine stimulates intestinal smooth muscle but depresses SA node in heart. The psychopharmacological agents or psychotropic drugs are those having primary effects on psyche (mental processes) and are used for treatment of psychiatric disorders. Many novel and atypical antipsychotics, selective serotonin reuptake inhibitors (SSRIs) and other antidepressants have been introduced since the 1980s. It is sadness, loss of interest and pleasure, worthlessness, guilt, physical and mental slowing, melancholia, self-destructive ideation.Antidepressants used for minor as well as major depressive illness, phobic states, obsessive-compulsive behaviour, and certain anxiety disorders [3].Medicinal plants have played a crucial role in world health and the use of natural products with therapeutic properties is as ancient as human civilization. According to the World Health Organization (WHO), about 65-80% of the world's population in developing countries depends essentially on plants for their primary health care. Many plants have been reported to have antidepressant activity and can be effective therapeutic alternatives for treatment of depression [4].Phytochemicals derived from herbs are known to decrease the risk of some severe disorders including autoimmune and cardiovascular diseases as well as neurodegenerative diseases. Indeed, popular polyphenols such as curcumin, ferulic acid, proanthocyanidin, quercetin, and resveratrol have shown potent anti-inflammatory and antioxidant properties. These phytochemicals repeatedly have demonstrated their neuroprotective effects, strongly suggesting that they can improve the symptoms of depression. Today, many people are searching for natural remedies to overcome depression. These have fewer side effects and are easily obtainable. Plant as antidepressant Hypericum perforatum (HP), more commonly known as St. John's wort is a plant which has been used for centuries as a medicinal herb[11].Theextracts of Morinda officinalis, Mimosa pudica Linn, Piper methysiticum Forst, Rhazya stricta and Siphocamphylus verticillatus, Kav-kava [5].*Quercus infectoria* oliv. is well-known since ancient times. Early study showed that as part of postpartum care, the Arabs, Persians, Indians, Malays and Chinese have traditionally used *Quercus infectoria* oliv. after childbirth to treat vaginal discharge and related postpartum infections [6]. The galls are spherical or pear-shaped with a smooth and shining surface bearing spinous projection. It is chestnut brown in colour. The galls collected before the emergence of the insects are the best such galls have inner soft tissue of a deep greenish yellow colour. Those collected after the escape of the insects will have perforationa. The galls generally vary in size, colour and appearance.The galls are astringent, acrid, cooling, haemostatic, constipating, vulnerary, expectorant, digestive, febrifuge, trichogenous and tonic. They are useful in vitiated conditions of pitta and kapha, internal haemorrhages, haemoptysis, diarrhea, dysentery, ulcerative stomatitis, cough, bronchitis, dyspepsia, fever, gonorrhea, leucorrhoea, menorrhrosis, impetigo, eczema, haemorrhoids, pharyngodynia, diabetes, hyperhidrosis, tonsillitis and general debility. It is very useful for blackening the hair and in the antidotal treatment in cases of poisoning by aconite, *Datura, Nux-vomica* and antimony [7].

Experimental
Collection, authentification and extraction
Collection of galls: The galls of *Quercus infectoria* were collected from Ayurvedic crude drug shop Bhopal (M.P). The galls were collected in the dried form. It was completely clean and dustfree. The galls were grinded by an electric mill to produce coarse powder. Powdered drug werekeptinairtightcontainers. Botanical identification is necessary because it ensures the safety and efficacy of the naturalplant. The collected material is compared with the published description of the drug and withauthentic specimen and identification is verified by an acknowledged expert.

Pharmacognostic study: In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay, and in view of lack of pharmacognostic study the present investigation was undertaken with an objective to evaluate galls of *Q. infectoria* on various pharmacognostic parameters, such as macroscopic, microscopic, physiochemical, fluorescence and phytochemical studies of the plant. Fresh galls were taken for morphological and histological studies. Coarse powder was used to study the microscopic characters and physicochemical investigations [8]. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Table 1: Macroscopic characteristics of *Q. infectoria* Olivier

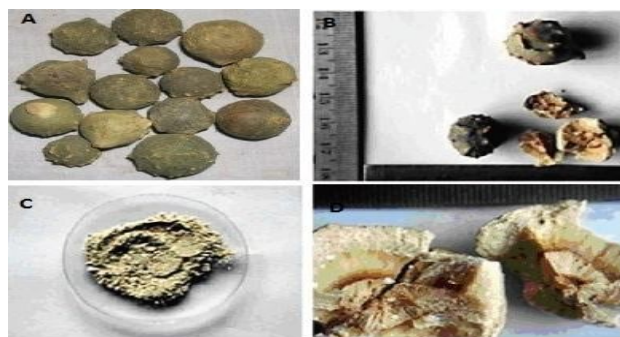| Q. infectoria Olivier Characteristics		
Crude Drug	Shape	Globose with horny appearances on external surface
	Size	1.4-2.3 cm in length and 1-1.5 cm in diameter
	Surface	Smooth with numerous horny protuberances giving rough touch
	Colour	brownish-black in colour externally and dark brown buff coloured
	Odour	Not characteristic
	Taste	Bitter astringent but at end sweetish sensation
	Fracture	Short granular
Powder	Nature	Mixture of coarse and fine
	Colour	Creamish-white
	Touch	Rough to smooth
	Odour	Not characteristic
	Taste	Bitter astringent

Figure 1: Macroscopic characteristics, A-*Q. infectoria*; B-Crude drug; C-Powder form; D-Internal surface in broken drugs

Figure 2: Outer surface of Galls of *Q. infectoria*;
Figure 3: Inner surface of Galls of *Q. infectoria*

Physicochemical analysis
Physicochemical values such as % of ash values and extractive values were determined according to the well established protocols[37]. The following Physicochemical analysis was investigated for the powder drug.

Table 2: Physiochemical parameters of *Q. infectoria*

Parameters	Value
Foreign matter (% w/w)	0.1
Loss on drying (% w/w)	9.5
Total ash value (% w/w)	5.02
Acid insoluble ash (% w/w)	0.11
Water soluble ash (% w/w)	2.22
Sulphated ash (% w/w)	0.21
Swelling index (mL)	2

Florescence analysis:
Powdered gall extracts were treated with various chemical reagents and exposed to visible, ultraviolet light (short UV) to study their fluorescence behavior [9].

Table 3: Florescence analysis of *Q. infectoria* Olivier

Treatment	Visible	Long Wavelength UV Light	Short Wavelength UV Light
1N NaOH (Aqueous)	Royal Red	Brown Dark	Brown
1N NaOH (Alcoholic)	Light Golden Yellow	Fluorescent Yellow	Fluorescent Dull Green
1N HCl	Orange Yellow	Light Brown	Greenish Yellow

Acetic Acid	Yellow	Fluorescent Light Green	Fluorescent Light Green
Ethyl Alcohol	Yellow	Fluorescent Orange	Fluorescent Dull Green
H_2SO_4 (50%)	Golden Yellow	Light Brown	Fluorescent Green
Powder as such	Reddish-brown	Yellow	Orange Yellow

Extraction of plant material
The two fractionates of *Quercus infectoria* was prepared with methanol and aqeous.
MethanolicExtract-
Coarsely powdered 100g galls of *Quercus infectoria* was macerated and extracted with 250 ml methanol at room temperature for 7 days and the extract was concentrated, frozen and lyophilized by lyophilizer.
Aqueous Extract- Coarsely powdered 100g galls of *Quercus infectoria* was macerated and extracted with at room temperature for 7 days and the extract was concentrated by placed in oven at not more than 40°C for about 24 hours.
Physical characterization of extract: Different physical parameters of extracts including their colour and percentage yield we reobtained and extracts were weighed and percentage yields were calculated.

Table 4: Physical characterization of extract

S. N o.	Extracts	Extraction Time (days)	Colour	Yield in gm	% Yield
1	Methanolic extract	7	Buff	12.5	25
2	Aqueous extract	7	Brownish	15.1	30.2

Note – 100 g of crude plant material was taken for extraction, The extract was in semisolid- solid form. The extract was stored in tightly packed container.

Preliminary phytochemical screening:
Phytochemical screening means to analyze the plant material for its chemical constituents. It involves the isolation of active constituents and their qualitative identification.
Qualitative chemical test: Extracts of the *Qurecus infectoria* were subjected to qualitative chemical

test to assess the presence of alkaloid, glycosides, proteins, amino acids, steroids, tannins, carbohydrates, phenol compounds by using standard screening procedure [10].

Table 5: Preliminary phytochemical screening of *Q infectoria*

Chemical constituents	Methanolic extract	Water extract
Phenols	+	+
Flavonoids	+	+
Steroids	-	-
Triterpenes	-	-
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Glycosides	-	-
Carbohydrates	+	+

+Denotes the presence of the respective class of compounds.
Chromatographic studies thin layer chromatography: The sample and the standard were applied on silica gel plates. Thin layer chromatograms were developed by using Toluene: ethyl acetate: Formic acid (6:4:0.8 v/v/v) as mobile phase. The development was stopped when the solvent front had advanced about 7.5 cm. After drying plates in air, 10% solution of ferric was used as a spraying agent for the detection. Gallic acid in the sample was identified by comparison with the spot of the reference standard [11].
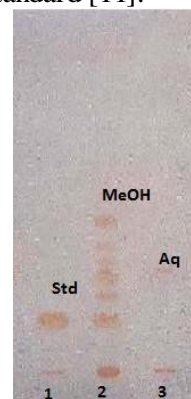

Figure 5: Silica gel thin layer chromatography for *Quercus infectoria*

withstandardgallicacid; 1-Standardgallic acid;2-Methanolic extract,3- Aqueousextract in-vitro Animal study (Anti-depressant activity)

Forced swim test (FST):

Animals: Albino Swiss mice (20-25 g) male

Extract preparation: Suspension of all extracts was prepared in 1% acacia solution. **Standard Preparation:** Suspension of powder fluoxetine tablet equivalent to 2mg/mL was prepared in 1% acacia solution.

Drug treatment: The animals were pretreated orally with 1% acacia suspension of extracts of *Quercusinfectoria* for 7 days daily at the doses of 100, 200 mg/kg/day. All the experimental procedures were started on day 4 and 7day, 1 H after the drug administration. Other group served as control and standard received vehicle (1% acacia solution) and fluoxetine (10mg/kg body weight) respectively orally.

Procedure: All the experimental procedures were started on day 4 and 7day after 1 hof the drug administration. Mice were individually forced to swim in an open cylindrical container (diameter 20 cm, height 30 cm), with a depth of 15cm of water at $25\pm1°C$. The water in the containers was changed after each trial.

Evaluation: The immobility time, defined as the absence of escape-oriented behaviors, such as swimming was scored during 6min. Each mouse was judge to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water [12-13].

Table 6: Effect of extracts of *Quercus infectoria* and fluoxetine on immobility time in the forced swim test in mice

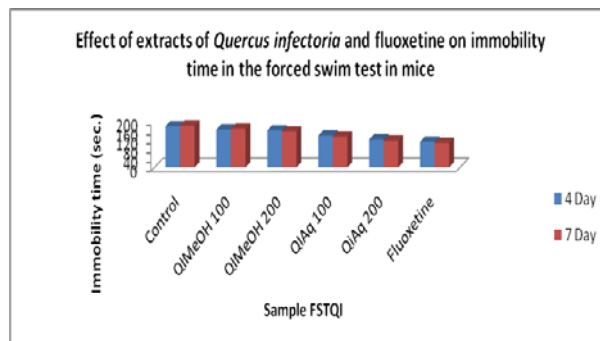| Sample FSTQI	Immobility time (sec.) (Mean±SEM)	
	4th DAY	7th DAY
Control	178.08±3.52	181.11±3.761
QIMeOH 100	164.1±2.195	167.22±5.259
QIMeOH 200	160.20±3.22***	155.20±6.149*
QIAq 100	137.3±4.316***	133.10±4.763***
QiAq 200	121.10±8.89	116.2±2.36***
Fluoxetine	111.02±4.87***	106.13±2.83***

Figure 6: Effect of extracts of *Quercus infectoria* and fluoxetine on immobility time in the forced swim test in mice. Values are expressed as mean±S.E.M.(n=6). *P≤0.05P≤0.01***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)**

Tail suspension test (TST) Animals: Albino Swiss mice (20-25g) male

Extract preparation: Suspension of all extracts was prepared in 1% acacia solution. **Standard Preparation:** Suspension of powder imipramine tablet equivalent to 2mg/mL was prepared in 1% acacia solution.

Drug treatment The animals were pretreated orally with 1% acacia suspension of extracts of *Quercusinfectoria* for 7 days daily at the doses of 100 and 200 mg/kg/day. All the experimental procedures were started on day 4 and 7day after 1 hof the drug administration. Other group served as control and standard received vehicle (1% acacia solution), Imipramine (10mg/kg body weight) respectively.

Procedure: Mice are suspended 50cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail.

Evaluation: The total immobility period was scored manually during 6 minutes test session with the help of stop-watch. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. The parameter obtained was the number of seconds spent immobile. Parameter used was the number of seconds spent immobile [14-15].

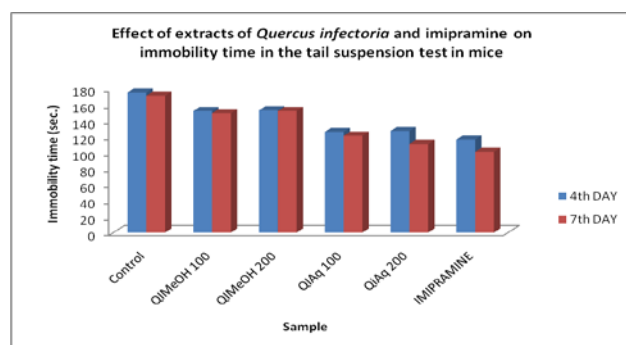**Table 7: Effect of extracts of *Quercus infectoria* and imipramine on immobility time in the tail suspension test in mice**

Sample	Immobility time (sec.)

	(Mean±SEM)	
	4th DAY	7th DAY
Control	175.11 ± 5.2	171.12 ± 2.23
QIMeOH 100	152.2 ± 3.041	149.3± 4.044
QIMeOH 200	153.10 ±3.107*	152.20 ±2.176
QIAq 100	126.20 ± 3.12***	121.20 ± 3.315***
QiAq 200	127.37 ± 4.71***	110.8 ± 2.18***
IMIPRAMINE	116.20 ± 4.140***	101.20 ± 3.116***

Figure 7: Effect of extracts of *Quercus infectoria*and imipramine on immobility time in the tail suspension test in mice. Values are expressed as mean±S.E.M. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Open-field test (OFT) Animals: Albino Swiss mice (20-25g)male

Extract preparation: Suspension of all extracts was prepared in 1% acacia solution. **Standard Preparation:** Suspension of powder fluoxetine tablet equivalent to 2mg/mL g was prepared in 1% acacia solution.

Drug treatment The animals were pretreated orally with 1% acacia suspension of extracts of *Quercusinfectoria* for 7 days daily at the doses of 100, 200 mg/kg/day. All the experimental procedures were started on 7day after 1 hof the drug administration. . Other group served as control and standard received vehicle (1% acacia solution)and fluoxetine (10mg/kg body weight) respectively.
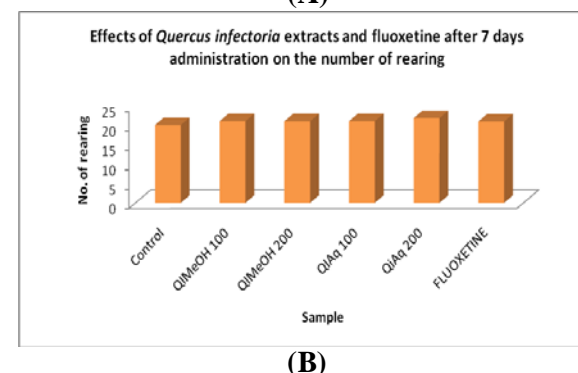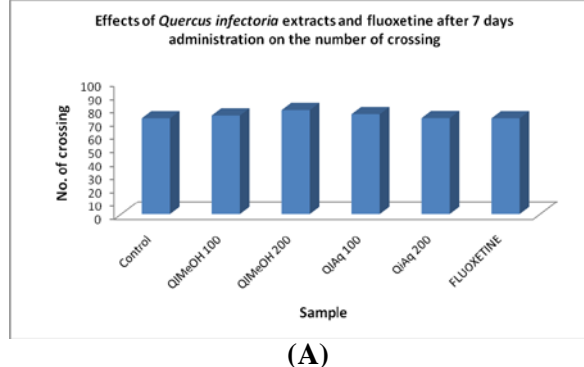
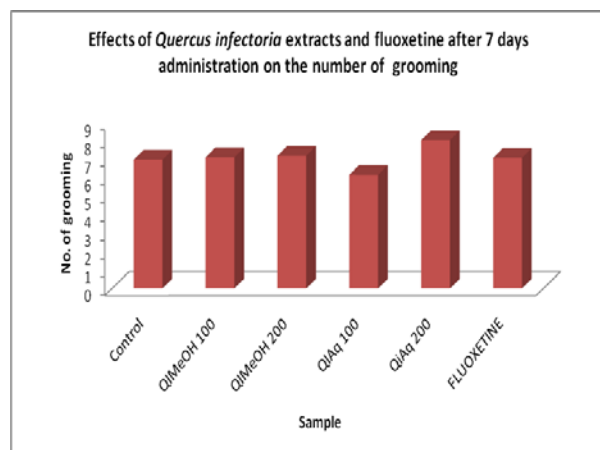**Procedure**: Animals were individually placed in a box (30×30×15cm), with the floor divided into 9 equal squares. The box was cleaned with 10% ethanol after each mouse exposition.

Evaluation: After the habituation to the arena for 5 min, ambulation/Locomotor (the number of squares crossed with all paws), number of grooming and rearing events were observed for 5 minutes [16].

Table 8: Effects of *Quercus infectoria*extracts and fluoxetine for 7 days administration on the number of crossing, rearing and grooming in open field test

Sample	No. of crossing	No. of rearing	No. of grooming
Control	72.13±2.045	20.1±0.904	7.0±0.23
QIMeOH 100	74.20±3.361	21.10±0.526	7.13±0.39
QIMeOH 200	78.20±2.128	21.07±0.92	7.217±0.39
QIAq 100	75.17±2.248	21.10±0.87	6.13±0.381
QiAq 200	72.24±2.370	21.87±0.883	8.07±0.514
FLUOXETINE	72.10±2.120	21.01±0.912	7.10±0.270

(A)

(B)

(C)

Figure 8: Effects of *Quercus infectoria* extracts and fluoxetine after 7 days administration on the number of crossing (A), rearing (B) and grooming (C) in open field test. Values are expressed as mean±S.E.M. (n=6). * P≤0.05 ** P≤0.01 *** P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Results and Discussion
Pharmacognostic study Macroscopic characteristics:
Q. infectoria is a small tree or shrub growing to 4 to 6 feet tall, crooked, with smooth and bright leaves, acorn long and narrow, scaly and downy. The branches often slender and drooping, leaves are elliptical, glabrescent and up to 4 cm long. Petioles are up to 4mm long. Flowers are in axillary fascicles, pedicels filiform. Fruits are baccate and 8mm in diameter. It becomes black while ripening. Root is cylindrical, branched and shows fibrous fracture, 6-10cm long and 4-8mm in thickness. The crude drug is globose with horny appearances on external surface with size of 1.4-2.3 cm in length and 1-1.5 cm in diameter, with brownish-black in colour externally and dark brown buff coloured. Surface is smooth with numerous horny protuberances giving rough touch, and with unpleasant odour (**Table 6.1**). The galls are globular (2 inch), with uneven surface with pores (indicates infection), with hallow structures and inner surface is yellow (**Figure 1**).
Microscopic characteristics: Vascular strands are present at places. Radially elongated sclereids were found touching the lower epidermis. They are

4-7 layered and are interrupted with parenchyma cells at places. Deposition of colouring matter was more concentrated towards the lower epidermis (**Figure 2 – 3**).
Physiochemical constants: The percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash were shown in **Table 2**. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The loss on drying and foreign matter was 9.50 and 0.10 respectively. The extractive values are primarily useful for the determination of exhausted drugs.
Fluorescence analysis:
Fluorescence analysis of the drug powder is presented in **Table 3**.
Extraction of plant material: The yield of methanolic extract of *Qurecus infectoria* was found to be 12.5 g (% Yield-25%) with buff colour mass after 7 days and the yield of aqueous extract *of Qurecus infectoria* was found to be 15.1 g (% Yield-30.2%) with brownish colour mass after 7 days. The extract was in semi-solid form.
Preliminary phytochemical analysis:
Investigations on the preliminary phytochemical screening of *Quercus infectoria* galls extracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and carbohydrates in methanolic and aqueous extracts respectively (**Table 5**).
Chromatographic studies: As the gallotannic acid is hydrolysable tannin which, on dissolution in water hydrolysed to gallic acid. So, gallic acid was used as a standard for thin layer chromatography. Retension factor (Rf) value obtained for standard gallic acid was 0.36 & 0.36 with methanolic extract of galls (**Figure 5**). The retension factor was same for the standard & test compound reflecting the presence of gallic acid which was formed from gallotannic acid.
Antidepressant Activity:
Effects of *Qurecus infectoria* extracts on the immobility time in the Force swim test: The methanol extract of *Qurecus infectoria* induced a significant antidepressant effect in the FST because it significantly reduced the immobility time compared with the vehicle treated group (181.11±3.761) (**Figure**

6).The methanol extract of *Qurecus infectoria* the immobility time was found to be 167.22±5.259, 155.20±6.149 and for aqueous extract was 133.10±4.763, 116.2±2.36 for the doses of 100 and 200mg/kg/day on 7th day respectively. The standard group treated with fluoxetine(10mg/kg)exhibited powerful activity (106.13±2.83). No significant difference observed in immobility time of *Qurecus infectoria* extracts on 4th day and 7th day in FST.

Effect of *Qurecus infectoria* extracts on the immobility time in the tail suspension test: In the TST, the chloroform extract of *Qurecus infectoria* showed a significant effect on decreasing the immobility time, compared with the vehicle-treated control group(171.12 ± 2.23 sec)(**Figure 7**). The mean immobility time of the methanol extract of *Qurecus infectoria* treated group for 100 and 200mg/kg dose was 149.3± 4.044 and 152.20 ±2.176 sec.,respectively. While aqueous extract of *Qurecus infectoria* showed significant effect for 100 and 200mg/kg dose was 121.20 ± 3.315 and 110.8 ± 2.18 sec.,respectively when compared with control. The standard group treated with imipramine (10 mg/kg), also significantly diminished the immobility time(101.20 ± 3.116 sec.). No significant difference observed in immobility time of *Qurecus infectoria* extract on 4th days and 7th days in TST.

No significant differences were observed in the number of squares crossed, rearing and grooming between vehicle treated group and *Qurecus infectoria* extracts as well as standard treated groups (P <0.05) (**Figure 8**). Although *Qurecus infectoria* has been used to treat nervous shock in traditional medicine, its specific neuropharmacological activities have not been demonstrated yet. Principal value of UDP is to minimizing the number of animals required to estimate the acute oral toxicity of *Qurecus infectoria* extract and estimating the median lethal dose. *Qurecus infectoria* extract not showed toxicity. The forced swim test and tail suspension test are the most common animal models of depression used for antidepressant screening. In both tests,animals are placed in an inescapable situation and the antidepressant like activity is expressed by the decrease of immobility time (Hurley et al.,

2014; Porsolt et al.,2001).In the FST,mice are forced to swim in a restricted space from which they cannot escape and are induced to assume a characteristic behavior of immobility.This behavior reflects a state of despair or lowered mood, which can be reduced by several agents that are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in the FST. The chloroform extract of HPS decreases immobility time while methanol extract not showed any effect in TST as well as FST. Methanol extract of *Qurecus infectoria* showed dose dependent activity. This immobility in TST and FST referred to as behavioral despair in animals is believed to reproduce a condition similar to human depression. The compounds which able to increases locomotor activity in OFT including psychostimulants, convulsants and anticholinergics give a false positive result in TST and FST (Farah Idayu et al., 2011).In general, hyperkinesis also produces false positive effect in TST and FST by shortening the immobility time(Freitas et al.,2010). Therefore, OFT was used to exclude these false effects that could be associated with psychostimulants,convulsants and anticholinergics or hyperkinesis activity(Kwon et al.,2010).The main difference between antidepressants and psychostimulants is that antidepressants would not increased locomotor activity.

Conclusion
Depression means selective diminution of activity of specialized cells, e.g. barbiturates depresses CNS, quinidine depresses heart. Certain drugs stimulate one type of cells but depress the other, e.g. acetylcholine stimulates intestinal smooth muscle but depresses SA node in heart. Thus, most drugs cannot be simply classed as stimulants or depressants.The psychopharmacological agents or psychotropic drugs are those having primary effects on psyche (mental processes) and are used for treatment of psychiatric disorders. During the past 60 years psychiatric treatment has witnessed major changes due to advent of drugs which can have specific salutary effect in mental illnesses. Depression is the second leading psychiatric disorder where 21 % of the world population suffers from this disease. However, the neuropharmacological activity of this plant is not

investigated extensively which influenced us to design our study. The present study investigated the antidepressant-like effect of *Qurecus infectoria* in different behavioral model of depression in mice. The result was concluded that thatreductionofimmobility time elicited by methanolic extract of *Qurecus infectoria* in FST as well as in TST was specifically arises viaits antidepressant mechanism. In TST and FST chloroform extract of *Qurecus infectoria* decreasesimmobility time which is not due to any psychostimulant, anticholinergic, convulsanteffector hyperkinesis activity.

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