ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

PHYTOCHEMICAL STANDARDIZATION AND ANTIDEPRESSANT ACTIVITY OF LEAVES OF *LAWSONIA INERMIS*

[1]Vivek Kumar Seth, [1]Shilpi Mishra, [1]Ashish Mishra

[1]Department of Pharmacognosy, Faculty of Pharmacy, Advanced Institute of Biotech and Paramedical Sciences, Kanpur, UP, India

Corresponding Author e-mail id: Vivekseth88@gmail.com

ARTICLE INFO

Key words:

Lawsonia inermis leaves
In-vivo antidepressant activity

Access this article online
Website:
https://www.jgtps.com
Quick Response Code:

ABSTRACT

Mental depression is a distresses person's mood, thoughts, physical health and behaviour with chronic illness. The biological and emotional components are also attached with symptoms of depression. The retardation of thought, action and appetite are biological symptoms & emotional indicators include mystery, apathy and pessimism, low self- esteem consisting of feeling of guilt, inadequacy and ugliness, indecisiveness and loss of motivation. Patients with major depression have symptoms that reflect changes in brain, monoamine neurotransmitters, specifically nor epinephrine, serotonin, dopamine. The reasons for the disease include stimulation of MAO-A, inhibition of NA and 5-HT. Symptoms include the diminished interest of pleasure, feelings of worthlessness or inappropriate guilt, a decrease in appetite and libido, insomnia, and recurrent thoughts of death or suicide. Many scientists are researching plant material for treating this disorder and there are lots of publications on it. Several drug-drug interactions can also occur. These conditions create an opportunity of alternative treatment for depression by the use of medicinal plants. Since all the synthetic drugs available for the treatment of depression have various adverse effects associated with problematic interactions, Although a number of synthetic agents are being used as the standard treatment for depression. They have adverse effect that can compromise the treatment like dry mouth, fatigue, anxiety, agitation, drowsiness, cardiac arrhythmia, gastrointestinal or respiratory problems and several dug –drug interactions can also occur. These conditions create an opportunity for alternative treatment of depression by used of medicinal plants and herbs.

INTRODUCTION

Mental health is a dynamic state of inner stability which enables individuals to use their abilities in harmony with universal values of society. Necessary cognitive and social skills; ability to recognize, express and modulate one's own emotions, as well as identify with others; flexibility and ability to cope with adverse dealing with life and function in social roles; and harmonious association between body and mind represent essential components of mental health which contribute to differ degrees, to the state of internal stability. Various researchers found that mental illness and weak economic status are related. The relationship between poverty and mental disorders is universal and found across societies irrespective of levels of development. Hopelessness, insecurity, rapid social change, the risks of violence and disease are factors responsible for the vulnerability of poor people to mental illnesses [1]. Depression is the 4th leading contributor to the global burden of disease that affects 17-20% of the people resulting it contribute major social as well as economic problems. Fortunately, It's treatment is also available. It can increase the risk of variety of

physical problems as well as emotional problems. It can also inhibit your daily life style activity like function at home or work. The symptoms of depression are including feelings of sadness, suicidal thoughts, loss of appetite, irregular sleep pattern, loss of energy, feeling of hopelessness and despair, loss of interest and pleasure, loss of confidence or feeling guilt and poor concentration [2]. Mental disorders include various types of problems, with different symptoms. However, they are usually characterized by some combination of abnormal thoughts, emotions, behaviour, and relationships with others. Examples are schizophrenia, depression, intellectual disabilities, and disorders due to drug abuses. Mental disorders today are conceptualized as behavioral or psychological syndromes that occur in a person in response to the distress, disability or suffering, not merely the expectable or usual response to a particular event [3]. National survey of Americans found that 18.5% percent of adults (18 or older) experienced a mental illness in any one year. This result is equivalent to 43.8 million people [4]. Depression is a severe problem of every age group in world wild but In Western countries depression and schizophrenia are most often seen by the public as caused by the social environment, particularly recent stressors [5-8]. While psychiatric epidemiologists would concur about the importance of stressful life procedures in depression, in schizophrenia life events are additional of a trigger than a cause. Biological factors are seen by the public as less significant than environmental ones, while relations of people with schizophrenia are more likely to see biological factors as required provided that the label schizophrenia‘ to a vignette has also been found to raise the likelihood that biological rather than psychosocial cause is seen as responsible. In some non-Western cultures, supernatural phenomena, such as witchcraft and possession by evil spirits, are seen as significant causes of mental disorders [9], although this is uncommon in the West [10]. Medicinal plants are being widely used, either as single drug or in combination in health care delivery system. Lawsonia inermis (henna), which is recognized in traditional system of medicine. It has been traditionally reported in use of headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilitis, sores, amenorrhea, scabies, diseases of the spleen, dysuria, bleeding disorder, skin diseases, diuretic, antibacterial, antifungal, anti-amoebiasis, astringent, anti-hemorrhagic, hypotensive and sedative effect. Several studies and researches are being carried out towards it’s activities and The researcher reveals the cytotoxic , hypoglycaemic , nootropics, antimicrobial, antibacterial , trypsin inhibitory , wound Healing , antioxidant, hepatoprotective, anti-parasitic,anti-inflammatory, analgesic and antipyretic , tuberculostatic , protein glycation inhibitory , anti-tumoral activity. With all these potential benefits, this plant is not widely investigated as antidepressant agent. The further research is proposed as antidepressant activity of this plants drug to explore the hidden potential and its uses, towards the benefit of mankind**.** our aim is to explore the potential of medicinal plants in the management of depression. The present study is proposed *Lawsonia inermis* leaves have more potent activity for management of depression due to presence of more phytochemical constituents. These phytochemical constituents have antidepressant activity as previous scientist work. Thus, the proposed part of plant have maximum potent phytochemical constituent for justified the proposed work. *Lawsonia* inermis is a traditional plant of the lythraceae family. *Lawsonia* is named after Isaac Lawson (a Scottish army doctor who was a friend of Linnaeus in 18th century and inermis means unarmed without spines). It is also known as Henna, is basically indigenous to Middle East but found to many other parts of the world. Historians claim that henna has been used for 5000 years as a medicine and cosmetic. In pre-history, decorating of human body parts (hair, hands etc) and ritual painting was started and henna is mostly used for this art. Henna is used to celebrate weddings, births, festivals and circumcisions. Henna is applied to the skin and hair. Its active principles provide cooling and astringent effect and also provide protection against

many fungi and bacteria. It can also help in reduce the elevated body tmperature, to soothe headaches, burning feet. Leaves contains Lawsone (2-Hydroxy-1, 4-napthoquinone) is the principle natural dye contained at 1.0-1 .4 %. Other related compounds are: 1, 4dihydroxynaphthalene.1, 4- naphthoquinone, 1,2-dihydroxy-glucoyloxynaphthalene and 2-hydroxy-1,4-diglucosyloxynaphthalene,Flavonoids (luteolins, apigenin, and their glycosides),Coumarins (esculetin, fraxetin, scopletin). Steroids (β-sitosterol). The leaves of Lawsonia inermis also contain soluble matter tannin, gallic acid, glucose, mannitol, fat, resin and mucilage. Bark contains napthoquinone, isoplumbagin, triterpenoids-Hennadiol, aliphatics. Flower on steam distillation produce an essential oil (0.02 %) rich in ionones (90 %) in which β-ionones predominated.

MATERIAL AND METHODS

All chemicals and solvents were of analytical grade (AR Grade) and were purchased from Sigma Aldrich, Ranbaxy fine chemicals Ltd., LOBA chemicals Ltd., S.D. fine chemicals Ltd., Spectrochem chemicals. Pre-coated TLC plates having silica gel 60 F254 thickness 0.2 mm were purchased from Merck. All the solvents used for HPLC analysis were purchased from JT Baker and Fischer scientific Ltd.

Collection and authentication of plant material: The selected plant material *Lawsonia* inermis **leaves** were collected from local area of Lucknow, (U. P.) India, and authenticated by botanist.

Macroscopic studies: The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Physicochemical Evaluation: Physiochemical qualities, for example, ash values and extractive values were researched for chose plant as the official strategies and as per WHO guidelines. Ash values (Total ash, Acid insoluble ash and Water soluble ash), Extractive values, Loss on drying and pH were determined for *Lawsonia inermis leaves*.

Extraction Process of Drug: Extraction includes partition of bioactive segment of the plant tissues from the latent moiety by utilizing specific solvents in standard extraction systems. Plant herbs were extracted successively with hexane, and ethanol utilizing maceration method of extraction. The totally dried leaves of ***Lawsonia inermis*** was coarsely powdered and afterward extracted with non polar solvent hexane for defatting of plant material. Leaves powder (100g) were stuffed in vessel and kept with hexane for 24 hours and procedure was repeated till complete extraction. The plant material then kept with ethanol for 24 hours and procedure was repeated till complete extraction. The obtained ethanol extract were filtered and concentrated on rotatry evaporator to get methanol extract.

Prelimnary phytochemical analysis of extracts: Qualitative test as Phytochemical examination of any plant species is a vital procedure as it give the starter data about presence of different chemical constituents and furthermore gives further possibilities of the specific plant species in its future research examinations. The extracts acquired by extraction methods were exposed to different chemical tests to recognize the presence of a class of chemical constituents i.e. are presence of alkaloid test, carbohydrates tests, glycoside tests, phytosterols and Triterpenoids test, protein and amino acids test, phenolic and tannins test, flavonoids test, saponins test.

***In-vivo* pharmacological screening (Anti-depressant activity):**

Drugs and Chemicals: All the biochemicals employed in these investigations were of highest purity and procured from Sigma company USA, Merck Germany, Sisco Research Laboratory, Mumbai, Qualigens Mumbai, Across Organics Mumbai, Spectrochem, Mumbai or S.D. Fine chemicals Mumbai. All the organic solvents were of AR grade. Spectrophotometer (Schimatzu model UV 1601) double beam, spectroflurometer (Elico model), refrigerated super speed centrifuge (Sorvall RC-5B model), light microscope (Lynx, Lawrence and Mayo) were used for the preparation and estimation of biological samples.

Experimental animals: The animal experiments, male mice weighing about 100-125g were used. These mice had can be able to access laboratory feed and water under standard laboratory conditions. The animals used in the present study were maintained in accordance with the guidelines of National Institute of Nutrition, India and approved by Institutional Animal Ethics Committee (IAEC). Experiments performed by an observer who was unaware of the each treatment, were carried out between 1- 3p.m. For the behavioral test, different doses of the extract were separately suspended in a vehicle comprising 1% (w/v) tween 20 in distilled water and a standard drug (amitriptyline and fluoxetine) were given by gastric gavage once a day over a period of 1,3, 7, 14 and 21 days. Behavioral test was conducted 1 hour after the last treatment/administration.

Forced swimming test (FST):

Animal groups: The activity was performed with 42 total mice. We were randomly divided into 7 groups, each group having 6 mice. The mice of each group were treated accordingly treatment given in **Table 4** and treated as follows: Group 1 act as normal control and Group 2 have tween-20 suspensions and act as experimental control (FST group). Group 3-5 were orally administered with various doses of ethanol extract of Lawsonia inermis having three different doses. Group 3 treated as 100 mg extract/kg of body weight, Group 4 treated as 200 mg extract/kg of body weight, Group 5 treated as 300 mg extract/kg of body weight. The animals present in Group 6 and 7 received standard anti- depressant drug-amitriptyline and fluoxetine (10 mg/kg body weight).

Experiment design: The FST conducted in mice as all the groups of mice were subjected to swimming test except group 1 in a cylindrical glass aquarium (50 x 30 cm diameter), containing 25±2ºC water. Mice were allowed to swim for 6 min and the duration of immobility was measured during the final 4 min interval of the test using a video tracking system. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. Following swimming sessions, they were then towel dried. In order to determine the time-dependent effects on immobility time, oral treatments with LIEOH for 1, 3, 7, 14 and 21 consecutive days were investigated [11-12]

Tail suspension test (TST):

Animal groups: The activity was performed with 42 total mice. We were randomly divided into 7 groups, each group having 6 mice. The mice of each group were treated accordingly treatment given in **Table 5** and treated as follows: Group 1 act as normal control and Group 2 have tween-20 suspensions and act as experimental control (TST group). Group 3-5 were orally administered with various doses of ethanol extract of Lawsonia inermis having three different doses. Group 3 treated as 100 mg extract/kg of body weight, Group 4 treated as 200 mg extract/kg of body weight, Group 5 treated as 300 mg extract/kg of body weight. The animals present in Group 6 and 7 received standard anti- depressant drug-amitriptyline and fluoxetine (10 mg/kg body weight).

Experiment design: A box having each wall side with 35cm was used for the tail suspension test. The front surface of the apparatus was open and each mouse was suspended by fixing the tail in the centre of the upper surface using a tail hanger and non-irritant adhesive tape with the head 5 cm to the bottom. The experiment was performed in darkened room with minimal background noise for duration of 5 min. The total duration of immobility (total immobility time) was observed and measured during the final 4 min interval of the test period. All test sessions were recorded by a video camera positioned directly above the box. Mice were considered immobile only when they hung passively and completely motionless [13-14].

Elevated plus maze test: Elevated plus maze test (EPMT) is most broadly utilized as well as approved method to quantify anxiety in animal models. Mechanical assembly comprised of 4 arms of which 2 were remained open along with 2 shut. Open arms

(35 cm2 x 5 cm2) were crossed with shut arms (35 cm3 x 5 cm3 x 20 cm3) at an inside point (5 cm2 x 5 cm2). Behavioral testing was performed under dim light in a noise-attenuated room. Animals were treated with separate treatment groups and following half-hour, they were exclusively put on EPM device at the middle, confronting one of the shut arms. Duration (in a moment or 2) spent by every one of the animal on open and shut arms was noted for 300 seconds. Evaluation of the anxiolytic-like effects was based on behavioral measures of the time spent in the open and closed arms or in the center platform (expressed as a percentage of total test time), and on the number of open arm entries (OAE). An entry into a specific arm was scored when a mouse placed all four paws into the arm. The other parameters studied were the number of head dipping (DIP, exploratory movement of head/shoulders over the sides of the maze), and stretch-attend postures (SAP, exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward) [14].

RESULTS AND DISCUSSION

Macroscopic studies: A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc. *Lawsonia* inermis leaves occur as short, smooth, compound, ovate-lanceolate, acute, symmetrical, entire, pinnate, opposite, sweet smelling, characteristics or bitter in taste and varies in length, Colour green and No adulterants have been found as foreign organic matter. Lawsone is mainly present in the marginal vein or petiole in large quantity**.**

Physicochemical Evaluation: Physicochemical parameter such as Ash values (Total ash, Acid insoluble ash and Water soluble ash), Extractive values, Loss on drying and pH of all selected plant drugs were performed. Ash values of crude drug provide an idea about the inorganic composition or earthy matter and other impurities present in drug. All parameters of selected drugs found within the limit as per API. Results of physicochemical parameters are shown in **Table 1.** Physicochemical parameters are total ash (12.09 %), acid insoluble ash (2.98 %), and water soluble ash (4.21 %). Loss on drying was found to be (4.91 %) w/w. Alcohol soluble extractive value and aqueous extractive value was 4.21 % w/w and 5.48 % w/w respectively. The extractive values are mainly useful for the determination of adulterated or exhausted drug and extractive values are shown in **Table 2.**

Extraction process of drug: Methanol extract of *Lawsonia* inermis leaves drugs obtained by maceration method after defatting of leaves with hexane. Defatting of leaves with nonpolar solvent cause removal of chlorophyll and fatty material which can further hindered the activity of plant extract.

Preliminary phytochemical analysis of extracts: Extract (ethanol extract) of selected plant *Lawsonia* inermis leaves drugs obtained by maceration method was subjected to qualitative phytochemical tests to identify the presence of secondary metabolite (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. In ethanolic extract carbohydrate, glycosides, tannins, phenolic compounds and gums and mucilage were present and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent. Results are presented in **Table 3.**

***In-vivo* antidepressant activity (Anti-depressant activity):**

Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on body weight mice (FST and TST groups): The effect of extract on the body weight change is presented in Table 6. All the result was showed that there was no difference in body weight gain by the animals among all the groups subjected to 1-day, 3-days and 7-days treatment. As the treatment began from 7 days to two to three week or 14 days to 21 days, a slight increase in weight gain was observed after oral administration. The weight gain of mice may be the normal weight gain of rats. It is confirmed that, administration of LIEOH

did not have any effect on the weight of animals.

Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST and TST groups): In FST, mice are forced to swim in a restricted space from which they cannot escape and are induced to a characteristic behavior of immobility. This behavior, reflecting a state of despair is reduced by several agents; these are therapeutically effective in human depression. The TST also induces a state of despair in animals like that in FST. This immobility referred to as behavioral despair in animals and also recognized as a condition similar to human depression, this was investigated by possible time-dependent effects on immobility time. The oral treatments with LIEOH for 1, 3, 7, 14 and 21 consecutive days respectively were investigated under the standardized application schedule preceded by the appropriate vehicle control application. A reduction in the duration of immobility of animals in the FST reflects their anti-depressant-like performance. LIEOH administration showed a significant activity to reduce the immobility time at doses of 100, 200 and 300 mg/kg in forced swimming test in dose dependent manner in mice. The effects of LIEOH, amitriptyline and fluoxetine on immobility in mice FST are presented in **Table 6 -7** and **Figure 1** and TST are presented in **Table 8 - 9** and **Figure 2** respectively. The clinical anti-depressant effects often appear after chronic treatment, in mice in FST and TST. The result indicated that the slight decrease in immobility time showed after 3rd days and 7th days treatment and the decrease was non-significant at $p<0.05$. The mice were able to swimming after LIEOH pre-treatment for 14th days and 21st days. There was a reduction in the duration of immobility started compared with stress control and the effect was observed with the classical anti-depressant drug fluoxetine and amitriptyline. LIEOH at 300 mg/kg b. wt. exhibited significant decrease in immobility duration after oral treatment for 14-days. After 21-days treatment of LIEOH, there was a significant treatment effect for dose in immobility time. The maximal effect was observed at 300mg/kg b.wt. allowed to reduction in immobility time with reference as anti-depressants drug as amitriptyline and fluoxetine.

Elevated plus maze test: Elevated plus maze test (EPMT) is most broadly utilized as well as approved method to quantify anxiety in animal models. Evaluation of the anxiolytic-like effects was based on behavioral measures of the time spent in the open and closed arms or in the center platform (expressed as a percentage of total test time), and on the number of open arm entries (OAE). An entry into a specific arm was scored when a mouse placed all four paws into the arm. The other parameters studied were the number of head dipping (DIP, exploratory movement of head/shoulders over the sides of the maze), and stretch-attend postures (SAP, exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward). There was a reduction in the duration of immobility started compared with stress control and the effect was observed with the classical anti-depressant drug fluoxetine and amitriptyline. LIEOH at 300 mg/kg b. wt. exhibited significant decrease in immobility duration after oral treatment for 14-days **(Table 10 and Figure 3).**

Table 1: Physicochemical parameters of *Lawsonia inermis* leaves

S. No.	Physicochemical parameter values (% w/w)	Lawsonia inermis leaves
1	Total ash	12.09
2	Water soluble ash	4.21
3	Acid insoluble ash	2.98
4	Loss on drying	4.91
5	Foreign organic matter determination	1.1

Table 2: Solvent extractive values (%w/w) of *Lawsonia inermis* leaves

S. No.	Name of extract	Extractive value *Lawsonia inermis*
1	Alcohol soluble extractive value	4.21 % w/w
2	Water soluble extractive value	5.48 % w/w

Table 3: Phytochemical analysis of *Lawsonia inermis leaves* extracts

S. No.	Phytochemical	Indication test	Ethanol extract
1	Alkaloid	Dragendorff test	-
2	Napthoquinone	Juglone test	+
2	Steroid	Salkowaski test	-
3	Carbohydrates	Molish test	+
4	Triterpene	Vanillin-sulphuric acid test	-
5	Tannin	Ferric chloride test	+
6	Glycosides	Keller-killani test	+
7	Protein	Biuret test	-
8	Flavonoid	Shinoda Test	+
9	Saponin	Lead acetate test	-
10	fixed oils, fats		-

Where + is Present and – is Absent

Table 4: Experiment animal groups (FST)

Animal groups	Treatment
Group 1	Normal control
Group 2	Tween-20 suspensions + FST
Group 3	LIEOH (100 mg/kg b wt.) + FST
Group 4	LIEOH (200 mg/kg b wt.) + FST
Group 5	LIEOH (300 mg/kg b wt.) + FST
Group 6	Amitriptyline (10 mg/kg b wt.) + FST
Group 7	Fluoxetine (10 mg/kg b wt.) + FST

Table 5: Experiment animal groups (TST)

Animal groups	Treatment
Group 1	Normal control
Group 2	Tween-20 suspensions + TST
Group 3	LIEOH (75mg/kg b wt.) + TST
Group 4	LIEOH (150mg/kg b wt.) + TST
Group 5	LIEOH (300mg/kg b wt.) + FST
Group 6	Amitriptyline (10mg/kg b wt.) + TST
Group 7	Fluoxetine (10mg/kg b wt.) + TST

Table 6: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on body weight in mice (FST groups)

Body weight (g) during different treatment period							
Days	Groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	107.8±2.1	106.6±1.5	109.2±2.1	110.5±2.1	108.5±3.6	106.4±1.4	104.3±1.4
3	108.4±1.2	108.8±1.7	111.6±2.5	116.2±1.1	110.5±2.6	107.9±2.1	106.1±1.6
7	110.6±21	111.4±3.2	113.4±3.4	117.4±2.4	111.4±3.8	110.2±2.1	107.5±1.6
14	111.5±1.9	115.4±2.1	116.1±3.3	119.5±3.6	113.3±3.3	111.7±2.1	109.7±2.2
21	114.3±2.1	118.9±2.3	122.8±1.9	121.4±2.1	113.8±2.1	112.8±1.3	112.4±1.2

Values are presented as the mean ± SD (n=8). There were no significant differences at p<0.05.

Table 7: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST groups)

Group	Dose mg/ kg b.wt	Duration of immobility (s)				
		Days				
		1	3	7	14	21
Group 1	-	-	-	-	-	-
Group 2	-	112.1±7.1	102.1±3.1	108.1±6.3	103.4±5.1	105.4±3.9
Group 3	75	103.4±6.6	87.1±4.1[a]	89.1±3.6	75.4± 4.1[a]	66.2±3.6[a]
Group 4	150	98.2±8.3	84.5±3.3[a]	76.2±4.3[a]	59.7±5.1[a]	57.4±5.7[a]
Group 5	300	89.3±86.1[a]	76.1±4.1[b]	66.1±4.7[b]	41.8±2.1[b]	24.2±4.6[b]
Group 6	10	73.1±7.2[b]	66.3±6.1[c]	56.2±5.1[c]	29.2±5.4[b]	15.4±2.9[c]
Group 7	10	79.4±9.2[a]	69.6±3.1[b]	67.1±3.1[b]	36.0±4.6[c]	22.5±2.2[b]

Values are presented as the mean ± SD (n=8). Values bearing different superscripts in the same column are significantly different (p<0.05) (ANOVA).

Table 8: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on body weight in mice (TST groups)

Body weight (g) during different treatment period							
Days	Groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	102.1±2.2	102.1±11	103.1±1.3	106.1±1.1	103.1±1.6	100.1±1.1	100.3±1.1
3	105.7±1.7	103.8±1.1	105.4±1.2	107.1±2.1	104.2±1.1	100.7±1.5	101.2±1.3
7	108.6±11	105.1±2.2	106.3±1.4	109.1±1.4	105.1±1.3	101.1±1.7	102.1±1.1
14	111.1±1.2	107.3±2.1	106.9±2.3	110.2±2.1	108.7±1.2	102.2±2.2	103.2±1.1
21	115.5±2.3	109.2±1.1	107.2±2.1	112.1±1.1	109.2±1.8	103.2±2.1	104.1±1.1

Values are presented as the mean ± SD (n=8). There were no significant differences at p<0.05.

Table 9: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (TST groups)

Group	Dose mg/ kg b.wt	Duration of immobility (s)				
		Days				
		1	3	7	14	21
Group 1						
Group 2	-	93.3±3.9	92.7±6.1	93.4±4.3	91.4±3.7	89.1±5.7
Group 3	75	87.5±3.6	85.6±6.8[a]	84.4±4.3	78.7±4.7[a]	75.2±1.7[a]
Group 4	150	86.1±3.1	77.8±6.6[a]	73.7±3.2	74.6±3.6[a]	69.6±2.2[a]
Group 5	300	86.6±3.4	71.4±5.1[b]	66.1±4.4[a]	65.8± 4.7[b]	60.2± 3.7[b]
Group 6	10	79.5±3.1	67.7±6.4[b]	59.7±3.1[a]	56.4±3.4[b]	51.4±2.4[b]
Group 7	10	74.8±3.6	67.0±4.6[b]	55.4±4.6[a]	49.5±3.7[b]	46.5±4.1[b]

Values are presented as the mean ± SD (n=8). Values bearing different superscripts in the same column are significantly different (p<0.05) (ANOVA).

Table 10: Effect of LIEOH, on time spent in open arms and time spent in closed arms in elevated plus maze model

Group	Dose mg/ kg b.wt	Time Spent in Open Arms (s)	Time Spent in Closed Arms (s)
Group 1		32.15±3.05	216.17±3.11
Group 2	-	101±7.92	189.04±4.19
Group 3	75	51.02±4.12	241.21±2.14
Group 4	150	71.02±5.02	210.11±3.84
Group 5	300	93.04±6.02	207.14±4.24

Values are presented as the mean ± SD (n=8). Values bearing different superscripts in the same column are significantly different (p<0.05) (ANOVA).

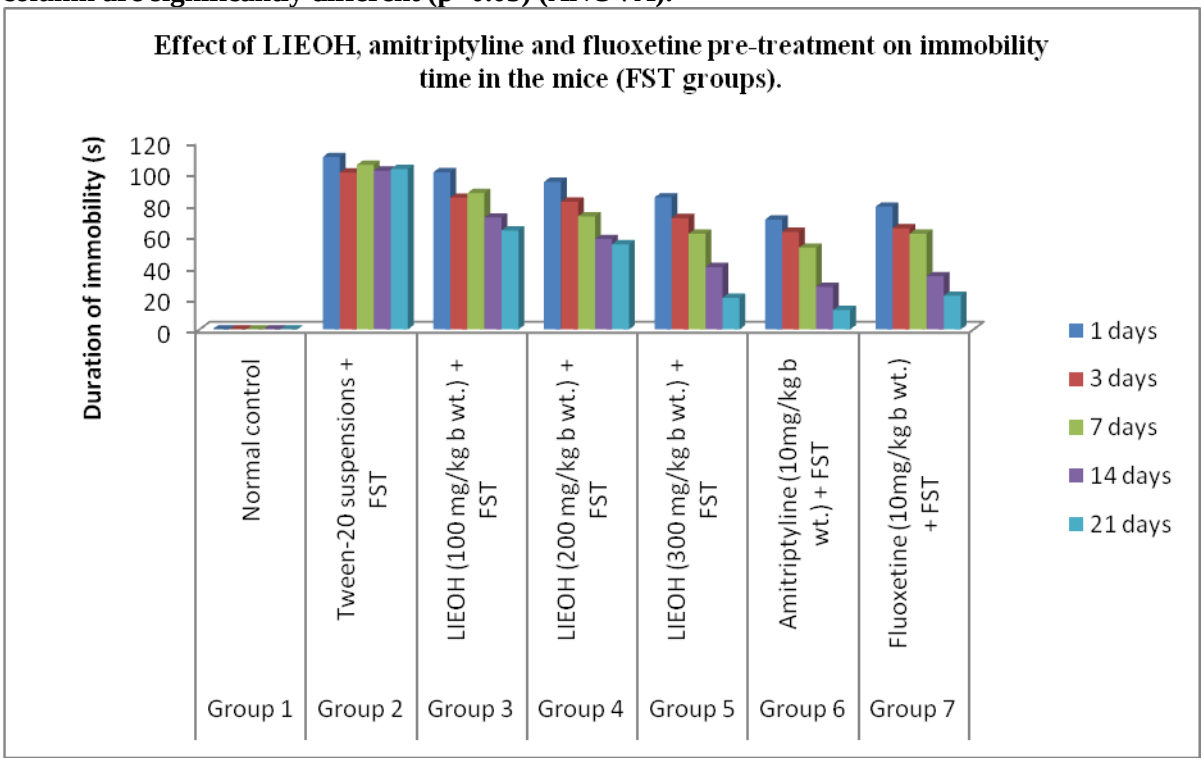

Figure 1: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (FST groups)

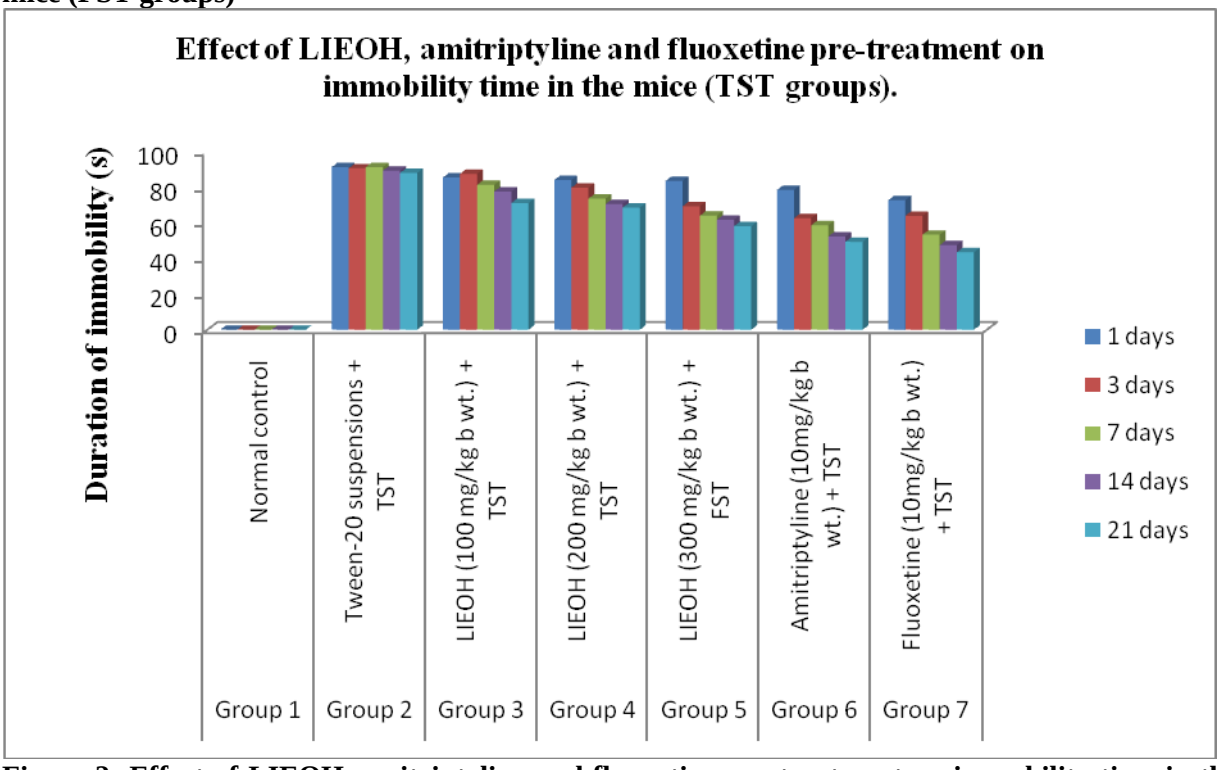

Figure 2: Effect of LIEOH, amitriptyline and fluoxetine pre-treatment on immobility time in the mice (TST groups)

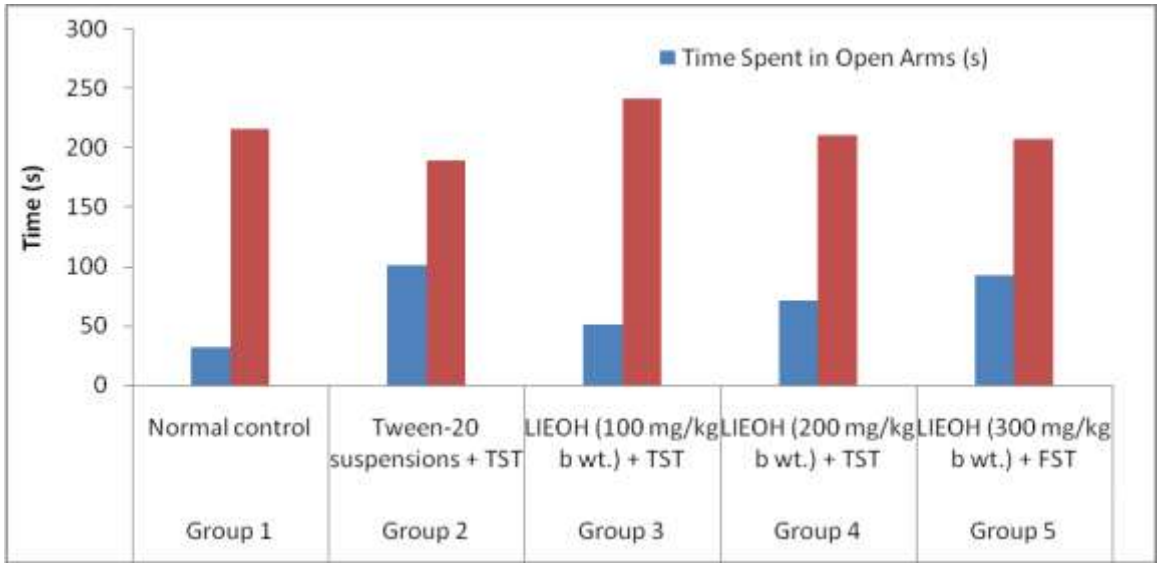

Figure 3: Effect of LIEOH on time spent in open and closed arms in the mice (Elevated plus maze)

SUMMARY AND CONCLUSION

Mental depression is biological and emotional components are also attach with symptoms of depression include mystery, apathy and pessimism, low self- esteem consisting of feeling of guilt, inadequacy and ugliness, indecisiveness and loss of motivation. Patients with major depression have symptoms that reflect changes in brain, monoamine neurotransmitters, specifically nor epinephrine, serotonin, dopamine. Several drug-drug interactions can also occur. These conditions create an opportunity of alternative treatment for depression by the use of medicinal plants. Since all the synthetic drugs available for the treatment of depression have various adverse effects associated with problematic interactions, our aim is to explore the potential of medicinal plants in the management of depression. The present study is proposed *Lawsonia* inermis leaves have more potent activity for management of depression due to presence of more phytochemical constituents have maximum potent phytochemical constituent for justified the proposed work. The treatment of ethanol extract of *Lawsonia* inermis leaves with effective dose to 150 mg to 300 mg, there was a produce significant effect for dose in immobility time of all animals present in different groups.

The maximal effect was observed at 300mg/kg body weight allowed to reduction in immobility time with reference as anti-depressants drug as amitriptyline and fluoxetine. So, it was concluded that the ethanolic extract of *Lawsonia* inermis leaves was able to treat depression produce in mice and as well as in human with effective concentration justified in present study.

REFERENCES

1. Patel V, Kleinman A. “Poverty and common mental disorders in developing countries.” Bulletin of the World Health Organization, 2003 81, 609-615.
2. Galderisi S, Heinz A, Kastrup M, Beezhold J, Sartorius N, “Toward a new definition of mental health.” World psychiatry : Official journal of the world psychiatric association (WPA), 2015, 14, 2, 231–233.
3. Taube-Schiff M, Lau MA. In Major depressive disorder. Hersen, M.–Rosqvist, J.(szerk.) Handbook of psychological assessment, case conceptualization, and treatment, 2008, 1, 319-351.
4. Addis ME, Jacobson NS, “Reasons for depression and the process and outcome of cognitive–behavioral psychotherapies.” Journal of consulting and clinical psychology, 1996, 64, 6, 1417.

5. McKeon P, Carrick S. "Public attitudes to depression: A national survey. Irish journal of psychological medicine." 1991, 8, 2, 116-121.
6. Matschinger H, Angermeyer MC. "Lay beliefs about the causes of mental disorders: a new methodological approach." Social psychiatry and psychiatric epidemiology, 1996, 31, 6, 309-315.
7. Jorm AF, "Mental health literacy: Public knowledge and beliefs about mental disorders." The British Journal of Psychiatry, 1990, 177, 5, 396-401.
8. Jorm AF, Korten AE, Jacomb PA, Christensen H, Rodgers B, Pollitt P, "Public beliefs about causes and risk factors for depression and schizophrenia." Social Psychiatry and Psychiatric Epidemiology, 1997, 32, 3, 143-148.
9. Razali SM, Khan UA, Hasanah CI, "Belief in supernatural causes of mental illness among Malay patients: impact on treatment." Acta psychiatrica scandinavica, 1996, 94, 4, 229-233.
10. Angermeyer MC, Matschinger H, Riedel-Heller SG, "Whom to ask for help in case of a mental disorder? Preferences of the lay public." Social psychiatry and psychiatric epidemiology, 1999, 34, 4, 202-210.
11. Slattery, D., Cryan, J, "Using the rat forced swim test to assess antidepressant-like activity in rodent",. Nat Protoc 7, 2012 1009–1014.
12. Castagné V, Moser P, Roux S, Porsolt RD, "Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice", Curr Protoc Pharmacol. 2010, 5, 5, 8.
13. Steru L, Chermat R, Thierry B, Simon P, "The tail suspension test: a new method for screening antidepressants in mice", Psychopharmacology (Berl). 1985, 85, 3, 367-70.
14. Pan H, Dohn M R., Kingston R, Carneiro AMD, "Integrin αVβ3 Function Influences Citalopram Immobility Behavior in the Tail Suspension Test", Frontiers in Neuroscience, 2019, 13, 70.