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PHARMACOLOGICAL EVALUATION OF POLYHERBAL SUSPENSION AS ANTIDEPRESSANT IN RAT

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ABSTRACT: The ethanolic extract of *Moringa oleifera* leaves & methanolic extract of rose hips of *Rosa damascena* were first subjected to various phytochemical tests and further assessed for their antioxidant activity by DPPH method. Different ratio of two drugs in the polyherbal formulation were based on antioxidant activity in albino Wistar rats. Thirty rats (180 -250 g) of either sex were randomly divided into five groups. **Group I:** Normal Control received 0.9% Normal Saline. **Group II:** Disease control, received Isotretinoin 60 mg/kg p.o single dose daily for 11 days without any treatment. **Group III:** Received Isotretinoin 60 mg/kg p.o single dose daily for 11 days and treated with standard drug Imipramine 10-20 mg/kg p.o for the next 45 days. **Group IV:** Received Isotretinoin 60 mg/kg p.o single dose daily for 11 days and treated with Polyherbal (Moringa leaves & Rosehips) suspension (100 mg/kg p.o.) for next 45 days. Group V: Received Isotretinoin 60 mg/kg p.o single dose daily for 11 days and treated with Polyherbal suspension (200 mg/kg p.o) for the next 45 days. The antidepressant activity was evaluated by Tail Suspension Test, Forced Swim Test, Locomotor activity, and Hole Board Test. It was found that the polyherbal formulation possess profound CNS stimulant like activity as the treated animals were significantly have more tendency to struggle and are active as compared to non-treated animals. Based on that intervention, this formulation used for the treatment of depression, anxiety. Our study suggests the polyherbal suspension has a neuroprotective effect that can be used as novel antidepressant drug.

INTRODUCTION: Now a day's depression is a major health problem of the whole world. About 350 million people suffer from the disease, and it will become the second cause of death till 2020. Depression is a condition of psychological illness. It is characterized by feelings of sadness or despair.

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Depression can change an individual's thinking capacity and also affects his/her social behavior and sense of physical well-being. Peoples of any age group can be affected by depression, including young children and teens. It can be hereditary, run in families, and usually starts between the ages of 15 and 30 years [1-2].

Some of the common factors that cause depression are genetic (hereditary), trauma, and high levels of stress, mental illnesses such as schizophrenia, substance abuse, and postpartum (after childbirth) depression. Some other reasons that contribute to depression are serious medical conditions such as

cardiac disease, cancer and HIV use of certain medications, alcohol and drug abuse, individuals with low self-esteem, trauma and high levels of stress due to financial problems, the breakup of a relationship or loss of a loved one.

Depression can be reduced by regular exercise, a healthy diet, and stable relationships. They help keep stress low and thereby reduce the chances of feeling depressed again. With correct treatment, a depressed person can return to a happier life [3].

Moringa oleifera is an excellent remedy for malnourishment belonging to the family Moringaceae. Moringa is a rich source of nutrients a variety of necessary phytochemicals present in its leaves, pods and, seeds. Moringa provides seven times more vitamin C than oranges. *Moringa oleifera* can grow in an adverse situations also & requires little care. It requires loamy mud with a slightly acidic to slightly alkaline pH [4].

Moringa oleifera is widely found in the Indian sub-continent. It is a deciduous tree, fast-growing, drought resistance, having a height of 10-12 meters [5]. Moringa possesses many necessary nutrients such as vitamins, minerals, amino acids, beta carotene, antioxidants, anti-inflammatory nutrients & omega 3&6 fatty acids.

Moringa oleifera is mentioned as an important medicinal plant in traditional medicine. Numerous pharmacological studies have exposed the ability of this plant to show analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, hepatoprotective, gastroprotective, anti-ulcer, cardiovascular, anti-obesity, antiepileptic, anti-asthmatic, anti-diabetic, diuretic, anesthetic, anti-allergic, anthelmintic, wound healing, antimicrobial and antidiarrheal properties. Moringa oleifera has various pharmacological uses in numerous pathophysiological conditions [6].

Rosa damascena mill L. is a well-known plant of the Rosaceae family. Rose is well recognized ornamental plant & has been discussed as the king of flower [7]. The *R. damascena* has also used for therapeutic purposes; several products & isolated constituent as of flowers, petals, and hips (seed pot) of this plant have been located for study in a variety of *in-vivo* & *in-vitro* studies. Numerous constituents were obtained from flowers, petals, and hips (seed-pot) of *Rosa damascena* together with terpenes, glycosides, flavonoids, and anthocyanins. This plant contains carboxylic acid, vitamin C, kaempferol, and quercetin Flowers also include a bitter principle, tanning matter, fatty oil, and organic acids [8].

R. damascena is also used in the treatment of abdominal & chest pain, treatment of menstrual bleeding, strengthening the heart & digestive problems & as an anti-inflammatory.

Rosehip possesses seeds of the rose plant. Dried rosehip and the seeds are used together for the formation of medicine. Fresh rosehip is rich in vitamin C, so it can be used in cold flu, and vitamin C deficiencies [9].

MATERIALS AND METHODS:

Solvents and Chemicals: Solvents and chemicals were used in the experiments are Methanol, ethanol, n-hexane, and butanol are used in extraction. DPPH (2-2 diphenyl-1-picrylhydrazyl), ascorbic acid for analysis of antioxidant activity. Other reagents like HCL, ammonia, NaOH, chloroform, sulphuric acid, lead acetate, 1N Sodium Hydroxide, *etc.* are used for phytochemical screening.

Plant Collection and Authentication: The leaves of *Moringa oleifera* L. were collected from the local area of Jankipuram, Lucknow, on 29 November 2018. The sample of *Moringa oleifera* L. family- Moringaceae was authenticated by macroscopic observations and confirmed by comparing it with local flora and authentic samples.

The plant material *Rosa Damascena* was purchased from the local market of the chowk, Lucknow. The sample of *Rosa damascenes* Herm. Family-Rosacea was authenticated by macroscopic observations and confirmed by comparing it with flora and authentic samples.

Preparation of Plant Extract:

Extraction Process of *M. Oleifera*: The leaves are cleaned by tap water & the portion was dried in the shade for a few days. The dried sample was ground in a mortar pestol. The powdered drugs were kept in sealed containers & protected from light until used. Dried powdered leaves were separately placed into a thimble & were extracted with 30 to

40 °C with ethanol in a Soxhlet apparatus. Extraction was carried out at five cycles/hour until exhaustion. The combined extract from each extraction was dried under reduced pressure at 50 °C using a Rotary Vacuum evaporator. The crude extract was weighed & kept in a tight container protected from light [10].

Extraction process of *Rosa damascena*: The petals were separated & fresh rose hips are collected and stored in a dark place at room temperature the dried sample was ground in mortar pestle. 200 gm. of the dried rosehip was placed in Soxhlet apparatus & extracted with methanol up to 60 to 85 °C containing glass beads in a volumetric flask. Extraction was carried out at five cycles / h until exhaust. The extract was concentrated in hot air cabinet at 60 °C and then added n-hexane to the crude extract up to (500 ml) by vigorous shaking.

The extract was filtered, and the residue marc is obtained. N-butanol (20 to 25) ml was added by vigorous shaking again; the extract was filtered. Filtrate (n-butanol soluble extract) Marc (Final extracted Methanolic fraction)

Phytochemical Analysis: Qualitative phyto-chemical tests for the identification of anthocyanins, coumarins, steroids, tannins, saponins, quinones, phenolic compounds, and flavonoid were carried out for the methanolic and ethanolic extract of the test drug.

Preparation of Suspension of the Herbal Drug: Weigh 5 gm of an extracted solution of *Moringa oliefera* and Rosehip, triturate it properly. Add about 1 gm of tragacantha solution; add propyl paraben as preservative up to 0.1 gm. All the ingredients are triturated properly now add 2, 5 ml glycerin, at last, make up the volume with distilled water about 100 ml [11].

Experimental Animals: Mouse (3-4 months old) of either sex were selected for the study and followed by acclimatization for two weeks by maintaining standard environmental conditions and fed with standard pellet diet & water. The experiment will be carried out following the CPCSEA guidelines and Institutional animal ethical clearance. Then all animals will be randomized into five groups with six animals in each group.

Experimental Designs:

- **Group I:** Normal Control (0.9% Normal Saline), continued with a diet for 45 days.
- **Group II:** Disease control (Isotretinoin 60 mg/kg p.o) single-dose continued with a diet for the last 11 days.
- **Group III:** Standard drug Imipramine 10-20 mg/kg p.o continued for the next 45 days.
- **Group IV:** Isotretinoin 60 mg/kg p.o + Polyherbal (Moringa leaves & Rose hips) suspension (100 mg/kg p.o.) continued for next 45 days.
- **Group V:** Isotretenoine 60 mg/kg p.o + Polyherbal (Moringa leaves & Rose hips) suspension (200 mg/kg p.o) continued for next 45 days.

Acute Toxicity Studies: Acute toxicity studies were performed, permitting the organization for economic cooperation and development (OECD) guidelines. The acute toxicity study of herbal drug suspension was analyzed after oral administration at different doses as per the OECD guideline no. 423 method was accepted for toxicity studies. The extracts were administered orally at the treatment of 2000 mg/kg, 4000 mg/kg observed for seven days.

Assessment of *in-vivo* Antidepressant activity: Isotretinoin (drug) induced depression in the rat: Isotretinoin, a retinoid, is available as Accutane in 10-mg, 20-mg and 40-mg soft gelatin capsules for oral administration. Each capsule contains beeswax, butylated hydroxyanisole, edetate disodium, hydrogenated soybean oil flakes, hydrogenated vegetable oil, and soybean oil.

Gelatin capsules contain glycerin and parabens (methyl and propyl). Chemically, isotretinoin is 13cis-retinoic acid and is related to both retinoic acid and retinol (vitamin A).

It is a yellow to orange crystalline powder with a molecular weight of 300.44 Accutane may cause serious brain problems; Accutane can increase the pressure in your brain. This can lead to permanent loss of eyesight &, in rare cases, death.

Physical Parameters for the Evaluation of Antidepressant Activity:

Tail Suspension Test: Tail suspension test is a mouse behavioral test useful for the screening of antidepressant drugs, assessing depression-related behavior by immobility.

Procedure: The tail suspension test involves suspending the mouse above the ground by their tails, for conducting the procedures only requires a suspension bar or shelf ledge, & tape. In a laboratory, we use tail suspension boxes made of wood with the dimensions (58 height × 60 widths × 11.5 cm depth).

The tape is generally used to strong enough to carry the weight of the mouse being tested. Tape fragments that will be used during the session should be cut & prepared for the course. The tape is generally applied to the end of the tail with 2-3 millimeters of a rear, once the tape is applied, start the recording & identify the session before the mice are suspended.

This session is generally carried out for 6 min. During the behavioral analysis, the time that each mouse recorded the mobility time is measured, while it is possible to measure the immobility time directly [12].

Forced Swim Test: Forced swim test is commonly used for the evaluation of antidepressant drugs & the study of depressive behavior in rodents. This test is based on the fact that when animals are placed in a beaker filled with water & forced to swim, they try to escape by forceful movements. The animal will first efforts to escape to move out that time will consider immobility time.

This test is carried out for the exposure of the animal to stress. In this test, a transparent cylindrical glass container which should be 50 cm in height & a diameter should be 20 cm, for observation of animals prepare a video camera in front of a container for a view of animal behavior. The procedure is different for rats and mice. For mice, there is one session for 6 min, divided in first (2 min) & lasts for 4 min.

For Rats 2 session is conducted, the first session is the pretest for the 15 min; the second session is approx. for 5 min. Fill the container with tap water at 23 ± 1 °C adjust the water depth according to the Rat size. The rat hind legs cannot touch the bottom of the container. Place each rat in the water-filled cylinder container for 6 min. After 15 min removes the rat from the container & place it in the transient drying cage. Change the water after every session to avoid any influence on the next rat. During the behavioral analysis note, the Mobility time (struggling) climbing observed the front paws [13].

Locomotor Activity: In a digital actophotometer, a continuous beam of light falls on photocells present inside the equipment. These photocells are activated when the rays of light falling on the photocells are obstructed/cut off due to the movement of animals crossing the path of a light beam.

This cut off the quantity of light beam is measured electronically. This instrument measures the active exploratory movement of the animal. In our present study, the high dose of test drugs shows a significant increase in locomotor activity.

Disease control group shows the least movement in actophotometer. This equipment is used to measure the effect of the drug on motor activity of the rat and useful in screening & evaluation of drug for pharmacological & toxicological experiments.

Hole Board Test: Box with holes is used in this method, which has been used to measure the tendency of sniffing through holes. Hole poking is normal rodent behavior Animals who are in depression have a reduced tendency of poking the holes.

The apparatus is made up of one box in which the holes are spaced evenly on the floor of the equipment; there are 12 to 14 holes for a test. Rats are placed one by one in the boxes, frequency, and duration of responses are measured with these parameters several times. Rate of head dipping is the condition which is measured in case of depression, frequency of hole poking is an exploration condition also known as an investigatory behavior.

Statistical Analysis: Results of the study were represented by mean ± SD (Standard deviation). Data were analyzed by one-way ANOVA, followed by Dennett's test P values. ****$p<0.0001$

Histopathological Evaluation:

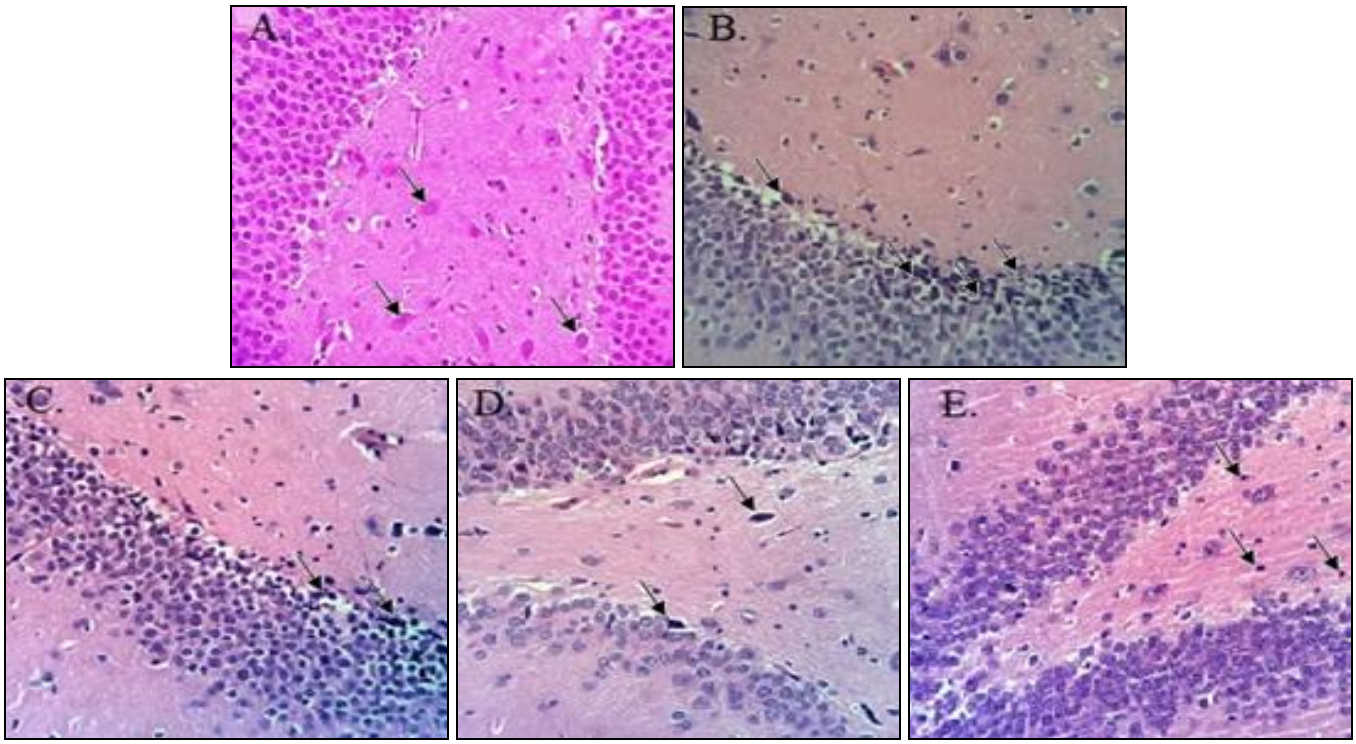

FIG. 1: A BRAIN CORTEX NORMAL B. HIPPOCAMPUS DEGENERATION OF GRANULAR NEURONS OF DENTATUS GYRUS. (ARROW) C. HIPPOCAMPUS: DEGENERATION OF GRANULAR NEURONS OF THE DENTATE GYRUS (ARROW) BUT MOST OF THE CELLS ARE PROTECTED. D HIPPOCAMPUS: MAJORITY OF NEURONS ARE PROTECTED. E HIPPOCAMPUS: PROTECTED DENTATE GYRUS

RESULTS AND DISCUSSION: Now, we can discuss the significant action of the polyherbal formulation by the observations seen during the experimental protocol. The various parameters observed are struggled during tail suspension and forced swim test, locomotor activity in actophotometer, and sniffing response in hole board test.

In the case of drug-induced depression, the statistical results of ANOVA show that the polyherbal formulation has demonstrated significant response as compared to the standard drug.

The result of the present study suggested that the ethanolic leaf extract of *Moringa oliefera* possesses antidepressant activity in TST, FST, HBT, and Actophotometer test.

Qualitative phytochemical analysis of *Moringa Oliefera* leaf extract shows the presence of steroids, saponins, coumarins, quinones, flavonoids, *etc*. *Moringa oliefera* at a dose of 200 mg/kg shows significant response ($P<0.0001$) by one way ANOVA followed by Dennett's multiple tests and demonstrated the significant value, ****$p<0.0001$. In the case of TST, there is a reduced duration of immobility of rats if compared to disease control. Histopathological report of brain tissues also supports that polyherbal formulation has a neuroprotective effect.

FST, TST, HBT, Actophotometer are the most commonly used models for the screening of new antidepressant drugs. Depressive symptoms are observed due to functional deficiency of noradrenaline, serotonin, or dopamine neurotransmitters in the limbic system, prefrontal cortex, hippocampus, and amygdala areas of the brain.

The primary target of antidepressant drugs to increase the level of these neurotransmitters in mind. Finally, based on the above result, we can conclude that the polyherbal formulation shows dose-dependent action and is helpful in the treatment of both drug-induced and stress-induced depression. In the future, this formulation can be used as a novel and effective formulation to treat depression and to save our society.

TABLE 1: OBSERVATION TABLE OF PHYTO-CHEMICAL TESTING

S. no.	Phytochemical Testing	Moringa leaves	Rose Hip
1	Tannins	Absent	Present
2	Steroids	Present	Present
3	Saponins	Present	Present
4	Coumarins	Present	Present
5	Quinones	Present	Absent
6	Flavonoids	Present	Absent
7	Anthocyanins	Absent	Absent
8	Phenolic	Absent	Present

TABLE 2: OBSERVATION TABLE OF WEIGHT VARIATION BETWEEN INITIAL WEIGHT & FINAL WEIGHT

Group of Treatment	Initial weight	Final weight
NC	169.33 ± 14.334	196.33 ± 4.633
DC	184.166 ± 12.624	137.33 ± 19.054
SD	172.166 ± 10.438	164.333 ± 10.4412
TLD	171.166 ± 8.612	150.333 ± 13.441
THD	186.166 ± 11.513	172.1667 ± 13.377

TABLE 3: OBSERVATION TABLE OF ANTIOXIDANT ACTIVITY OF M.O AND R.D

Concentration (µg/ml)	Ascorbic acid		Moringa oliefera		Rosa damascena	
	Absorbance	%Inhibition	Absorbance	%Inhibition	Absorbance	%Inhibition
100	0.031	95.36	0.307	100	0.031	95.36
200	0.027	95.95	0.287	200	0.027	95.95
300	0.025	96.25	0.266	300	0.025	96.25
400	0.023	96.55	0.245	400	0.023	96.55
500	0.021	96.86	0.227	500	0.021	96.86
IC 50	12538		52.58		31.76	

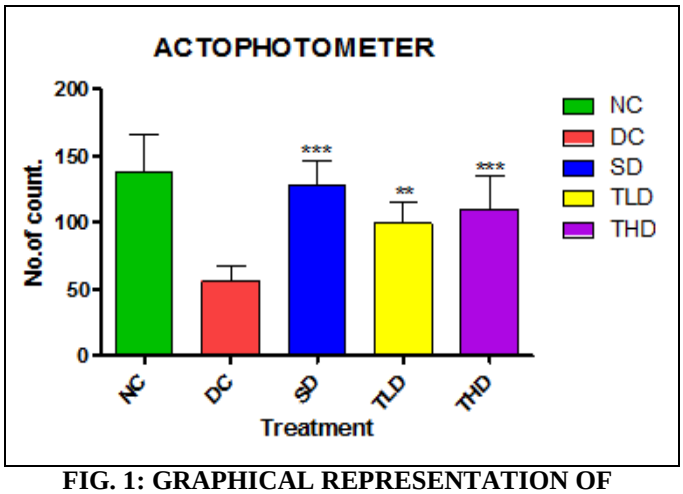

FIG. 1: GRAPHICAL REPRESENTATION OF LOCOMOTORY TEST BETWEEN NO. OF COUNT *vs.* TREATMENT

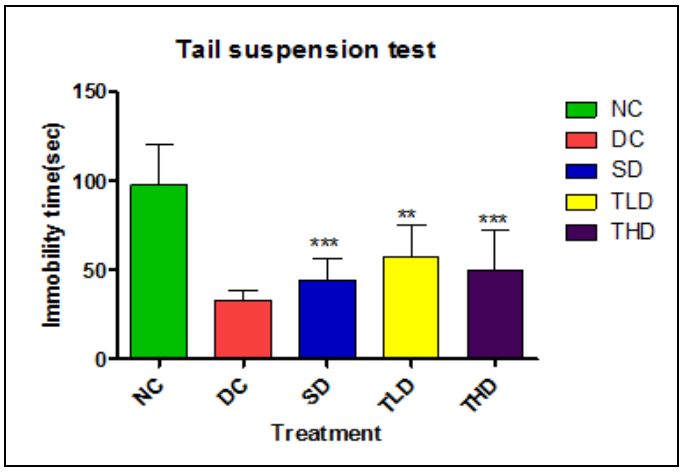

FIG. 2: GRAPHICAL REPRESENTATION OF TST BETWEEN IMMOBILITY TIME *vs.* TREATMENT

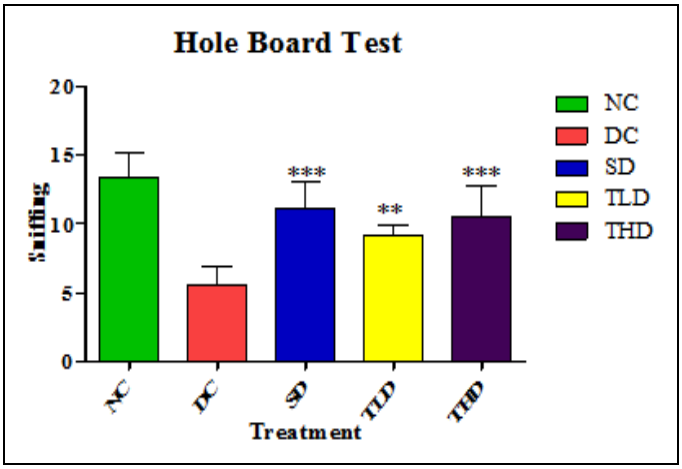

FIG. 3: GRAPHICAL REPRESENTATION OF HOLE BOARD TEST BETWEEN SNIFFING *vs.* TREATMENT

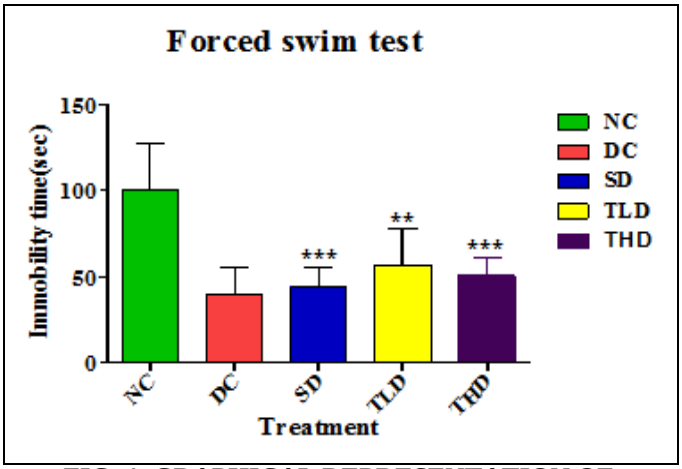

FIG. 4: GRAPHICAL REPRESENTATION OF FORCED SWIM TEST BETWEEN IMMOBILITY TIME *vs.* TREATMENT

TABLE 4: OBSERVATION TABLE FOR DIFFERENT BEHAVIOUR MODEL

Group drug treatment	Actophotometer (No. of count)	Forced swim test (Immobility test)	Tail suspension (Immobility test)	Hold board (Sniffing)
NC (Normal saline)	137.6667±28.6273	100.833±26.278	97.533±22.713	13.3±1.75
DC (isotretonoin)	55.500 ±11.4224	39.50±15.9394	33.333±5.5377	5.5±1.37
SD (imipramine)	127.5±19.3261	43.833±12.1559	44.666±11.5181	11.1±1.94
TLD (M.O. + R.D.)	99.16667±15.753**	56.666±214631**	57.166±18.23**	9.16±0.75**
THD (M.O. R.D.)	110.1667±25.380***	50.666±10.9117***	49.5±22.7222***	10.5±2.25***

CONCLUSION: The observations obtained during the experimental protocol suggest that polyherbal formulation has a significant antidepressant action. Various behavioral models used in this study are FST, TST, and HBT & locomotory activity. The present study suggests that Moringa leaves & rose hips are neuroprotective in nature because of various phytochemicals present in these plants. Increased time of struggle in FST & TST shows that polyherbal formulation has antidepressant action.

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CONFLICTS OF INTEREST: The author(s) confirm that this article content has no conflict of interest.

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