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Mimicking human antidepressant activity in Swiss Albino Mice using *Madhuca Longifolia*

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***Abstract*---**The present study was evaluated to screen the antidepressant potential of *Madhuca longifolia* in different animal models like forced swim test (FST), tail suspension test(TST), reserpine induced hypothermia (RIH). Adult swiss albino mice weighing 25- 30 gm.Standard drug used in above study was Fluoxetine(10mg/kg,p.o). The antidepressant activity of *Madhuca longifolia* was compared to the standard drug. The model shows closely mimics symptoms & signs of human depression. The high dose of *Madhuca longifolia* showed extremely high significant values over standard drugs with a decrease in immobility and ptosis. The results obtained in the present study suggest that *Madhuca longifolia* proves to be a potential therapeutic drug for treating depression along with anti-inflammatory, anti-psoriatic and antioxidant activity.

***Keywords*---**Antidepressant activity, *Madhuca longifolia*, Forced swim test, Tail suspension test, Reserpine induced Hypothermia.

Introduction

Mental issues is a state of low mood and drastically affects the person's thoughts, behavior, feelings and sense of well-being". The change in mood is observed in all age group from children to adult. On surveying of the World Health Report(Abbey LR *et al,* 2008), almost 450 million people are suffering from a mental or behavioral disorder. This burdens the 12.3% of the global state of disease (Reynolds EH*et al*, 2008). This illness creates the feelings of the suicide and in lockdown of covid-19 it is increased drastically. The standard formulation available in the themarket are tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs) (Thase*et al*, 1995). Neuroscience provideshelp to solve the issues related to mental disorders. Nowadays probiotics are also used to treat some psychological disoders as it keeps the gastrointestinal region at its best (Lerer*et al*, 2002). Based on nutraceutical studies various plants are being used as complementary alternative medicines for the management of neural-disorders (Patil *et al*, 2015, Patil 2020, Patil 2020, Patil 2019, Patil 2019, Patil 2018, Patil 2020, Patil 2020, Patil 2019, Patil 2019, Patil 2020, Patil 2020, Patil 2020, Patil 2020, Patil 2021).

Based on the above information, the leaves of *Madhuca longifolia (MEML)* were selected for evaluating its antidepressant activity. Chemical constituents in *Madhuca longifolia* such as alkaloids, bioflavanoids, quercetine are found responsible for antidepressant activity.

Materials and Methods

Animals

Balb/c strain male & female mice weighing between 25-30 gm were used for the study. Animals procurement was done 7 days before the study and was housed under standard laboratoy conditions of temperature (25±2 ^{0}C) and relative humidity (55±5%) with a 12:12 light-dark cycle in polypropylene cages. All the animals were fed a standard diet and water was supplied ad libitum under strict hygienic conditions. All the experimental protocols are permitted by the Institutional Animal Ethical Committee **(IAEC)** (DYPIPSR/IAEC/15-16/P-03)of Dr. D.Y. Patil College of Pharmacy. All the animal studies were performed as per the rules and regulations following the guidelines of CPCSEA with the registered number **CPCSEA.** (198/99/CPCSEA) All the animals have fasted 3hrs before oral administration of vehicle/standard/test compounds during the experiment. All the experiments were carried out during the light period(9:00 to 17:00 hrs) to avoid circadian rhythms (as per OCED Guidelines 2001)

Collection and Authentication of Plant material

The leaves part of *Madhuca longifolia* (Moha) was collected from a local distributor in Yavatmal, Maharashtra, in November 2015. The plant material was identified and authenticated with the Botanical Survey of India, Western Regional centre, Koregaon Road, Pune 411001;Herbarium was submitted under specimen:no.BSI/WRC/iden./2015/539.

Preparation of extract

The dried leaves were crushed into fine particles (powder) using a mixer (Narongchai*et. al.* 2007) The powdered leaves were packed in a thimble in a soxhlet apparatus and subjected to continuous hot percolation at a temperature 60-80 °C using methanol as solvent till clear solvent was observed in siphon tube. The extract was concentrated in a rotary vacuum evaporator. The concentrated extract was dried and packed in an air-tight container.

The % yield of various extracts was evaluated using the formula:

$$\% \text{ Yield} = \text{Weight of extract (g)} / \text{Weight of dry powder(g)} \times 100$$

Preliminary phytochemical screening

The preliminary phytochemical investigations were carried out with the methanolic leaves extract of Madhucalongifolia for qualitative identification of phytochemical constituents like alkaloids, carbohydrates, gums, proteins and amino acids, glycosides, steroids, flavonoids and triterpenoids by using standard procedures.

Pharmacological Evaluation
Acute oral toxicity studies

Toxicity studies of extract were carried out in albino mice weighing between 25-30g.Four groups of mice comprising 3 animals in each group were treated with 5, 50, 300 and 1000mg/kg of the extract orally, via gastric catheter. Other groups of mice comprising three animals each were treated with 20, 250,750,1000 mg/kg.p.o .via gastric catheter.The animals were then observed continuously for the first 4hrs for any behavioral changes and mortality if any at the end of 72 hrs was observed.

Drugs and Chemicals

Fluoxetine was purchased from Sigma Life Sciences, Bangalore, Reserpine from Sigma Life Sciences, Bangalore.

The experiment was carried out in a narrow glass cylinder (75 cm in width,58 cm high) containing water (26°C)±1°C to a depth of 28 cm, from which they cannot escape. All the micehave fasted for 3 hrs before the administration of vehicle/standard/test compounds. Mice of either sex are individually forced to swim in an open container containing water. The first group was assigned as control receiving only a vehicle.Other two groups receivedan acute dose of methanolic extract of *Madhuca longifolia.* The last group received standard drugs. The total demonstration of immobility is recorded during the last 4 min of the 6 min period.Thirty minutes later, The animals were subjected to swimming. The test was carried out for 6 minutes.(Porsolt*et al*,1978)

Tail Suspension Test (TST):

The Tail-Suspension Test is a mouse behavioral test useful in the screening of potential antidepressant drugs and assessing other manipulations that are expected to affect depression-related behaviors. Mice are suspended by their tails with tape, in such a position that they cannot escape or hold on to nearby surfaces. (Vangeois*et al*,1997).

Reserpine Induced Hypothermia (RIH):
Procedure:

All the animals have fasted for 3 hrs before oral administration of vehicle/standard/test compound. The basal rectal temperature was measured by inserting an electronic thermometer to a constant depth of 3 cm. On the day before testing, animals were injected with 2 mg/kg body weight of animal subcutaneously with reserpine. The animals were housed in a climate-controlled animal colony and had free access to food and water for 18 hrs.After 18 hours of the administration of Reserpine, once again rectal temperature was measured.Rectal temperature was measured every 1/2 hr, 1 hr, 2 hr & 4 hrs after oral administration of the compounds.(Yu ZF*et al*,1997)

Scoring of ptosis in mice is as follows: Normal – 0; 1/4th closed eyes – 1; 1/2 closed eyes – 2; ¾ th closed eyes – 3; Fully closed eyes–4

Statistical Analysis
Results were presented as Mean ± SEM. The data were subjected to statistical analysis by one-way analysis of variance (ANOVA) followed by Dunnett's t-test and $P<0.05$*, 0.01** and 0.001*** were considered significant, $P>0.05$ was considered non-significant (ns) Vs Control group.

Results
Percentage Yield :

500g of the dried powder was placed in soxhlet apparatus (Perfit, India) and subjected to successive extraction using petroleum ether (60°-80°C), and methanol. The percentage yields of methanolic extract were found to be 25.22 % w/w.

Phytochemical screening:

The preliminary phytochemical screening was found to be the presence of active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, steroids, proteins, cardiac glycosides, carbohydrates,sesquiterpenes, fixed oils and fats. (Table.1)

Acute Toxicity Study:

The LD50 of *Madhuca longifolia*was found 1000 mg/kg.

Forced Swim Test:

In this test:animals treated with three doses of MEML (75mg, 350mgand 750mg/ kg, i.p) showed decreases in their immobility times, which wassignificant respectively; p<0.01). (Table.2 and 3)/ (Fig. 1)

Tail Suspension Test

In this test MEML (350, 750 mg/kg, i.p) showed decreases in their immobility tim es (p< 0.01) as compared to respective control group. (Table.4 and 5)/ (Fig. 2)

Reserpine Induced Hypothermia

In this test antidepressant effect of *Madhuchalongifolia* after oral administration was
studied in RIH.model. Animals treated with three doses of MEML (350, 750 mg/k g, i.p) exhibited a decrease in hypothermiawhich was significant (p< 0.01) as compared to the respective control group. Significant Increase in ptosis micee& significant reduction of temperature. (Table.6 and 7)/ (Fig. 3)

Discussion

The present work was subjected to investigation for the evaluation of the antidepressant activity of Methanolic leaves extract of *Madhucalongifolia*in experimental laboratoryanimals like mice. The extract was primarily subjected to phytochemical investigation and acute oral toxicity study. In acute oral toxicity study, MEML did not show any lethal effect even up to the doses of 1000 mg/kg, p.o and complete absorption of drug through GIT was observed. The effect of MEML was investigated for its putative antidepressant activity by using various experimental models in mice viz. Tail Suspension test, Forced Swim test and Reserpine induced Hypothermia.

Forced Swim test & Tail Suspension test are the most commonly used preliminary screening tests for characterizing potential antidepressant drugs. In these models MEML at doses of 350 mg/kg, p.o and 750 mg/kg, p.o showed a significant increase in the motor activity of mice which elevate depressed mood by decreasing the immobility time of mice (BorsiniF*et al*,1988). The parameters observed in this model are the immobility time of mice. Drugs that decrease immobility time lead to an increase in the motor activity of mice which inhibits depression developed due to swimming and tail suspension of mice in these tests andoffers protection against depression induced by thesemethods. In the present study, MEML (350& 750 mg/kg, p.o) has shown a significant dose-dependent activity i.e. increase in the dose of the drug proportional to a decrease in the immobility time threshold and offers good percentage protection as compared to the control group. Similarly, the standard drug Fluoxetine (10 mg/kg, p.o) had significant percentage protection. Fluoxetine was a selective serotonin reuptake inhibitor that works on the serotonin balance by inhibiting a transporter that selectively pumps serotonin back into the neurons(Narong P*et al*,2007).

In Reserpine-induced hypothermia model, Reserpine induces hypothermia which cannot be antagonized by the MEML (350 & 750 mg/kg, p.o) and Fluoxetine (Standard- 10 mg/kg-p.o) which reveals that MEML might be strongly acting through serotonin reuptake inhibition like Fluoxetine. The parameters observed in this model are rectal temperature readings and Ptosis Score.The extract might affect the serotonin reuptake inhibition like Serotonin reuptake inhibitors to exert their antidepressant activity.

Conclusion

In the present study, Methanolic extract of *Madhuca longifolia* (MEML) was evaluated by using various experimental models. MEML at doses of 750 mg/kg, p.o showed significant increase in the motor activity of mice which elevate depressed mood by decreasing immobility time of mice in Forced Swim Test and of mice in the Tail Suspension test. In Reserpine induced hypothermia model, MEML shows antagonism against hypothermia produced by the Reserpine. From all the above findings, the present investigation suggests that the methanolic extract of *Madhuca longifolia* may possess antidepressant activity by inhibiting the reuptake of Serotonin which acts through serotonergic receptors (G-protein coupled receptors) (Soulimani*et al*,2007) as a mood elevator. However, an extensive Pharmacological study of this plant is required for a complete understanding of the antidepressant activity of MEML. Further investigation should be carried out to isolate and identify the chemical constituent which is responsible for its antidepressant activity.

Conflict Of Interest

The authors declare no conflict of interest which is documented.

References

Abbey LR. Characterization of animals of behavioural models using juvenile mices for screening paediatric antidepressants efficacy. United States: University of Nebraska - thesis publicaton; 2008. 185: 396-404.

Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity *Psychopharmacology.*1988, 94, 147-60.

LererB.,Macciardi, F. Pharmacogenetics of antidepressant and mood stabilizing drugs: a review of candidate-gene studies and future research directions. *The International Journal of Neuropsychopharmacology*. 2002, 5, 255-275.

Narongchai P, Omboon L and Leena S. Rapid reversed-phase high performance liquid chromatography for vitexin analysis and fingerprint of Passiflora foetida. *Current science*. 2007, 93,378-382.

OECD 2001-gudeline on Acute Oral Toxicity (AOT) Environmental health safety monograph series on testing and adjustment No.425.

Patil A., *et al.* "Complete Genome Sequence of *Lactobacillusplantarum* Strain JDARSH, Isolated from Sheep Milk". *MicrobiologyResourceAnnouncements*9.2 (2020).

Patil A., *et al.* "Probiotic potential of *Lactobacillus plantarum*with the cell adhesion properties". *Journal of Global PharmaTechnology*10.12 (2018): 1-6.

Patil A., *et al.* "Shelf-life stability of encapsulated lactic acidbacteria isolated from

Sheep milk thrived in different milk asnaturalmedia".*SmallRuminantResearch*170(2019):19-25.
Patil AR and Disouza J. "Isolation and characterisation of *Lactobacillus*speciesfromsheepmilk".*InternationalJournalofInnovativeScienceEngineeringandTechnology*1.1(2020):9-17.
Patil AR, Patil P. "The Insight of Body's Immune System,Inflammation and Damages in Wound Healing- The review".*InternationalJournalforResearchinAppliedScienceandEngineeringTechnology*9.8 (2021):2777-2780.
Patil AR. "*Lactobacillus rhamnosus*ARJD as a Functional Foodwith Potential Antioxidant and Antibacterial Abilities". *ActaScientificPharmaceuticalSciences*3(2019):63-70.
Patil AR. "The role of the food and fertilizers in antimicrobialresistance in human and its preventive measures". *International Journal of Innovative Science, Engineering and Technology*1.1 (2020): 31-37.
Patil AR. "Use of Probiotics as a Functional Food against Cancer". *Acta Scientific Pharmaceutical Sciences* 3.8 (2019): 103-105.
Patil AR., et al. "Lactobacillus Model Moiety a New Era DosageForm as Nutraceuticals and Therapeutic Mediator". In Biotechnology and Bio forensics Springer, Singapore (2015): 11-21.
Patil AR., *et al.* "Nanotechnology based upgradation in tuberculosisdiagnosis andtreatment" (2020):395-414.
PatilA, DisouzaJ."Genomic-BasedRestrictionEnzymeSe-lection for Specific Detection of *Lactobacillus rhamnosus*and*Lactobacillusplantarum*strainby16SrDNAPCR-RFLP".*International Journal of Innovative Science, Engineering and Technology*1.1 (2020): 91-99.
PatilA.,*etal.*"Bananafiberscamouflagingasagutwormina 6-month-old infant". *Iberoamerican Journal of Medicine* 3(2020): 245-247.
PatilA.,*etal.*"Evaluationof*Lactobacillusplantarum*growthinmilkofIndianbuffalobreedsbasedonitsphysico-chemicalcontent".*BuffaloBulletin*38.2(2019):345-352.
PatilAR."Theoriginofnovelcoronavirus:COVID-19".*International Journal of Innovative Science, Engineering and Technology*1.1 (2020): 18-23.
Porsolt R, Anton G, Jafre M. Behavioural despair in mices: A new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* 1978, 47, 379-391.
Reynolds E H. Brain and mind: a challenge for WHO. Lancet 2003, 361, 1924-1925.
Shoba.S, Patil.P.A, Vivek.V. Hepatoprotective activity of *Madhuca longifolia.* aqueous extract against paracetamol, isoniazid and alcohol induced hepatotoxicity in male wistarmices, Int J of Phytopharmcol. 2008, 23, 291-296
Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R and Mortier F. Behavioral effects of Passifloraincarnata L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *JEthnopharmacol.* 1997; 57: 11-20
Thase ME, Howland RH. Biological processes in depression: an update and integmiceion. In: Beckham EE, Leber WR, Handbook of Depression, 2nd ed., New York, Guilford, 1995, 3,213–279.
VangeoisM., PasseraG., ZuccaroF. and CosteninJ., Individual differences in response to imipramine in the tail mouse us pension test, *Psychopharmacology*. 1997, 134, 387-391.

Yu ZF Kong LD and Chen Y. Antidepressant activity of aqueous extracts of Curcuma longa in mice: *Ethnopharmocol.* 2002, 83, 161- 65.

Tables

Table1: Phytochemical analysis of *Madhucha longifolia*

Sr.no	Name of the Test	Procedure	Observation	Inference
1	Alkaloids	Extract + Dragendorff reagent Extract +Mayer's reagent Extract + Hager's reagent	Orange colour White ppt Yellow ppt	+ + +
2	Glycosides	Anthrone + $H2SO_4$ + Heat	Purple or green	-
3	Carbohydrates	Extract + Molish's reagent + conc.H_2SO_4 Fehling's solution A&B	Purple colour Brick red colour	+ +
4	Phytosterols /triterpenoids	Liebermann Test Salkowski Test Noller's test	Bluish green Red fluorescent Pink colour	+ + +
5	Proteins & Amino acids	Biuret test Xanthoprotein test Millon's reagent test Ninhydrin test	Violet colour Orange colour White ppt White ppt	+ + + +
6	Saponins	Extract + water + shaking	Formation of honey comb like froth	+
7	Flavonoids	Shinoda's test Zn-HCI acid reduction test	Red colour Magenta colour	+
8	Fixed oils & Fats	Spot test	Stains appear after drying	-

9	Gums/Mucilage	Extract + water	No thickening of the substance	-
10	Phenolics/ Tannins	$FeCl_3$ + Extract + lead acetate + water	Intense colour Formation of white ppt	+/+

A. Forced Swim Test:

Table 2:Percentage inhibition of MEML in Forced Swim Model in Mice

Group	**Percentage Inhibition (%)**			
	30min	**60min**	**120min**	**240min**
Control	50.5	51.4	52.3	50.1
Fluoxetine (10mg/kg,p.o)	41.4	69.6	47.2	49.2
MEML1 (350mg/kg,p.o)	28.85	36.3	32.6	34.72
MEML2 (750mg/kg,p.o)	55	59.6	47.4	46.48

B. Tail Suspension Test

Table 3: Effect of MEML on immobility time in Tail Suspension Model in Mice

Group	Immobility Time (MEAM ± SEM), N=5
Control	126.2±10.02
Fluoxetine (10 mg/kg,p.o)	59.6±6.22**
MEML1 (350mg/kg,p.o)	72.8±12.80*
MEML2 (750 mg/kg,p.o)	60.9±8.40**

[Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at **p < 0.01, *p<0.05 Non Significance (#) at p >0.05 Vs control.]

Table 4: Percentage inhibition of MEML in Tail Suspension Test in Mice

Group	Percentage Inhibition (%)
Control	51.57
Fluoxetine (10 mg/kg,p.o)	68.47
MEML (350 mg/kg,p.o)	59.60
MEML (750mg/kg,p.o)	57.44

C.Reserpine Induced Hypothermia

Table 5: Effect of MEML on the temperature in Reserpine induced hypothermia model in mice

Group	Initial	Rectal Temperature (MEAN ± SEM) ; N=5			
	0min	**30min**	**60min**	**120min**	**240min**
Control	37.9±0.28	30.2±0.56	30.85±0.67	31.2±0.78	31.8±0.67
Fluoxetin (10mg/kg,p.o)	38.1±0.12**	32.6±0.30**	34.8±0.26**	35.6±0.29**	36.8±0.35**
MEML (350mg/kg,p.o)	39.25±0.9	30.6±0.60*	31.2±0.58	32.8±0.41**	33.3±0.4*
MEML (750mg/kg,p.0)	39.12±0.11**	30.83±0.42	32.66±0.48**	33.2±0.38**	34.8±0.28**

[Results were analysed by one-way ANOVA using Dunnett's multiple comparison test; Significance at **p < 0.01, *p<0.05 Non Significance (#) at p >0.05 Vs control.]

Table 6: Effect of MEML on the degree of hypothermia in Reserpine induced hypothermia model in mice

Group	Degree of Hypothermia			
	30 min	**60 min**	**120 min**	**240 Min**
Control	-8.2	-6.9	-6.5	-6
Fluoxetine (10 mg/kg,p.o)	-7.55	-4.05	-3.45	-4.05
MEML1 (350 mg/kg,p.o)	-7.58	-6.58	-6.41	-6.1
MEML2 (750 mg/kg,p.o)	-8.7	-8.7	-8.5	-7.6

Table 7: Effect of MEML on Ptosis in Reserpine induced hypothermia model in mice

Group	Initial	Ptosis Score : N=5			
	0 Min	**30 min**	**60 min**	**120 min**	**240 Min**
Control	2	2	3	4	4
Fluoxetine (10 mg/kg,p.o)	2	3	3	4	4
MEML (250 mg/kg,p.o)	2	2	3	3	4
MEML (750 mg/kg,p.o)	3	3	4	4	4

[Results were analyzed by one-way ANOVA using Dunnett's multiple comparison test; Significance at **p < 0.01, *p<0.05 Non-Significance (#) at p >0.05 Vs control.]

Figures

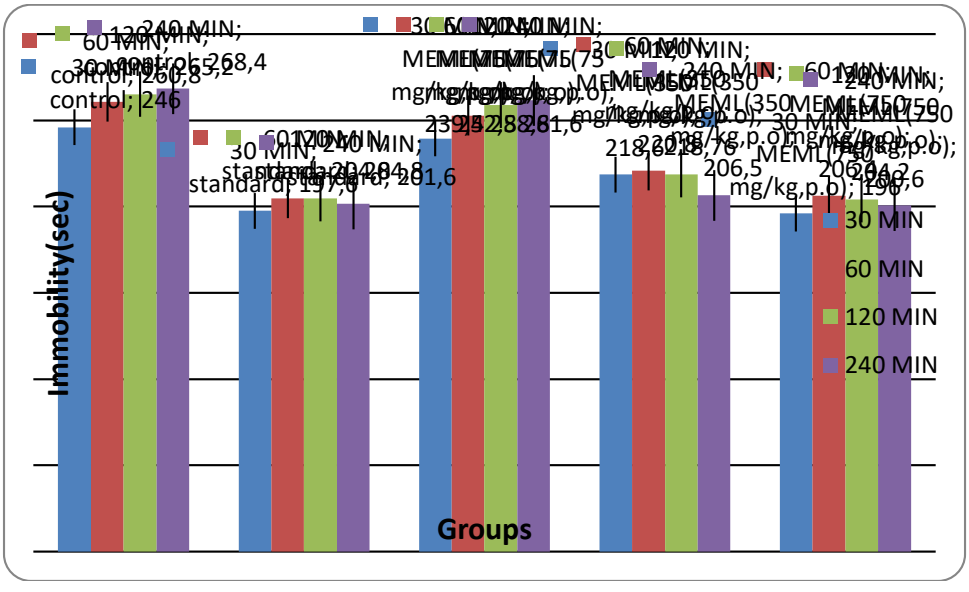

Fig.1. Graph showing a decrease in immobility in Forced Swim Test.

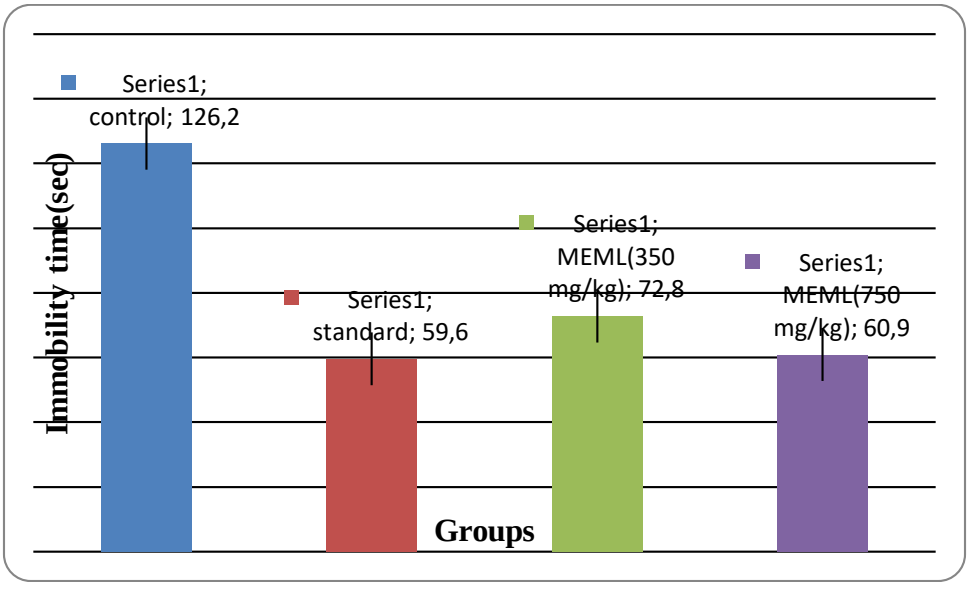

Fig.2. Graph showing decrease in immobility in Tail Suspension Test

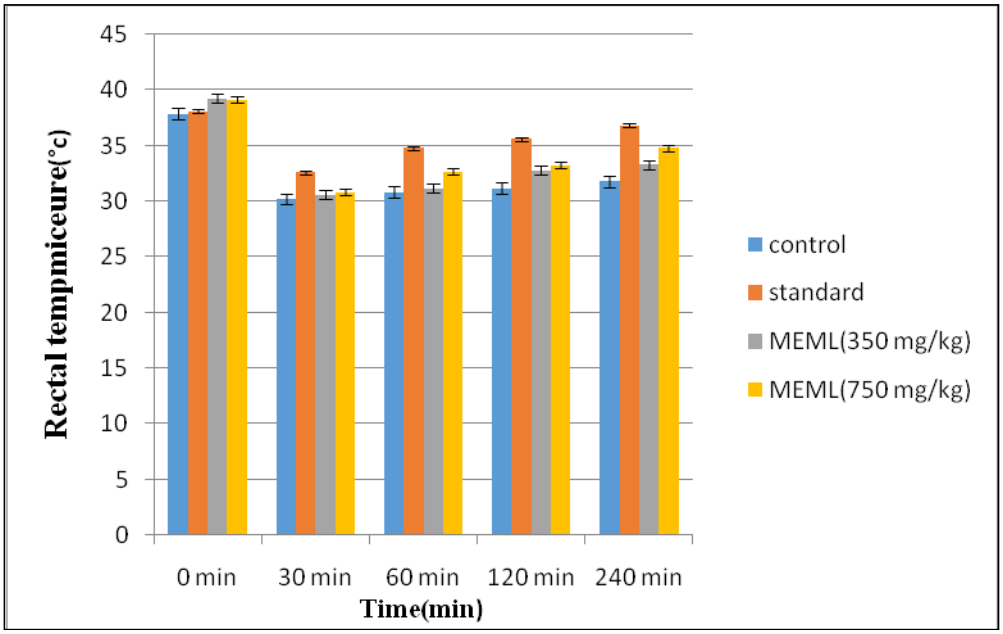

Fig.3. Graph showing increase in temperature in Reserpine induced hypothermia