EVALUATION AND COMPARISON OF ANTIDEPRESSANT ACTIVITY OF MARKETED AYURVEDIC FORMULATIONS

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ABSTRACT:

Depression is referred as an affective disorder which is described by alteration in mood, absence of interest in the surroundings, psychomotor retardation and melancholia. The aim and objectives of present research work is to assess the antidepressant activity and compare the effectiveness of marketed Ayurvedic formulations in mice by using Despair Swim Test and Tail Suspension Test and also estimate the concentration of Nor adrenaline from mouse brain by using Photoflurimeter. The experimental design for present work was the animals were divided into 08 groups and each group contains 06 mice and by using per oral route for 14 days of treatments the Immobility Period was noted on First, Seventh and Fourteenth day.

Forced Swim Test: Group I Control it contains distilled water having dose 10 ml / kg , Group II Standard (Imipramine), dose -15 mg / kg, Group III Formulation A having dose 1.3 ml / kg, Group IV Formulation B dose -1.56 ml / kg. Tail Suspension Test: Group V Control it contains distilled water having dose 10 ml / kg , Group VI Standard (Imipramine), dose -15 mg / kg, Group VII Formulation A having dose 1.3 ml / kg, Group VIII Formulation B dose -1.56 ml / kg. The conclusion of present studies are Formulation A and B possess significant antidepressant activity and Formulation B is highly effective as compared to Formulation A as observed in two models which are employed in this study. However, the precise mechanism of action by which the plants in the formulations shows the antidepressant like effect are not completely studied. So the further additional studies are necessary to isolate the exact active chemical constituents which are responsible for antidepressant action.

KEYWORDS: Depression, Antidepressant drugs, Forced Swim Test, Tail Suspension Test.

INTRODUCTION:

Depression is referred as an affective disorder which is described by alteration in mood, absence of interest in the surroundings, psychomotor retardation and melancholia.[1] Depression belongs to heterogeneous group of mental disorder which is considered by extreme exaggerations and disturbance of mood, which adversely affect cognition and psychomotor functions.[2] The main symptoms of depression are due to functional deficiency in concentration of monoaminergic neurotransmitters like Dopamine, Nor adrenaline, Serotoninin the brain. Those drugs which increases

the concentration of these neurotransmitters in the central nervous system such drugs shows the antidepressant activity. The direct antagonism at $5HT_3$ receptor site which may be associated with the antidepressant activity as conventional antidepressants also possess affinity for central $5HT_3$ binding site.[3]

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant source that are used in the treatment of psychiatric disorders. On one hand these agents have a less antagonistic effect profile, and on the other hand they have been shown to be similar in efficacy to their synthetic counterpart.[4] Almost all synthetic medicines are existing for therapeutic management of depression having serious adverse effects and number of drug interactions. The no. of Ayurvedic formulations are available in the market and it's pharmacological investigations are not done yet. Therefore, the aim of this study was to evaluate and compare the antidepressant activity of two Ayurvedic formulations.

MATERIALS AND METHODS:

Table No. 01 : List of Drug and Chemicals

Sr.No.	Drug / Chemicals	Company
01.	Imipramine	Torrent Pharmaceutical Ltd. Ahmadabad.
02.	Nor adrenaline	Research – lab fine chemindustries.Mumbai.
03.	Normal Saline	Claris Pharmaceutical
04.	Conc.HCL	Research – lab fine chemindustries.Mumbai.
05.	Butanol	Research – lab fine chemindustries.Mumbai.
06.	Glacial acetic acid	Research – lab fine chemindustries.Mumbai.
07.	Sodium acetate	Research – lab fine chemindustries.Mumbai.
08.	Sodium Hydroxide	Research – lab fine chemindustries.Mumbai.
09.	Potassium Iodide	Research – lab fine chemindustries.Mumbai.
10.	Sodium Sulphite	Research – lab fine chemindustries.Mumbai.
11.	Ethanol	Datta sugar factory, Shirol.

Table No.02 : List of Apparatus and Equipments

Sr.No.	Apparatus / Equipments	Company
01.	Forced Swim Test apparatus	Self Made
02.	Analytical Weighing Balance	Shimadzu Instru, Mumbai.
03.	Variable volume of micropipette	Bio system
04.	Homogenizer	Remi motors LTD
05.	Cooling Centrifuge	Remi motors LTD
06.	Ultrasonicator	D-120/1H
07.	Refrigerator	Haier
08.	Photoflurimeter	Equiptronics

A. Procurement of Formulations :

The Formulations were purchased from Dandekar ayurvedic shop, Sangli.

B. Calculation of Doses :

The dose calculations were done by using Paget and Barnes, 1964.

C. **Experimental Protocol :**

All the experiments were carried out using young (6 to 8 Weeks) female Swiss Albino mice having weight 18 – 24gm. Mice were kept in polypropylene cages with stainless steel lid. Animals were kept under standard housing conditions with free access to standard pellets *ad libitum*. The animals were acclimatized for 7 days prior to experiment. Form B protocol were prepared and submitted to Institutional Animal Ethics committee (IAEC). Approval for animal use was obtained from IAEC prior to experimental study. The experimental protocol (IAEC/ABCP/02/2017-18) was approved by the IAEC. The procedures involving laboratory animals were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

D. Experimental design :

The animals were divided into 08 groups and each group contains 06 mice and by using per oral route for 14 days of treatments the Immobility Period was noted on First, Seventh and Fourteenth day.

Table No.03: Groups for Forced Swim Test

Sr.No.	Group	Treatment	Dose
01.	Group I	Control (Distilled Water)	10 ml / kg
02.	Group II	Standard (Imipramine)	15 mg / kg
03.	Group III	Formulation A	1.3 ml / kg
04.	Group IV	Formulation B	1.56 ml / kg

Table No. 04: Groups for Tail Suspension Test

Sr.No.	Group	Treatment	Dose
05.	Group V	Control (Distilled Water)	10 ml / kg
06.	Group VI	Standard (Imipramine)	15 mg / kg
07.	Group VII	Formulation A	1.3 ml / kg
08.	Group VIII	Formulation B	1.56ml / kg

Evaluation of Antidepressant Activity

01. Forced Swim Test[5,6]

Despair Swim Test was proposed as model to test antidepressant activity by porsolt in 1977. Mouse was individually forced swim in a plastic cylinder having height : 40 cm ; diameter : 18 cm) containing fresh water of 15 cm height and maintained at 25^0c (± 3^0c). Mouse was placed in the cylinder for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min. activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The immobility period was recorded for 05 min After 05 min. in the water the mouse was removed and allowed to dry before being returned to their home cages.

02. Tail Suspension Test[5,7]

The "Tail Suspension Test" has been described by Steru et al. (1985) as a facile means of evaluating potential antidepressants. The mouse was suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 01 cm from the tip of the tail. The duration of immobility was recorded for a period of 05 min. Mice will be considered immobile when they hang passively and completely motionless.

Estimation of Nor adrenaline[8]

Preparation of Nor adrenaline Sample -

On the day of experiment mice were sacrificed, Whole brain was dissected out and sub cortical region (including the stritum) was separated. Weight tissue was weight and homogenized in 5 ml HCL – Butanol solution for about 01 min. The sample was then centrifuged for 10 min. at 2000 rpm. An aliquot supernatant phase (01ml) was removed and added to centrifuge tube containing 2.5 ml heptanes and 0.31 ml HCL of 0.1M. After 10 min. The content in centrifuge tube was centrifuged under the same condition as above in order to separate the two phases, and overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken either for estimation of Nor adrenaline.

Estimation of Nor adrenaline -

To the 0.2ml aqueous phase, 0.05ml 0.4 M HCL,0.1ml of Sodium acetate buffer (p^H – 6.9) were added, followed by 0.1 ml Iodine solution (0.1M Ethanol) for oxidation. The reaction stopped after 02 min. by addition of 0.1 ml Sodium sulphite(Na_2So_3) Solution. 0.1 ml Acetic acid is added after 1.5 min. The solution was then heated to 100° C for 06 min. When the sample again reached to room temperature, excitation and emission spectra were read from Photoflurimeter by using 485 nm filter.

Statistical Analysis

The values are expressed as Mean ± SEM for six mice in each group. The Statistical Analysis was performed using one way ANOVA followed by Dennett's test. (Graph pad prism version 7.04). p value <0.05 was taken as statistically significant.

Days	Immobility Period (Sec.) Mean ± SEM			
	Control	**Standard**	**Formulation A**	**Formulation B**
01st	142.2 ±1.83 (100%)	100.2± 0.79*** (70.46%)	90.50± 1.43*** (63.64%)	82.50± 0.99*** **(58.01%)**
07th	138.7±1.35 (100%)	95.33± 1.35*** (68.73%)	82.50± 0.99*** (59.48%)	76.17 ± 1.04*** **(54.91%)**
14th	135.5±1.28 (100%)	92± 0.44*** (67.89%)	79.33± 1.11*** (58.54%)	73.83± 1.70*** **(54.48%)**

RESULTS:

Evaluation of Forced Swim Test

Table No. 05 : Effect of formulations on immobility period in Forced Swim Test

All the values were expressed in a Mean ± SEM. The results were analysed statistically by the one – way ANOVA followed by Dennett's test (n = 6). ***$p < 0.05$ as compared to control group. The values in bracket indicates that the % of Immobility.

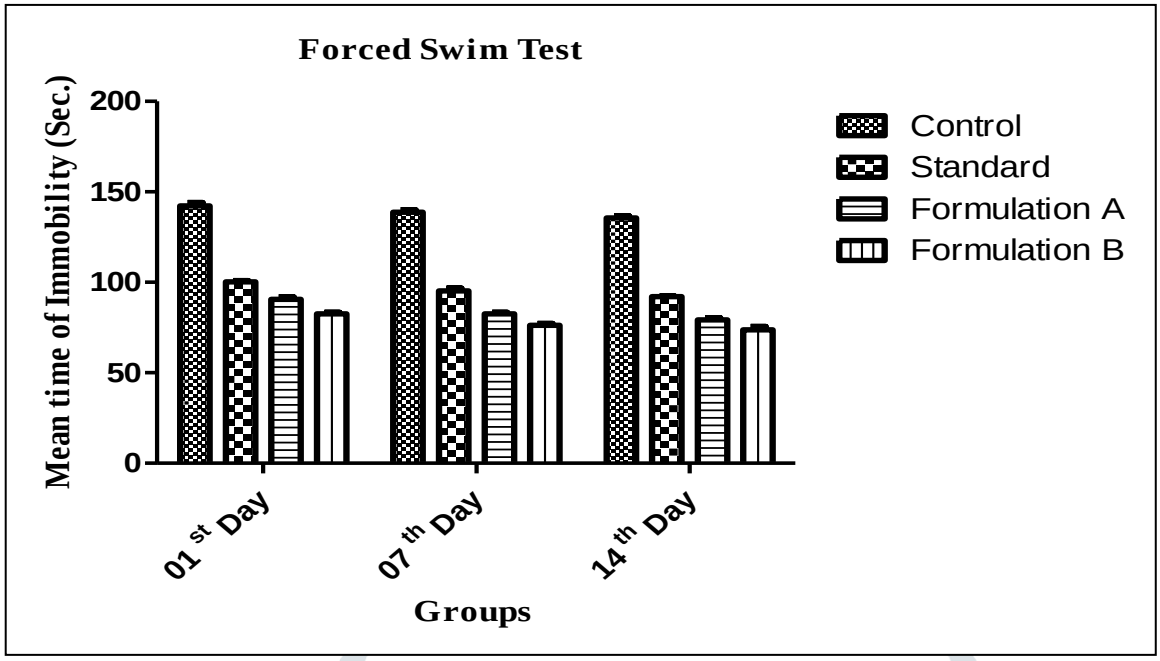

Figure No. 01: Effect of Formulations on the Immobility Period in Forced Swim Test

On the **first day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 36.36 & 41.99% respectively.

On the **seventh day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 40.52 & 45.09% respectively.

On the **fourteenth day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 41.46 & 45.52% respectively.

Evaluation of Tail Suspension Test

Table No. 06 : Effect of formulations on immobility period in Tail Suspension Test

Days	Immobility Period (Sec.) Mean ± SEM			
	Control	**Standard**	**Formulation A**	**Formulation B**
01st	202.2±0.74 (100%)	170.2±0.87** (79.01%)	154.5±1.60** (76.04%)	151.2±0.94*** (74.71%)
07th	190.7±1.14	145.3±1.14**	142.3±1.35**	139.8±1.19***

	(100%)	(76.31%)	(74.73%)	(73.15%)
14th	187.5±1.68 (100%)	142 ±0.73** (75.93%)	138.5±1.17** (74.06%)	134.7±1.02*** (71.76%)

All the values were expressed in a Mean ± SEM. The results were analysed Statistically by the one - way ANOVA followed by Dennett's test (n = 6). **p < 0.05, ***p < 0.05 as compared to control group. The values in bracket indicates that the % of Immobility.

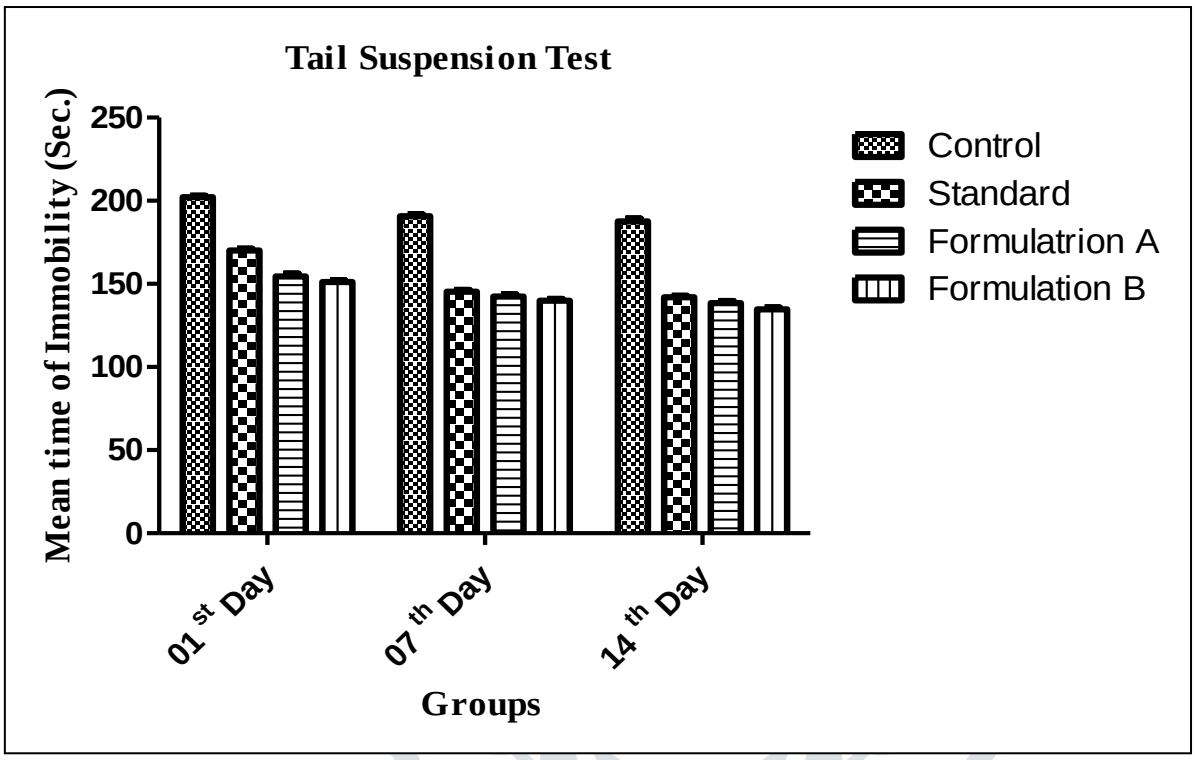

Figure No. 02: Effect of Formulations on the Immobility Period in Tail Suspension Test

On the **first day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 23.96 & 25.29 % respectively.

On the **seventh day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 25.27 & 26.85% respectively.

On the **fourteenth day** of treatment formulation A & B produced significant antidepressant activity in mice when compared to control group. The % reduction in immobility period by formulation A & B is 25.94 & 28.24% respectively.

Estimation of Nor adrenaline in FST model

Table No. 07 : Level of Noradrenalin in Brain tissue homogenate in FST model

Groups	Treatment	Dose	Concentration of Noradrenalin in ng / gm of Brain tissue (Mean ± SEM)
I	Distilled Water	10 ml/kg	212.5 ± 2.12
II	Imipramine	15 mg/kg	250.3 ± 1.38***
III	Formulation A	1.3 ml / kg	258 ± 2.38***
IV	Formulation B	1.56 ml / kg	268.5 ± 1.08***

Values are expressed in a Mean ± SEM. Statistical analysis of data was carried out by the one – way ANOVA followed by Dennett test (n = 6). ***p < 0.05 as compared to control group.

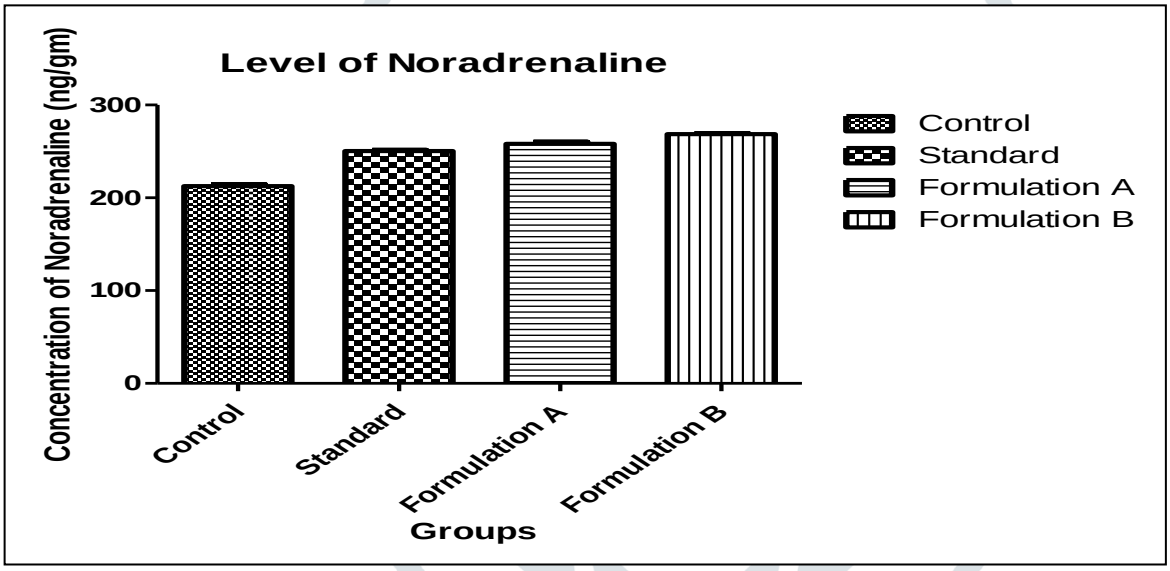

Figure No. 03 : Level of Noradrenalin from brain tissue homogenate in FST model.

In this level animals are treated with formulations showed significant ***p <0.05 ,increase in the level of Nor adrenaline when compared to control group similarly animals treated with Imipramine showed significant increase in the level of Nor adrenaline.

Estimation of Nor adrenaline in TST model

Table No. 08 : Level of Nor adrenaline in Brain tissue homogenate in TST model

Groups	Treatment	Dose	Concentration of Noradrenalin in ng / gm of Brain tissue (Mean ± SEM)
I	Distilled Water	10 ml/kg	183.5 ± 1.54
II	Imipramine	15 mg/kg	204.3 ± 1.64***
III	Formulation A	1.3 ml / kg	219.7 ± 1.28***
IV	Formulation B	1.56 ml / kg	226.2 ± 1.04***

Values are expressed in a Mean ± SEM. Statistical analysis of data was carried out by the one – way ANOVA followed by Dennett test (n = 6). ***p < 0.05 as compared to control group.

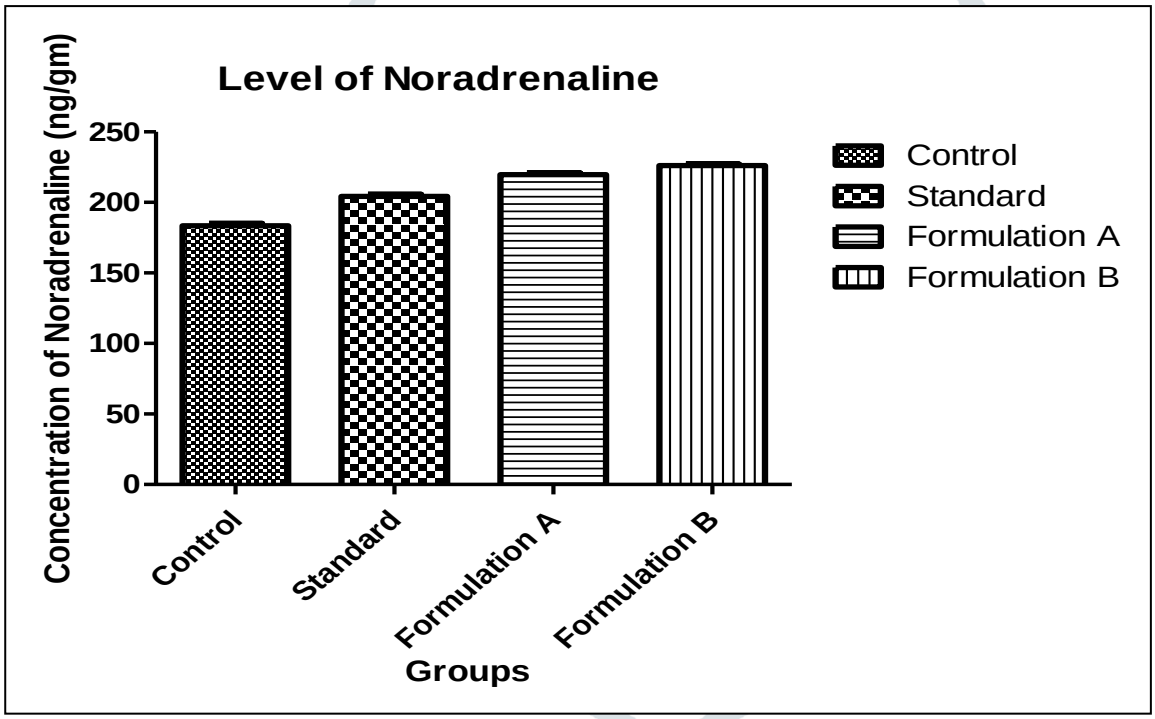

Figure No. 04 : Level of Noradrenalin from brain tissue homogenate in TST model.

In this level animals are treated with formulations showed significant ***p <0.05 ,increase in the level of noradrenalin when compared to control group similarly animals treated with Imipramine showed significant increase in the level of Nor adrenaline.

DISCUSSION:

Depressive disorder has long been associated with disturbances of brain biogenic amines such as Noradrenalin, Serotonin and Dopamine activity and data concerning with biogenic amines variations in depression have probably been the most widely studied.[9]

Forced Swim Test and Tail Suspension Test were selected for screening of antidepressant activity of two Ayurvedic formulations for present research work.

Since initial hypothesis of depression has been formulated long ago and proposing that symptoms of depression due to functional deficiency of cerebral monoaminergic transmitters such as Nor adrenaline, serotonin and dopamine located at synapses. Imipramine hydrochloride acts by inhibiting Noradrenalin and Serotonin reuptake and has been used as a standard drug in majority studies. The beneficial effect of Imipramine hydrochloride in the forced swimming test model seems to be due to increased availability of Nor adrenaline and serotonin at the post synaptic site following reuptake inhibition[10].

For present study we have selected two Ayurvedic formulations viz. Formulation A in a dose of 1.3 ml/kg and Formulation B in a dose of 1.56 ml/kg for evaluation and comparison of antidepressant activity with Imipramine.

Formulation A contains-

Convolvulus pluricaulis, Bacopa monnieri, Acorus calamus, Solanum xanthocarpum, Cyperus rotundus, Calastrus paniculata, Nardostachys jatamasi.

Formulation B contains-

Convolvulus pluricaulis, Bacopa monnieri, Acorus calamus ,Withania somnifera, Glycyrrhiza glabra, Inula racemosa, Rauwolfia serpentina.

The phytochemicals present in the plants of formulations are alkaloids, Glycosides, Steroids, Flavonoids, Triterpinoids and Saponins, Amino acids. Since the present study revealed the significant antidepressant effect of formulations in experimentally induced depression by Forced swim test and Tail suspension test models as compared to standard drug Imipramine hydrochloride, these constituents may be responsible for improving the level of vital neurotransmitters, nerve impulse transmission which were helpful in treatment of depression.

In **forced Swim Test** the percentage reduction in immobility of **formulation A** and **B** on 01st, 07th and 14th days of treatment were **36.36%, 40.52%** , **41.46% and 41.99%, 45.09**% , **45.52%** respectively.

In **Tail Suspension Test** the percentage reduction in immobility of **formulation A and B** on 01st, 07th and 14th days of treatment were **23.96%, 25.27%** , **25.94%** and **25.29%, 26.85%** , **28.24%** respectively.

In **Standard group** the percentage reduction in immobility by **Forced Swim Test** and **Tail Suspension Test** on 01st, 07th and 14th days of treatment were **29.54%** , **31.27%, 32.11%** and **20.99 %, 23.69 %, 24.07%** respectively.

From above data it was found that the activity of formulation A and B were more effective than standard drug (Imipramine) in both the models. Imipramine acts by inhibiting the reuptake of Nor adrenaline and Serotonin but formulations contains several plants and each plant acts by its own mechanism and showed that pharmacologically synergistic effect for antidepressant action. Therefore, might be this is the reason for formulations showed highly effective than Imipramine. Further it was observed that the antidepressant activity achieved after 07th days of treatment was maintain same till 14th days in all treated groups (Formulation A, B and Standard). Among two

Ayurvedic formulations, formulation B in dose of 1.56 ml/kg showed significantly (***p < 0.05) reduced immobility time in FST and TST as compared to control group and having more significant antidepressant activity than formulation A.

As per Vikas Kumar et al. (2008) the antidepressant activity of Anximin, a polyherbal formulation showed 25.7% reduction in immobility after 07th days of treatment which was less effective than our formulations A and B.

For present research work we have also estimated the level of Nor adrenaline in animal models of depression.

In **Forced Swim Test** the level of Nor adrenaline was found to be **258ng/gm** and **268.5ng/gm** of brain tissue by formulations A and B respectively.

In **Tail Suspension Test** the level of Nor adrenaline was found to be **219.7ng/gm** and **226.2ng/gm** of brain tissue by formulations A and B respectively.

Formulation B showed that best significant effect than Formulation A because of following reasons might be responsible-

- The dose of formulation B (1.56 ml/kg) is more than the formulation A (1.3ml/kg).
- The formulation B is contains more quantity of plant extract in 10 ml as compared to formulation A.
- Formulation B contains several plants among that Ashwagandha [11] and Liquorice[12] showed the presence of amino acid –Tyrosine which is precursor for the synthesis of Nor adrenaline.

ACKNOLEDGEMENT:

The author gratefully thanks to Principal Dr. S. A. Tamboli of Appasaheb Birnale College of Pharmacy, Sangli, for providing essential facilities and Associate Professor, Dr. P. L. Ladda and all faculty members of ABCP, Sangli for valuable suggestions during my research work.

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