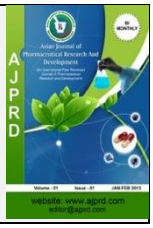
Available online on 15.08.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

Anti-Stress Activity (*in-vivo*) of Multi Herbal Capsule-Trasina® in Experimental Murine Model

Soumendra Darbar[1]*, Srimoyee Saha[2] and Shyamaprasad Chattopadhyay[1], Atiskumar Chattapadhyay[3]

[1]Research and Development Division, Dey's Medical Stores (Mfg.) Ltd.,62, Bondel Road, Kolkata-700019, West Bengal, India
[2]Department of Physics, Jadavpur University, 188, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
[3]Faculty Council of Science, Jadavpur University, 188, Raja S C Mallick Road, Kolkata-700032, West Bengal, India

A B S T R A C T

Background: In this modern era, stress has become a developing devil of human life.To maintain the healthy lifestyle measures adopted for smooth livelihood, stress should be kept under control.
Objective: The main aim and objective of the study was to find out the antistress activity of a multi herbal capsule Trasina® against anoxia stress tolerance, chemical induced stress, swimming endurance stress and immobilisation stress models in swiss albino mice.
Methods: Trasina® was screened for its antistress activity at 50 mg/kg, 100 mg/kg, 200 mg/kg, p.o. doses and diazepam was applied as reference standard drug at 2 mg/kg i.p. dose. Anoxia stress tolerance time, number of writhes, immobility time, organs weight of animals and estimating biochemical parameters such as glucose, cholesterol, blood urea nitrogen (BUN) along with triglyceride were measured to evaluate antistress activity of the formulation.
Results: Administration of Trasina® showed significant increases in anoxia stress tolerance time and significant decreases in number of writhes and immobility time in mice as compared with control untreated animals. In immobilisation stress model we observed that treatment with Trasina® significantly reduced glucose, cholesterol and blood urea nitrogen (BUN) and triglyceride levels. Moreover in stressful condition liver and adrenal gland weight were significantly increased whereas spleen weight was significantly decreased. Treatment with Trasina® maintained the normal organs weight as compared with stressful group.
Conclusion: In conclusion it clearly showed that Trasina® possessed significant antistress activity and maintain normal homeostasis.
Keywords: Poly herbal formulation, Antistress activity, Anoxia stress tolerance, chemical induced stress, Swimming Endurance Stress, Immobilisation Stress, Animal model

A R T I C L E I N F O: Received 04 July 2020; Review Completed 02 Oct. 2020; Accepted 08 Oct 2020; Available online 15 Oct. 2020

Cite this article as:

Darbar S, Saha S, Chattopadhyay S, Chattapadhyay A, Anti-Stress Activity (in-vivo) of Multi Herbal Capsule-Trasina® in Experimental Murine Model, Asian Journal of Pharmaceutical Research and Development. 2020; 8(5):52-58.
DOI: http://dx.doi.org/10.22270/ajprd.v8i5.839
***Address for Correspondence:**

Dr. SoumendraDarbar, Head Research and Development Division For M/s, Dey's Medical Stores (Mfg.) Ltd. 62, Bondel Road, Kolkata-700019,West Bengal, India

INTRODUCTION

In the modern lifestyle stress has become an integral part of human life[1]. Excessive stress disrupts the normal functioning of the body's homeostasis. Extreme stress not only suppress homeostatic mechanisms of the organism but also create question for survival of the organism[2,3]. Severity of the various diseases like headache, diabetes, immunosuppression, hypertension, reproductive dysfunctions and behavioural disorders are also alarming in stressful situation and are prone to central nervous system (CNS), endocrine system, and metabolic system which suppress smooth body's functions[4-6]. The Occupational Safety and Health Administration (OSHA)

declared stress a hazard of the workplace and decreased the work potentiality of the individuals[7].

Various allopathic antistress drugs are available in the market but produce severe adverse reaction for chronic consumption. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress[8,9]. Preclinical study clearly showed that intake of these drugs produce sever toxicity and produce organ damage[10]. So, medication through modern medicines to overcome the stress became very serious matter.

Last few decades Herbal formulations containing natural ingredients have been extensively use in both developed and under developed countries for their potential therapeutic effect in various diseases[11,12]. Scientist reviled that medical plants present in the herbal medicines possess several phytochemical active compounds those produce medicinal effects. Recently herbal formulations have growing demand in the world market. The concept of polyhedral formulation (PHF) is well documented in the ancient literature[13]. Compared to the single herb, the multiherbal formulation has better and extended therapeutic potential The herbal formulations claimed to enhance physical endurance; mental functions and non-specific resistance of the body have been termed as adaptogens[14-16]. Scientific research claimed that herbal medicine is cheap, easily available, fewer side effects, non-toxic and produce better synergistic action in comparison to synthetic modem medicines[17].

Trasina is marketed multi herbal capsulecontaining some Indian medicinal plants classified in Ayurveda, the classic Indian system of medicine, as Medhyarasayanas or drugs reputed to improve memory and intellect. Trasina is a combination of Shilajit, *Withaniasomnifera*, *Tinosporacordifolia*, *Ecliptaalba*, *Ocimum sanctum* and *Picrorrhizakurroa*. Bhattacharya et al in 1997 reported that the formulation has a memory-facilitating action. Research stated that after sub chronic administration of Trasina for 21 days on two rodent models had simulate some biochemical features known to be associated with Alzheimer's disease (AD)[18,19]. Our recent study confirmed that Trasina® have no toxic effects of animals and safe for therapeutic medication[20].

Thus, the present study was designed to assess the antistress ability of multi herbal capsule – Trasina® against anoxia stress tolerance, chemical induced stress, swimming endurance stress and immobilisation stress models in mice.

MATERIALS AND METHODS

Animals

Healthy, Swiss albino mice weighing 25 ±5 g were used for the study. The mice were housed in polypropylene cages and maintained under standard physiological conditions (12 h light and dark cycles, at 25±2 ºC and 50-60% humidity). They were feed with standard pallet diet and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Dey's Medical Stores (Mfg.) Ltd., 62, Bondel Road, Kolkata-700019 registered under CPCSEA, Govt. of India (Registration No. 50/po/98/CPCSEA).

Evaluation of antistress activity

a. Anoxia stress tolerance test in mice

Swiss albino mice weighing 25±5 g were selected and divided in to five groups of six animals each. Group I received only distilled water and served as control, Group II received diazepam at a single dose in each day (2 mg/kg, i.p.), Group III, IV and V received Trasina® at a dose of 50, 100 and 200 mg/kg in each day for four weeks. At the end of 1st, 2nd, 3rdand 4thweek i.e. on the 7th, 14th, 21st and 28thday one hour after the treatment stress was induced by placing each animal individually in the hermetic vessel of 1liter capacity to record anoxia tolerance time. The movement when the animal showed the first convulsions immediately removed from the vessel and resuscitated if needed. The time duration of entry of the animal in to the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from vessel may lead to death of the same[21].

b. Chemical induced stress in mice

Swiss albino mice were randomly divided in to five groups of 6 animals each. Group I received only distilled water and served as control, Group II received diazepam at a single dose in each day (2 mg/kg, i.p.), Group III, IV and V received Trasina® at a dose of 50, 100 and 200 mg/kg in each day. All the treatments were given continuously for 15 days. On day 15, one hour after the drug treatment all the animals received 0.1 mL of 6% (v/v) glacial acetic acid i.p. and number of writhes was observed in all the groups for 20 min[22].

c. Swimming endurance test in mice

Swiss albino mice were randomly divided in to five groups of 6 animals each. Group I received only distilled water and served as control, Group II received diazepam at a single dose in each day (2 mg/kg, i.p.), Group III, IV and V received Trasina® at a dose of 50, 100 and 200 mg/kg in each day. All the treatments were given continuously for 10 days. On day 10, one hour after the drug treatment all the animals were allowed to swim individually in a glass tank filled with water[23]. The immobility time of each mouse was recorded for 30 min [23].

d. Immobilisation stress in mice

Swiss albino mice were selected and divided in to six groups of six animals each. Group I Negative control (Unstressed, untreated), Group II Positive control (Stressed, received vehicle), Group IIIdiazepam (2 mg/kg i.p.), Group IVTrasina® (50 mg/kg p.o.), Group V Trasina® (100 mg/kg p.o.), Group VITrasina® (200 mg/kg p.o.). The treatment was made stated above for 10 days 1 h before the exposure of stress. Stress was induced by immobilising rats with head down, supine position by fixing the forelimbs and hand limbs to a wooden board inclined at an angle of 600, daily 2 h for a period of 10 days[24].

Determination of Biochemical parameters and Organs weight

At the end of the study the animals were sacrificed and blood was collected by retro-orbital for estimation of biochemical parameters. Blood were taken in a glass centrifuge tube and centrifuge at 6000 rpm. Clear serum were discarded carefully for biochemical parameters. Serum glucose, total holesterol (TC), blood urea nitrogen (BUN), and triglyceride (TG) were detected by using standard biochemical kits(Accurex Biomedical Pvt. Ltd, Thane, India). The weight of organs, such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100 g body weight of animal[25].

Statistical analysis

The data were presented as men ± standard error of mean (SEM) and analyzed using one –way analysis of variance (ANOVA) followed by Dunnett's and P< 0.05 was considered statically significant. Statistical Package for Social Science (SPSS 20.0) version software was used for statistical analysis.

RESULTS

Effect of Trasina® on Anoxia stress tolerance test in mice

Table 1 shows anoxia stress tolerance of 7th, 14th, 21st and 28th day in mice. The tolerance time was significantly enhanced on 7th, 14th, 21st and 28th day by dose dependent administration ofTrasina® at doses 100 mg/kg (P< 0.01) and 200 mg/kg (P< 0.001), p.o. and diazepam at 2 mg/kg (P<0.001) i.p. treated groups. However the effect Trasina® at 50 mg/kg dose on anoxia stress tolerance time in mice was not statically significant at the end of 1st, 2nd, 3rd and 4th week of treatment.

Table: 1 Ameliorative effect of Trasina® on anoxia stress tolerance time in mice

Groups	**Duration of anoxia stress tolerance (min.)**			
	Day 7	Day 14	Day 21	Day 28
Control	128.44±1.25	132.14±1.36	134.25±1.48	135.67±1.67
Diazepam 2 mg/kg	169.25±1.65**	171.95±0.99**	172.59±1.26**	174.56±1.95**
Trasina 50 mg/kg	136.25±0.95*	138.25±1.11*	140.54±1.91*	144.28±1.44*
Trasina 100 mg/kg	142.94±1.27*	148.39±1.15*	155.47±1.54*	160.27±1.36**
Trasina 200 mg/kg	155.21±1.06**	169.84±0.94**	175.64±0.96**	180.24±1.52**

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.001compared with control (one-way ANOVA followed by Dunnett's test).

Effect of Trasina® on Chemical induced stress in mice

Administration of glacial acetic acid 0.1mL of 6% (v/v) significantly elevated the number of writhes in mice. Pre-treatment with Trasina®at 50 mg/kg (P< 0.05) and 100 mg/kg (P< 0.01) doses were found to be significantly inhibit number of writhes in 20 minutes when compared to control group. HoweverTrasina®at high dose 200 mg/kg (P< 0.001) and diazepam at 2 mg/kg (P< 0.001) dose were found to be significantly inhibit number of writhes in 20 minutes when compared to control group i.e. effect of Trasina® was dose relative manner (Table 2).

Table: 2 Ameliorative effect of Trasina® on chemical stress tolerance time in mice

Groups	**Number of writhes (min.)**
Control	48±1.62
Diazepam 2 mg/kg	11±0.96***
Trasina 50 mg/kg	29±1.36*
Trasina 100 mg/kg	21±1.54**
Trasina 200 mg/kg	12±1.47***

Values are expressed as mean ± SEM, n=6, *P<0.01, *P<0.05, **P<0.001compared with control (one-way ANOVA followed by Dunnett's test).

Effect of Trasina® on swimming endurance test in mice

Effect of Trasina® on swimming endurance test in mice was shown in table 3. The immobility time was significantly increases in control group of mice; whereas pre-treatment with Trasina®at 50 mg/kg and 100 mg/kg doses were found to be significantly (P< 0.05) reduced immobility time in 30 minutes. However Trasina®at high dose 200 mg/kg (P< 0.01) and diazepam at 2 mg/kg (P<0.01) dose were found to be significantly reduced immobility time in 30 minutes.

Table: 3 Ameliorative effect of Trasina® on swimming endurance stress tolerance time in mice

Groups	**Immobility time (min.)**
Control	28.00±1.34
Diazepam 2 mg/kg	10.78±1.62***
Trasina 50 mg/kg	14.29±1.55*
Trasina 100 mg/kg	10.25±1.07**
Trasina 200 mg/kg	8.15±1.29***

Values are expressed as mean ± SEM, n=6, *P<0.01, *P<0.05, **P<0.001compared with control (one-way ANOVA followed by Dunnett's test).

Fig. A: Plants used in Trasina® Capsule

Fig. B: Trasina® Capsule

Fig. C: Dry Power of Trasina

Figure: 1 Main ingredients present in Trasina® (Fig.A);Trasina in solid doses form (Fig.B); Dry powder of multi herbal formulation Trasina® (Fig. C)

Effect of Trasina® on Immobilisation stress in mice

Effect on biochemical parameters

The results of biochemical parameters was showed in figure 2. The immobilisation stress caused marked increase in biochemical parameters like glucose, total cholesterol (TC), blood urea nitrogen (BUN) and triglyceride (TG) in mice. Pre-treatment with Trasina®at 50 mg/kg, 100 mg/kg p.o. doses were significantly (P< 0.05) reversed elevated levels of such biochemical parameters. However Trasina®at high dose 200 mg/kg were significantly (P< 0.01) reversed elevated levels of such biochemical parameters and showed the optimum protective effects.

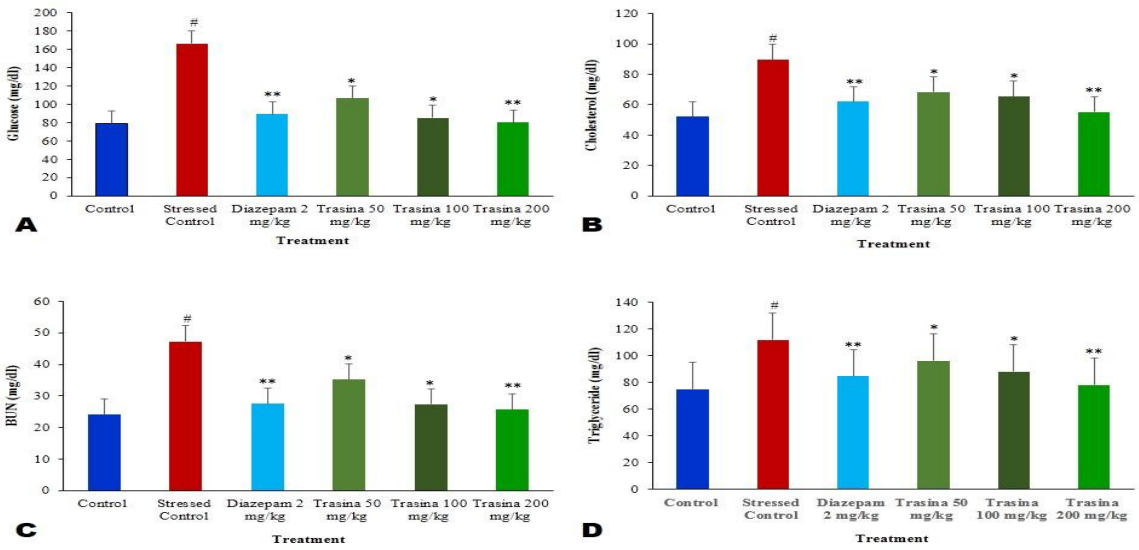


Figure: 2 Effect of Trasina® on biochemical parameters in immobilization stress in mice.Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals;*p<0.05, **p<0.001 compared with stressed control animals(one-way ANOVA followed by Dunnett's test).

Effect on organs weight

The results of organs weight was showed in figure 3.The immobilisation stress caused marked changes in organs weight i.e. weight of spleen was significantly reduced and weight of liver and adrenal gland was significantly increased in mice. However Trasina®at, 50 mg/kg and 100 mg/kg p.o. doses were significantly (P< 0.05) significantly increased weight of spleen and decreased weight of liver and adrenal gland. Whereas treatment with Trasina®at high dose i.e. 200 mg/kg p.o. were significantly (P< 0.01) increased weight of spleen and decreased weight of liver (P< 0.001) and adrenal gland (P< 0.01).

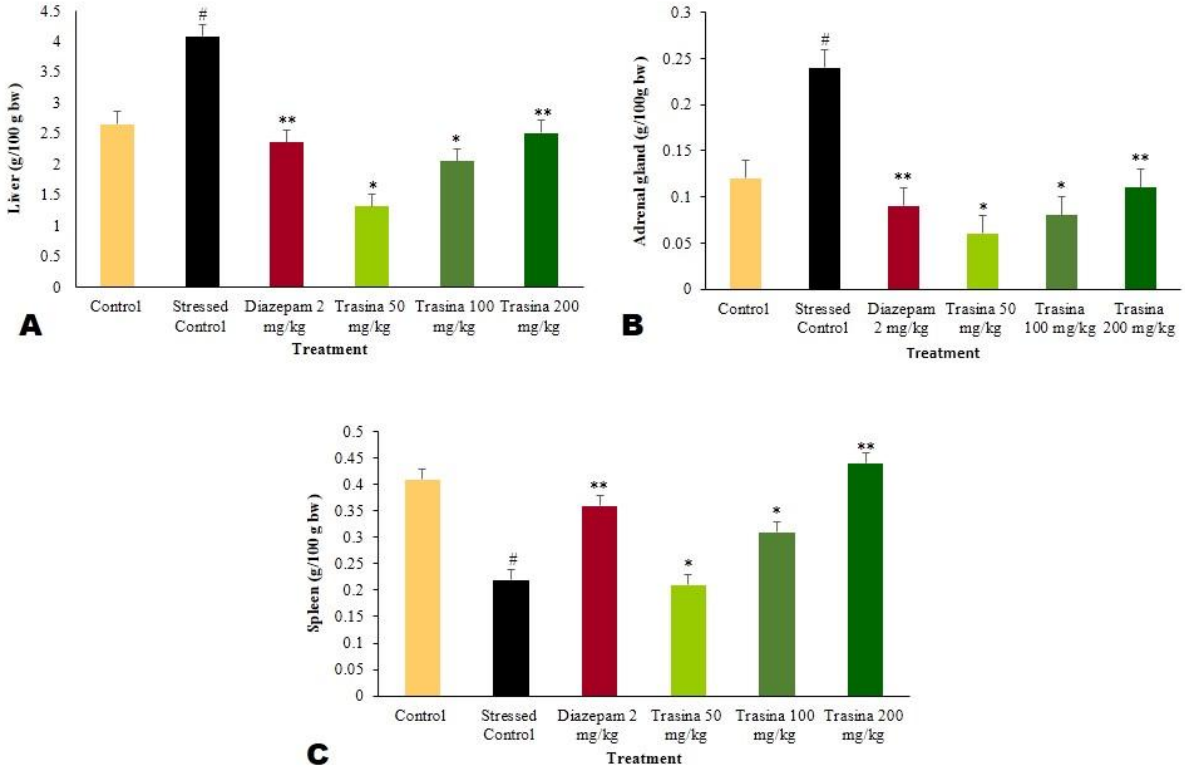


Figure: 3 Effect of Trasina® on organ weights in mice.Values are expressed as mean ± SEM, n=6, #p<0.001 compared with control untreated animals;*p<0.05, **p<0.001 compared with stressed control animals(one-way ANOVA followed by Dunnett's test).

DISCUSSION

Present study showed that in anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with Trasina® had increased the duration of stress tolerance indicating their antistress activity. Scientific study reviled that level of succinate dehydrogenase in brain is responsible for utilization and conservation of energy in the cellular system of the organisms, which helps adaptive process during stress[26]. Treatment with Trasina® may be increased the succinate dehydrogenase level which control the utilization and conservation of energy during stress condition. In swimming endurance test model administration of Trasina® significantly reduced the immobility time in animal clearly exhibited the antistress activity. Previous research study established that swimming endurance test elevate plasma adrenaline and noradrenaline level and decrease monoamine oxidase level decrease in the brain during stress[27]. The possible mechanism of antistress activity Trasina® against swimming endurance model might be due to the normalization of catecholamines and monoamine oxidase level. In immobilisation stress model significant increase in biochemical parameters such as glucose, cholesterol, blood urea nitrogen and triglyceride in stress control group when compared to the other groups.

Hyperglycaemia is very common in stressful condition which increase blood pressure and rate. In our result we also observed high sugar level in the blood in the chronic stress condition. Scientific reports stated that during stressful situation adrenal cortex secrets cortisol in man and corticosterone in laboratory animals which helps in maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis[28,29]. Application of Trasisn® significantly reduced the elevated blood glucose levels clearly indication the suppression of adrenal cortex's hyperactivity and maintained the homeostatic condition.

In stressed condition marked elevation of serum total cholesterol (TC), triglyceride (TG) and blood urea nitrogen (BUN) in experimental animals stated that stimulation hypothalamo-pituitary axis (HPA) and sympathetic system which release catecholamines and glucocorticoids inhibits the immune function at multiple sites like liver and kidney[30,31]. Administration of Trasina® significantly reduced the serum cholesterol, triglyceride and blood urea nitrogen (BUN) levels which may be happened due to the inhibition of stimulation of sympathetic nervous system.

Gross weight of the liver and adrenal gland were significantly increased in the stress condition and reverse to the normal condition by the treatment of Trasina®. This is happened due to the stress induced adrenomedullary response and decreased production of corticotropic hormone[32,33]. On the other hand splenic weight reduction in stress condition was observed which may be happen due to recruitment of lymphocytes to blood from spleen which results squeezing of spleen. In the study pre-treatment with Trasina® increase the splenic weight which indicates inhibition of recruitment of lymphocytes to blood from spleen.

However pre-treatment with Trasina® was found to be significantly reduces number of writhes in dose dependent manner. The possible mechanism for reduction in number of writhes by Trasina® might be due to the inhibition of pain and inflammatory processes. All these findings confirmed that antistress activity of Trasina® was significant and dose dependent manner. Our previous study demonstrate that herbs present in the Trasina® enrich with various phyto-constituents like polyphenols, flavonoids, triterpenes, tannins etc. may process variety of pharmacological action including antistress property.

CONCLUSION

It may be concluded that administration of Trasina® significantly increases anoxia tolerance time, significantly decreases immobility time and number of writhes in animals and this effect was dose dependent. Immobilisation stress induced changes in biochemical parameters and organs weight were copleately revert by Trasina® treated groups. The possible mechanism underlying this effect is mediated through normalization of catecholamines level. The results of present study demonstrated that Trasina® is a potent safe and nontoxic antistress medication and maintain homeostatic condition during stress.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgement

The authors are thankful to Prof. (Dr.) T.K.Pal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032 for his valuable suggestions and Mr. GautamDey, M.D., Mr. RanajitDey, Jt. M.D. and Mr. SubhartheeDey, Whole time Director, for facilities and encouragement during this investigation.

Conflict of Interest

We declare that we have no conflict of interest.

REFERENCES

1. Purane LM,Vidyadhara S. Antistress activity of *Diospyrosmalabarica*(Desr.) Kostel in mice and rats. International Journal of Pharmaceutical Sciences and Research. 2016; 7(8): 3299-3305.
2. Rai D, Gitika BG, Sen T,Patil G. Anti-stress Effects of Ginkgo biloba and Panax ginseng: a comparative study. Journal of Pharmacological Sciences. 2003; 93:458-464.
3. Subarnas A, Tadano T, Nakahata N, Arai Y, Kinemuchi H, Oshima Y, Kisara K,Ohizumi Y. A possible mechanism of antidepressant activity of beta-amyrinpalmitate isolated from Lobelia inflate leaves in the forced swimming test. Life Sciences. 1993; 52:289-296.
4. Anisman H,Zacharko RM. Multiple neurochemical and behavioural consequences of stressors: Implications for depression. In: Psychopharmacology of anxiolytics and antidepressants, Pergamon Press, New York, 1991: 57-82.
5. Sharma PK, Arora A. Herbal drug a twenty first century perspective. Jaypee brother's medicinal publishers Private Ltd: New Delhi, First Edition 2006.
6. Eills JM, Reddy P. Effect of *Panax ginseng* on quality of life. The Annuals of Pharmacotherapy. 2002; 36:375-379.
7. Lakshmi BVS, Sudhakar M. Screening of *Psidiumguajava* leaf extracts for antistress activity in different experimentalanimal models. Pharmacognosy Research. 2009; 1(6): 359-366.
8. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebanon Wissen Technology.1995; 28:25-30.
9. Anssari ZM, Fasiuddin M, Salman S, Nazer S, Imran M, Toufeeq M, Roshan S,Mahammed NL. Pharmacological screening of polyherbal formulation for anti-stress activity on Albino rats. IJPR.2015; 5(5): 125-128.
10. Adedapo AA, Jimoh OF, Koduru S, Afolayan JA, Masika JP. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complementary and Alternative Medicine.2008; 8:53-60.
11. Demiray S, Pintado ME, Castro PML. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tiliaargentea, Crataegi folium leaves and Polygonumbistorta roots. World Academy of Science, Engineering and Technology 2009; 54:312-317.
12. Darbar S, Chattopadhyay S. Antiulcer effect of livina, a herbal formulation against ethanol induced acute gastric ulcer in mice. Int. J. Pharm. 2010; 2(10):93-100.
13. Darbar S, Bose A, Chattaraj TK, Pal TK. Protective role of *Zingiberofficinale* Roscoe on aceclofenac induced oxidative stress in rat liver. Int J Pharm Tech Res. 2010; 2(1): 495-501.
14. Darbar S, Bhattacharya A, Chattopadhyay S. Ameliorative effect of Livina, a polyherbal preparation on Diclofenac-induced liver injury: A comparison with Silymarin. J Pharm Res.2010; 3(12): 2794-2798.
15. Darbar S, Chattopadhyay SP, Ghosh B, Chakraborty MR. Effect of a polyherbal liquid formulation on aceclofenac induced gastric mucosal damage in albino wistar rats. J Pharm Res. 2008; 7(2): 62-65.
16. Darbar S, Bose A, Bhaumik UK, Roy B, Chatterjee N, Pal TK. Antioxidant and hepatoprotective effect of andrographispaniculata leaf extract on diclofenac induced hepatotoxicity in rats. Pharmacologyonline. 2009; 2: 95-108.
17. Darbar S, Chakraborty MR, Chattarjee SP, Ghosh B. Protective effect of Livina, a polyherbal liquid formulation against ethanol induced liver damage in rats. Ancient science of life. 2009; 28(3): 14-20.
18. Bhattacharya SK, Kumar A. Effect of Trasina, an ayurvedic herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. J Altern Complement Med. 1997; 3(4):327-336.

19. Bhattacharya SK, Ghosal S. Effect of Shilajit on rat brain monoamines. Phytotherapy Research. 1992; 6(3): 163 – 164.
20. Darbar S,Chattopadhyay SP. Acute oral toxicity study of Trasina®, an Ayurvedic herbal formulation on experimental models. J. Pharm. Med. Res. 2019; 4(1): 84-86.
21. Vinod SP,Shivkumar H.Adaptogenic activity of *Trigonellafoenumgraecum*(Linn) seeds rodents exposed to anoxia and immobilization stress. Asian Pacific Journal of Tropical Biomedicine. 2012; S208-S211.
22. Nimbkar SR, Patki VP, Patki MP. Pharmacological evaluation of anti-stress and androgenic activity of Polyherbal formulation 'AP-300'containg *Panax ginseng*. Indian Drugs. 2001; 38:27.
23. Tanuj J, Sangeeta PS, Anita S. Anti-stress activity of ethanolic extract of *Asparagus racemosusWilld*roots in mice. Indian Journal of Experimental Biology. 2012; 50:419-424.
24. Sibi PI, Sajid KP.Antistress activity of *mikaniamicranthaKunth*roots in Wistar albino rats. Journal of Scientific and Innovative Research. 2013; 2(6):999-1005.
25. Kenjale RD, Shah RK,Sathaye SS. Anti-stress and anti-oxidant effects of roots of *Chlorophytumborivilianu.* Indian J Exp Biol. 2007; 4(3):974-979.
26. Kenjale RD, Shah RK,Sathaye SS. Anti-stress and anti-oxidant effects of roots of *Chlorophytumborivilian*y.Indian J exp Biol. 2007; 4(3):974-979.
27. Debnath J, Tigar P, Roopa K, Dupadahalli K, Praveen S. An Experimental Evaluation of anti-stress effects of *Terminaliachebula.* J Physiol Biomed Sci. 2011; 24(2):13-19.
28. Tsigos C,Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocreine factors and stress. J Psychosomatic Res. 2002; 53(4):865-871.
29. Kannur DM, Hukkeri VI,Akki KS.Adaptogenic activity of *Caesalpinabonduc*seed extracts in rats. J Ethnopharmacol. 2006; 108:327-331.
30. Schimmer BP, Parker KL. Adrenocortical steroids and their synthetic analogues. In: The Pharmacological Basis of Therapeutics. The McGraw-Hill Medical Publishing Division, New York, Edition 11, 2006: 1655-1662.
31. Nikunj BP, Varsha JG,Bharatkumar GP.Antistress activity of *Argyreiaspeciosa*roots in experimental animals. Journal of Ayurveda & Integrative Medicine. 2011; 2(3):129-136.
32. Pavia DL, Lampman GM, Kriz GS. Introduction to spectroscopy. 3rd edition, Thomson brooks Ltd, United State, 2001, 40-44.
33. Varsha G, Bharatkumar P, Nikunj P. Antistress activity of *Argyreiaspeciosa*roots in experimental animals, J Ayurveda Integr Med. 2011, 3: 129-136.