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Research Article

ADAPTOGENIC ACTIVITY OF METHANOLIC EXTRACT OF ANOGEISSUS LATIFOLIA WALL AND TABEBUIA ROSEA (BERTOL.) DC ON DIFFERENT EXPERIMENTAL MODELS

R. DEEPIKA[1], HEMAMALINI. K[2], AND DR. UMA VASIREDDY[3]

[1,2]Department of Pharmacology, TRR College of Pharmacy, Meerpet, Hyderabad, [3]IPA Treasurer. Email:deepikarumandla@gmail.com

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ABSTRACT

Objective: To investigate the adaptogenic activity of *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol.) DC against immobilization stress model, forced swimming endurance test and anti fatigue effect.

Method: Stress was induced by immobilization and forced swimming endurance test. The animals were divided into different groups. Group I served as negative control which received only vehicle. Group II rats served as positive control received stress with vehicle. Group III treated with Methanolic extract of *Anogeissus latifolia* wall(MEAL) (300mg/kg).Group IV treated with Methanolic extract of *Tabebuia rosea* (Bertol.)DC (METR) (500mg/kg).Group V treated with standard Ashwagandha. Stress was induced by placing the animal in supine position by fixing the forelimbs and hind limbs to a wooden board for two hrs for a period of ten days for immobilization stress model and for forced swimming endurance stress, the animals were subjected to swim daily once for a period of 7 days and on the end of the study period both the stress induced models were sacrificed and estimated for biochemical parameters. The biochemical parameters such as serum glucose (GOD-POD method), BUN (blood urea nitrogen), GLDH-UREASE method. The weight of organs such as liver, spleen, adrenal gland and testes after washing with alcohol is recorded per 100g body weight of animal. The anti-fatigue was measured by using Rotarod apparatus .The physical stress caused fatigue and motor coordination is measured for anti-fatigue activity.

Keywords: *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol) DC, Immobilization stress, Forced swimming endurance test and Anti fatigue effect.

INTRODUCTION

Adaptogens were initially defined as substances that enhance the "state of non-specific resistance a physiological condition that is linked with various disorders of the neuro endocrine-immune system .This definition has been updated as "*a new class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors*"[1] .The term adaptogen was used as a functional claim for certain botanicals and herbal medicinal products in Europe and the USA and the adaptogen concept is now a generally accepted concept .A number of clinical trials clearly demonstrated that adaptogens exert an anti-fatigue effect that increases mental work capacity against a background of stress and fatigue, particularly in tolerance to mental exhaustion and enhanced attention. Studies on animals and isolated cells have revealed that adaptogens exhibit neuroprotective, anti-fatigue, antidepressive, anxiolytic, nootropic, and CNS stimulating and tonic effects [2]. In contrast to conventional stimulants such as sympathomimetics (e.g., ephedrine, fenfluramine, phentermine, prolintane) and general tonics, adaptogens do not possess addiction, tolerance and abuse potentials, or impair mental function, or lead to psychotic symptoms with long term use .

If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions stress triggers a wide range of body changes called General Adaptation Syndrome (GAS).The stimuli, which produce GAS, are called the Stressors and range from physical to physiological factors including cold, heat, infection, toxins, major personal disappointment etc[3].Stress alters the equilibrium of various harmones which have significant impact on the immune response in general. The status of immune system-immunosuppression versus immunopotentiation will depend upon the net effect of these changes. Stress and depression have been shown to affect immune system functioning, with both immunosuppression and immune activation[4]. Correlations between depression and elevated susceptibility for infections or mortality rates have been observed and are associated with immune suppression [5].The physiological reaction to stress involves alteration in the autonomic nervous system, the endocrine system and the immune system. The secretion of glucocorticoids is a classic endocrine response to stress [6]. Stressful stimulation influences antigen-specific as well as non specific reaction [7].

MATERIALS AND METHODS

Plant Materials

The leaves of *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol.)DC were procured from Dr. K Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra pradesh, India. The plant was identified by a botanist, and voucher specimen was deposited in Sri Venkateshwara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute TRR College of Pharmacy (1447/PO/a/11/CPCSEA). After authentification, the leaves were cleaned and shade dried and milled into course powder by a mechanical pulverizer.

Preparation of Plant Extract

The leaves of these plants were dried under shade at room temperature (27-30ºC) for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powdered material (2 kg) was defeated with petroleum ether (60-80ºC) in a soxhlet extraction apparatus and marc was extracted with methanol (1000ml) overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30ºC under reduced pressure in a rotary evaporator. The crude extract was dissolved in 1% Tween 80 to required concentrations and used for the experiments.

Preliminary phyto-chemical evaluation

The MEAL and METR leaves were subjected to preliminary phytochemical screening for various plant constituents.

Animals

Albino rats weighing 160-250g were used. Albino mice weighing 18-30g were used. They were caged in a room under standard laboratory conditions i.e., temperature 23± 1ºc, relative humidity 55± 5% and lighting 08:00-20:00 hr. The animals were fed on a pelleted diet and water ad libitum. The animals were transferred to the laboratory atleast 1 hr before the start of the experiment. The

experiments were performed during the day (08:00-16:00hr).The ethical committee of the institute approved the protocol of the study.

Selection of dose for the study [8-9]

The dose for *Anogeisssus latifolia* wall was selected through the retrospective data 300mg/kg[8], and 500 mg/kg on dose for *Tabebuia rosea* (Bertol.) DC .

Adaptogenic activity [10-13]

Immobilisation stress model

Adult male albino rats of 150-200g were selected and divided into 5 groups of six animals each as Group I Negative control (Unstressed ,untreated), Group II Positive control(Stressed ,received vehicle), Group III extract (300mg/kg,p.o),Group IV extract(500 mg/kg p.o.),Group V standard withania somnifera (Ashwagandha)100 mg/kg p.o.). The treatment was made as stated above for 10 days 1 hour before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the fore limbs and hind limbs to a wooden board inclined at an angle of 600 daily, 2 hours (11am to 1pm) for a period of ten days .The animals were sacrificed at the end of specific period and blood was collected by cardiac puncture under mild ether anesthesia using disposable syringe and needle for estimation of biochemical parameters such as ,serum glucose(GOD-POD method),cholesterol (CHOD-PAP method),triglycerides(GPO-Trinder method),BUN (Blood Urea Nitrogen ,GLDH-UREASE method).The weight of organs , such as liver ,,spleen ,adrenal gland and testes after washing with alcohol was recorded per 100 g body weight of animal.

Forced swimming endurance test (Physical stress)

Rats of either sex (200-250) were used for forced swim endurance stress. Group I received 0.1% gum acacia in saline; (vehicle control).Group II mice were treated with 0.1% gum acacia in saline and stress; (negative control). Group III rats were treated with (methanolic extract of *Anogeissus latifolia* wall (300 mg/kg, p.o), and stress; (positive control).Group IV were treated with methanolic extract of *Tabebuia rosea* (Bertol.) DC (500 mg/kg, p.o.) and Group V mice were treated with water soluble powder of Ashwagandha (100 mg/kg, p.o.) The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37x 37x 30cm), filled with water to a height of 25cm.Extractswere given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the end point was taken when the animal started drowning. The mean swimming time for each group was calculated. Then animals were killed and blood was collected by cardiac puncture to estimate biochemical parameters like serum glucose, triglycerides, cholesterol, BUN, corticosterone and blood cell count (RBC, WBC and DLC).The weights of organs such as liver, adrenals, spleen were recorded after washing with alcohol.

Anti- fatigue effect in mice

Swiss Male Albino mice (weight 18-30g) were divided into 4groups of 6 animals each. The animals were kept at room temperature and had free access to food and water. Group I animals received control with stress. The extract was administered orally to group II in the dose of 300 mg/kg/day, group III in the dose of 500 mg / kg/daily for 7 days and group IV animals received standard ginseng marketed preparation in the dose of 100 mg/ kg/day. Animals in group-I were given the vehicle only that is distilled water in the same volume and served as control .On the 7th day 1 hr after the drug/vehicle administration, the animals were made to swim in a polypropylene tank (140x60x45cm) containing water up to 25cm height maintained at room temperature (30±2º) till they were exhausted by swimming continuously. The animals were placed on the Rota rod (UGO BASILE-7650 Italy) rotating at 15 rpm immediately after drying with tissue papers to monitor the anti- fatigue and muscle co-ordination effect. The duration of stay on the rod of every mouse was recorded.

Statistical Analysis

Values are presented a mean ± SD of 6 rats in each group. All the data were statistically evaluated by one way ANOVA followed by Dunnet's test, the limit of statistical significance was set at P- level < 0.05.

RESULTS

Preliminary phytochemical screening

In the Preliminary phytochemical screening of the MEAL and METR showed the presence of alkaloid, reducing sugars, carbohydrates, flavanoids, glycoside, saponins, terpenoids, steroids,protein, tannins, phenols and diterpenes.

Adaptogenic activity

The extracts of *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol.) DC showed significant reduction in immobilization stress when compared to the untreated group. The effect on biochemical parameters caused marked increase in serum glucose, cholesterol, triglycerides and BUN in rats. This stress induced elevated levels of biochemical parameters were significantly reversed by MEAL and METR in dose dependent manner. The effect on organs weight MEAL at doses of 300 mg/kg produced significant decrease in weight of liver and adrenal gland , but failed to increase spleen and testes weight significantly .However, METR at doses of 500mg/kg offered significant protection against change in weight of liver , adrenal gland spleen and testes when compared to stress control group in Immobilization stress model . The results are given in Table No: 1 & 2. In Forced swimming endurance test, stress induced elevated blood cell counts of RBC and DLC i.e. lymphocytes, neutrophils, eosinophils and monocytes. The animals significantly restore back forced swimming stress induced alterations in plasma corticosterone, glucose triglycerides, BUN and cholesterol. The results were compared to that of reference standard Ashwagandha in Table No: 3,4 &5. In Anti fatigue effect *Anogeissus latifolia* wall showed 28.96 % *Tabebuia rosea* (Bertol.)DC showed 45.82%, where the standard drug Ginseng (100 mg/kg) offered 68.78% protection from anti- fatigue effect. The 300 mg/kg doses of *Anogeissus latifolia* wall extract protected 3 out of 6 animals and 500mg/kg dose of *Tabebuia rosea* (Bertol.)DC protected 4 out of 6 animals. The standard drug Ginseng (100 mg/kg) protected 5 out of 6 animals from anti-fatigue effect and the significance is shown in the Table No: 6.

Table 1: Effect of MEAL and METR on immobilization stress induced biochemical parameters in rats

Group	Biochemical estimation(mg/dl)			
	Glucose	Cholesterol	Triglycerides	BUN
Negative Control	92.90±0.7522**	54.94±0.4704**	69.79±0.4543**	25.51±0.3486**
Positive Control	146.6±0.3735**	85.60±0.4950**	104.5±0.6274**	43.78±0.7240**
MEAL	115.3±0.4319**	66.26±0.3142**	86.36±0.3236**	35.53±1.968**
METR	106.7±0.3152**	65.21±0.3566**	84.06±0.4529**	32.52±0.5125**
Standard	94.81±0.3368**	58.34±0.3748**	76.38±0.4466**	31.23±0.4365**

The values are expressed as Mean ± SEM, n=6, *P<0.05**, P<0.01 as compared to positive control.

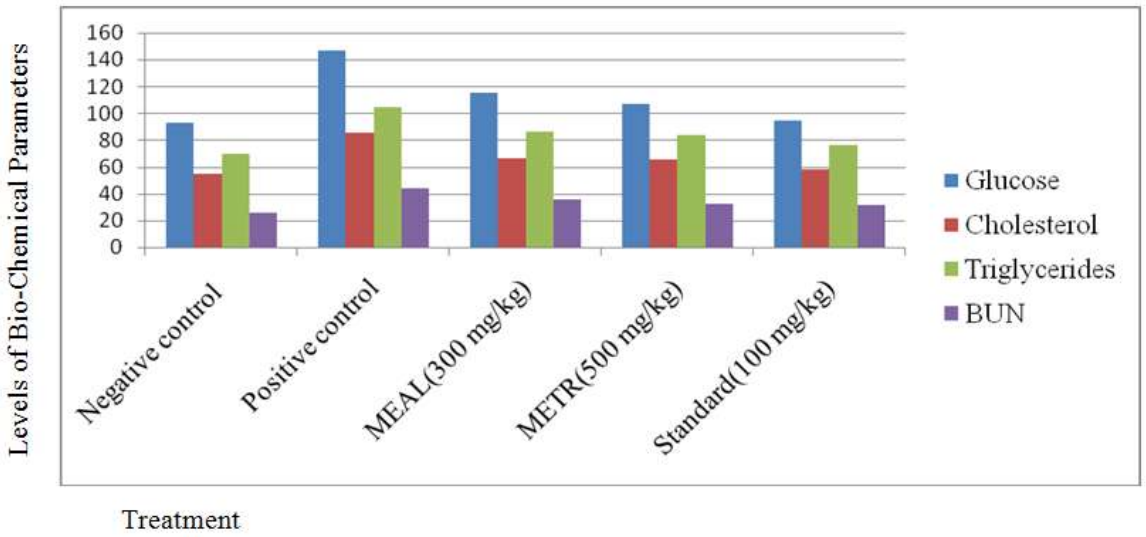

Fig. 1: Bar diagram for effect of MEAL and METR on immobilization stress Induced biochemical parameters in rats

Table 2: Effect of MEAL and METR on organ weight immobilization stress induced rats

Group	Organs weight gm/100 gm body weight			
	Liver	Adrenal gland	Spleen	Testes
Negative control	3.789±0.3894**	0.01333±0.004944**	0.3957±0.001647**	1.710±0.07234**
Positive control	7.771±0.5086**	0.03683±0.003936**	0.2357±0.003722**	1.385±0.4672*
MEAL	6.125±0.2947**	0.02433±0.002066**	0.2885±0.01789*	1.606±0.7678*
METR	5.804±0.1905**	0.02317±0.007923**	0.3673±0.01285**	1.857±0.4354*
Standard	3.981±0.2548**	0.02117±0.001302*	0.3885±0.01789**	1.874±0.5162*

The values are expressed as Mean ± SEM, n=6, significant at*P<0.05, **P<0.01 significant as compared to stress control, statistical test employed is ANOVA followed by Dunnet's test

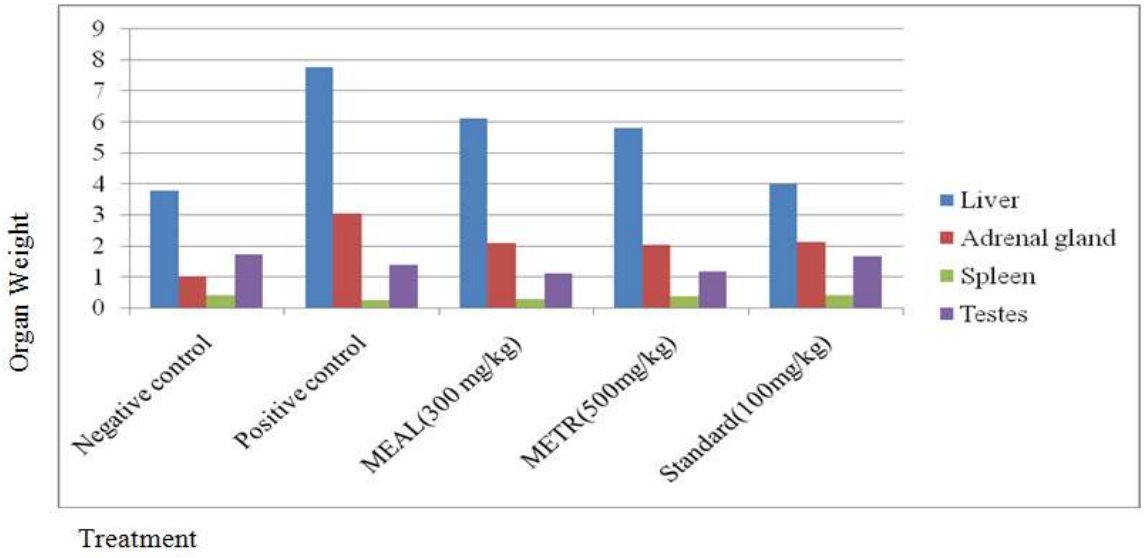

Chart No.2 Bar diagram for effect of MEAL and METR on organ weight in immobilization stress induced rats

Table 3: Effect of MEAL and METR on organ weights in forced swimming endurance stress in rats (Physical stress)

Treatment	Organ weight		
	Spleen (mg/100g)	Liver (g/100g)	Adrenal gland(g/100g)
Negative control	142.7 ±1.145**	3.503±0.2903**	14.01±1.337**
Positive control	127.5 ±1.607**	5.997±0.1458**	40.31±0.8774**
MEAL	175.7± 0.4944	5.002±0.3410**	29.92±0.8388**
METR	182.0 ± 0.2582*	4.927±0.2354**	29.18±0.8881**
Standard	189.3 ± 2.028**	4.907±0.2861**	19.04±0.3840**

The values are expressed as Mean ± SEM, n=6, significant at*P<0.05, **P<0.01 significant as compared to stress control, statistical test employed is ANOVA followed by Dunnet's test.

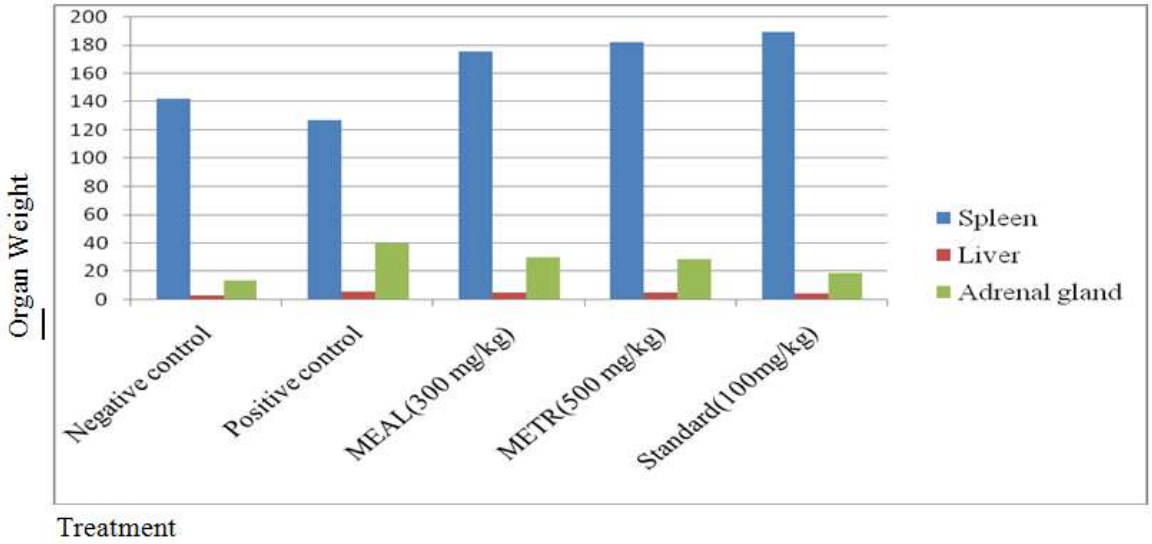

Chart No. 3: Bar diagram for effect of MEAL and METR on organ weights in forced swimming endurance stress in rats (Physical stress)

Table 4: Effect of MEAL and METR on blood cell count in forced swimming endurance stress in rats. (Physical stress)

Blood cell count						
Treatment	**RBC in millions**	**WBC (No. Of cells/mm3)**	**DLC No. Of cells**			
			Leucocytes	**Neutrophils**	**Eosinophils**	**Macrophages**
Control	6.057±0.4602*	6581±237.5**	4797±333.8**	2705±0.669**	61.23±0.6699**	8.610±0.2993**
Stress control	8.328±0.3216*	4411±493.7**	6747±225.0**	1367±0.610**	96.64±0.6107**	12.89±0.3790*
MEAL	6.097±0.5385**	4407±628.2**	4527±619.9**	1135±0.491**	89.31±0.4915**	11.42±0.2561**
METR	5.957±0.3278**	4300±384.1**	2375±273.8*	1108±0.707**	81.36±0.6384**	11.21±0.2791**
Standard	5.847±0.3019**	4249±418.8**	1877±236.9**	1067±0.638**	69.92±0.7070**	9.17±0.5065**

The values are expressed as Mean ± SEM, n=6, significant at *P<0.05, **P<0.01 significant as compared to stress control, statistical test employed is ANOVA followed by Dunnet's test.

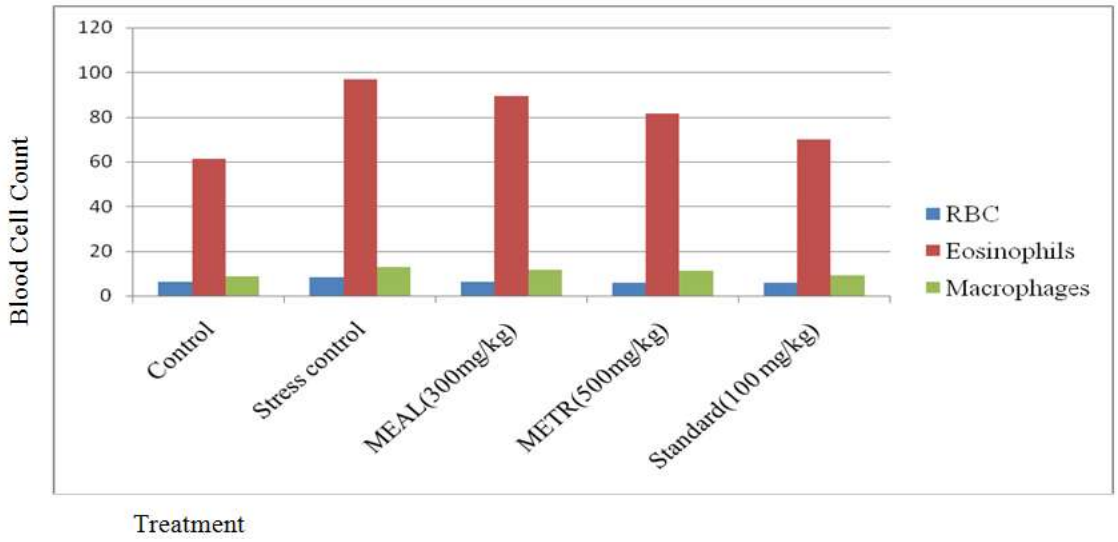

Chart No. 4: Bar diagram for effect of MEAL and METR on blood cell count in forced swimming endurance stress in rats (Physical stress)

Table 5: Effect of MEAL and METR on biochemical parameters in Swimming Endurance Stress in rat (Physical stress)

Groups	**Corticosterone µg/dl**	**Glucose mg/dl**	**Cholesterol mg/dl**	**Triglycerides mg/dl**	**BUN mg/dl**
Control	90.63±1.265**	119.8±0.4550**	36.33±0.5933**	61.91±0.3820**	30.59±0.5717**
Stress control	156.4±0.9746**	196.6±0.4309**	62.11±0.6520**	95.96±0.4076**	57.14±0.4520**
MEAL	127.1±0.5512**	126.0±0.5151**	53.77±0.2444**	83.21±0.4802**	52.88±0.3152**
METR	109.6±0.3396**	110.6±0.4726**	46.60±0.4363**	77.60±0.3878**	47.44±0.5696**
Standard	99.86±0.5508**	100.1±0.4392**	32.10±0.4735**	67.11±0.6555**	33.96±0.6256**

The values are expressed as Mean ± SEM, n=6, significant at **P<0.01 ***P<0.001 significant as compared to stress control, statistical test employed is ANOVA followed by Dunnet's test.

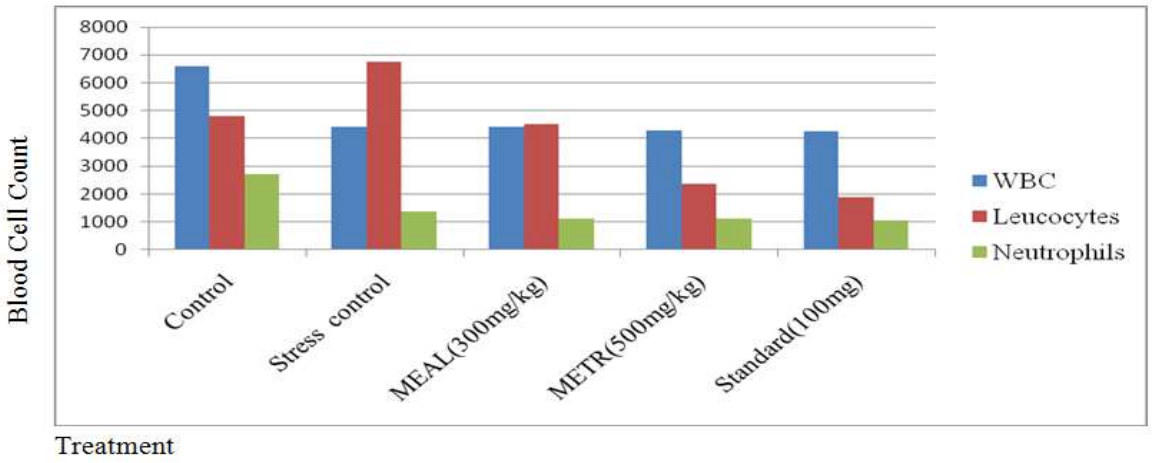

Chart No. 5: Bar diagram for effect of MEAL and METR on blood cell count in forced swimming endurance stress in rats

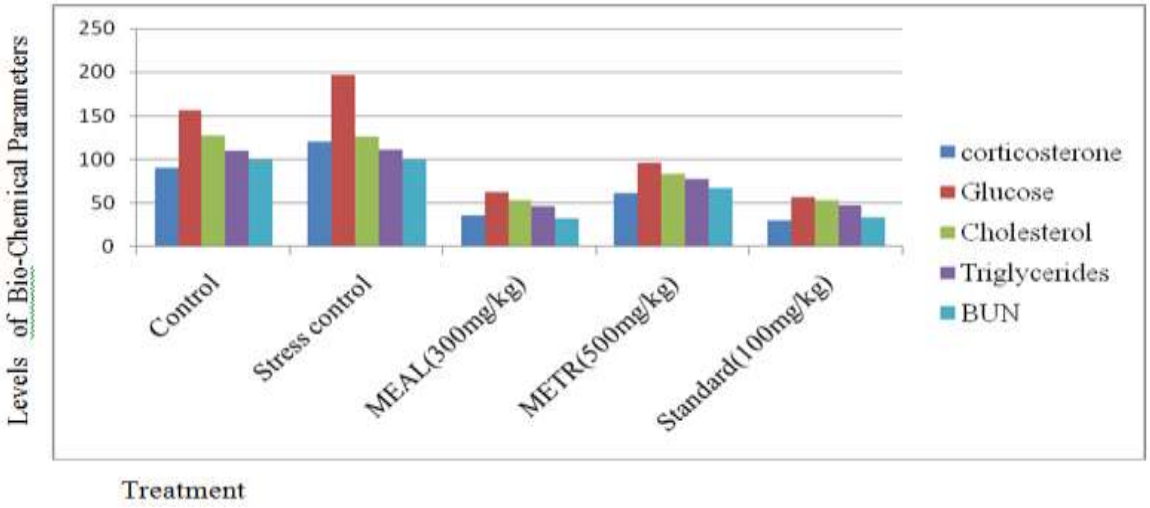

Chart No. 6: Bar diagram for effect of MEAL and METR on biochemical parameters in Swimming Endurance Stress in rats (Physical stress)

Table 6: Effect of METR and MEAL on anti-fatigue effect

Treatment	Dose (mg/kg, p.o.)	Anti-fatigue effect	% increase
		Duration of increase stay on Rotarod ; Mean ± SEM	
Control+ Stress	-	22.65±0.5736**	-
MEAL +Stress	300	29.21±0.4151**	28.96
METR +Stress	500	33.03±0.4221**	45.82
Ginseng +Stress	100	38.23±0.5942**	68.78

The values are expressed as mean ± SEM for 6 mice*P<0.05, **P<0.01 compared to respective control + stress group.

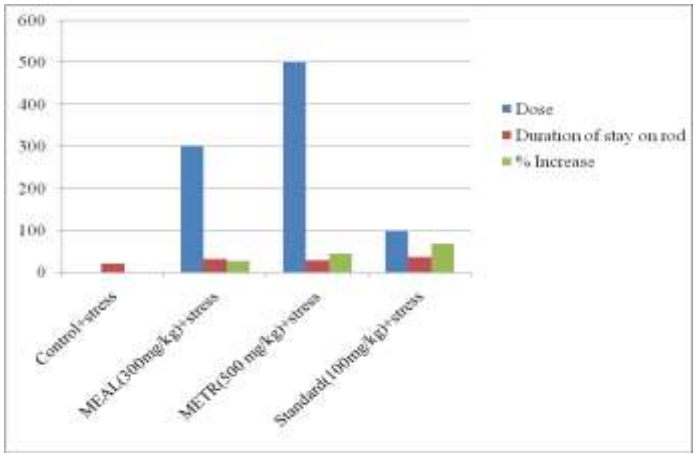

Chart No. 7: Bar diagram for effect of MEAL and METR on Anti-fatigue effect.

DISCUSSION

The results of the present study could suggest *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol.)DC the test extracts reduced the levels of serum biochemical parameters in dose dependent manner [14-17].Stress induces adrenomedullary response in man. Adrenaline in turn stimulates β2 receptors on the pituitary glands causing greater release of ACTH, which can stimulate the adrenal medulla as well as cortex. So adrenal gland weight increases. Cortisol increases m RNA levels in liver cells. This lead to increase in weight of liver . Spleen constricts to release more blood cells (RBC) during stress. So its weight decreases during stress[18-20] .This stress induced changes of organs weight were significantly reversed by the test extract at higher dose, whereas the lower dose of extract could able to exhibit protective effect on weight on liver and adrenal gland. But failed produce statistically significance increase in spleen and tessstes weight in immobilization stress model.

Withania somnifera (Ashwagandha) has a reputation of having a wide range of nonspecific actions that contribute to its potent adaptogenic activity. Withania somnifera is known to have an effect on the HPA axis including cortisol levels which are known to modulate the stress response and either improve or hinder adaptation .Withania somnifera has also been shown to suppress and down regulate IgE antibody response- IgE hypersensitivity is an example of poor adaptation to stress. Withania's mechanism of action may be due to its effect on the up regulation of anabolic process and its activity on catecholamines and mitochondrial processes which have been demonstrated in trials of animals under physical stress. Anabolic activity of Withania somnifera was attributed due to the presence of steroidal lactones called Withanolides. Anabolic effects may be due to the anti- serotonergic activity which would lead to an increase in appetite and therefore weight gain[21]. The potent antioxidant action is also thought to contribute to withania's adaptogenic effect.

The extract possess anti stress property as it significantly increased swimming time. Swimming endurance stress resulted in significant increase in adrenal gland weight and liver weight with concomitant decrease in spleen weight in stress control group, which was significantly reverted by *Anogeissus latifolia* wall at dose 300mg/ kg and *Tabebuia rosea* (Bertol.)DC 500 mg/kg. Stress induced elevated blood cell counts of RBC and DLC i.e. lymphocytes, neutrophils, eosinophils and monocytes have been significantly reduced by the methanolic extract in a dose dependent manner. The animals restored back forced swimming stress induced alterations in plasma corticosterone, glucose, triglyceride, BUN and Cholesterol [22].

The extracts possess anti fatigue property. Exhaustion theory and Radical theory have attracted most interest. Exhaustion theory suggests that during exercise, many energy sources, such as glucose and liver glycogen, will be exhausted, thus leading to physical fatigue [23] .Radical theory suggests that intense exercise can produce an imbalance between the body's oxidation system.

The methanolic extracts inhibited accumulation of free radicals. The accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs [24]. Several studies have shown that exogenous dietary antioxidants can decrease the contribution of exercise-induced oxidative stress and improve the animal's physiological condition[25-28]. In the past few decades, health scholars and athletic ability, postpone fatigue and accelerate the elimination of fatigue in human beings, but also have few side effects [29]. The physical stress caused significant fatigue and motor coordination. The significant reduction in anti-fatigue effect demonstrates the efficacy of *Anogeissus latifolia* wall and *Tabebuia rosea* (Bertol.)DC as an effective adaptogenic agent.

Experimental studies have confirmed the adaptogenic properties of ginseng and the effects are apparently due to presence of saponin glycoside content in the root. Literature survey indicate that flavonoids and tannins were reported to possess variety of pharmacological activities including antistress activity [30-31].In the present phytochemical screening on MEAL and METR gave positive tests for flavonoids ,tannins, saponins, glycosides ,this could be the reason for significant adaptogenic property of test extract.

CONCLUSION

The pharmacological and phytochemical investigations of the leaf extracts of the plants *Anogeissus latifolia* wall *and Tabebuia rosea* (Bertol.)DC has been represented here in this thesis. It deals with literature review, where previous research work of *Anogeissus latifolia* wall *and Tabebuia rosea* (Bertol.)DC has been discussed and also the ethnomedicinal documentation of this plants.The methodology of extraction, and the presence of phytochemical constituents in leaves of *Anogeissus latifolia* wall *and Tabebuia rosea* (Bertol.)DC were also reported. The methanolic extract of leaves of *Anogeisssus latifolia* wall and *Tabebuia rosea* (Bertol.)DC exhibited adaptogenic activity, but *Tabebuia rosea* (Bertol.)DC exhibited potent adaptogenic activity by Immobilization stress model, Forced swimming endurance test and Anti-fatigue effect. In addition, the extracts were found to contain a noticeable amount of flavanoids, tannins, saponins and glycosides which play a major role in anti stress activity. Thus the investigational works under taken and furnished in this thesis deals with the thorough evaluation of phytochemical and pharmacological profiles of the leaves of *Anogeissus latifolia* wall *and Tabebuia rosea* (Bertol.)DC.

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