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Comparative evaluation of adaptogenic and antioxidant activities of traditionally used Indian drugs

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ABSTRACT

The present study is aimed at evaluating the adaptogenic and antioxidant activity of traditionally used drugs in Ayurveda and Siddha system of medicine. Antioxidant and adaptogenic activity of methanolic extract of Muppu, Vidakanachoornam and Buchanania lanzan were evaluated using TBARS and GSH as biomarkers. Comparative results of the three formulations revealed that the Ayurvedic formulation Vidakanachoornam had maximum antioxidant potential as it was found to be most significant at the dose of 400 mg/kg body weight, followed by the extract of Buchanania lanzan.

Key words: Muppu, Vidakanachoornam, *Buchanania lanzan*, Antioxidant, adaptogenic activity

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INTRODUCTION

Modern man is exposed to stressors like highly ambitious and competitive lifestyle, food adulteration, atmospheric pollution, and synthetic drugs. Work-related stress results in health problems [1].Homeostatic mechanism is geared towards counteracting the everyday stressed living. If the stress is extreme, long lasting or unusual, the normal mechanism may not be sufficient. In this case the stress causes a wide set of bodily changes, called the general adaptation syndrome (G.A.S) [2]. There is abundant literature on the effects of stress on the physical and mental illness and infections. Reactive oxygen species (ROS) including superoxide anion radical, hydroxyl radical, and hydrogen peroxide, are formed during stress and, can cause oxidative damage of all major groups of biomolecules (DNA, proteins, lipids and small cellular molecules), which in turn leads to cardiovascular and neurodegenerative diseases[3]. Production of free radicals may be formed by exogenous factors like environmental pollutants, pathogens radiation, and drugs. These reactive oxygen species (R.O.S) create a homeostatic imbalance which generates oxidative stress and causes cell death and tissue injury. Involvement of R.O.S is implicated in neurodegenerative and other disorders Toxic effect of the free radicals causes oxidative stress and results in pathogenesis of diseases. In Indian system of medicine, there are a number of herbal drugs and formulations available, to withstand stress without changing any physiological function of the body. *Buchanania Lanzan* is a drug used in Ayurveda. It is known to have tonic, cardiotonic , antioxidant and astringent properties and is also used in the treatment of skin diseases.Muppu is said to be highly efficacious Siddha rejuvenating drug. The traditional Ayurvedic preparation – Vidakanachoornam, used in Alappuzha district, Kerela, for liver disorders, steatosis (fatty liver) and as an antioxidant. The constituents of the formulation are:*Piper longum* (Pippali);*Moringa oleifera* (Muringa) ; *Embelia ribes*(vidang) each 100 g in quantity.

MATERIALS AND METHODS

Plant material:
The plant material was collected from Yukka enterprises, Navi Mumbai. The material was shade dried, pulverized and preserved in air tight containers. Muppu was procured from, Intergrated Research Centre for Siddha Medicines, Bangalore and Vidakanachoornam was prepared under the guidance of an Ayurvedic Medical practitioner.

Chemicals
Chemicals were purchased from Sigma-Aldrich, while ascorbic acid was purchased from SD fine chemicals. All other reagents used were of analytical grade.

Animals
All animal experiments were performed after obtaining approval from The Institutional Animal Ethical Committee and the guidelines for the animal care were strictly adhered to during the experimentation as recommended by committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt of India. 10–12 weeks old albino mice weighing 18–22 g were used. The animals were maintained under standard conditions and were fed with commercial diet and water *ad libitum* during the entire experiment.

Estimation of blood glutathione and TBARS levels:
Male Albino mice 10–12 weeks old and weighing 18–22 g were obtained, divided into groups of six and employed for the study. These were maintained at a room temperature of 22 ± 2 °C with 12 h light/dark cycle and free access to pellet food and water. Group I: Naïve Control animals received (saline, p.o.),Group II:Stress control.,Group III: Animals received standard ascorbic acid at the dose of 100 mg/kg, p.o.,Group IV: Animals received Muppu at the dose of 100 mg/kg, p.o.,Group V: Animals received Muppu at the dose of 200 mg/kg, p.o.,Group VI: Animals received Muppu at the dose of 400 mg/kg, p.o.,Group VII: Animals received Vidakanachoornam at the dose of 100 mg/kg, p.o.,Group VIII: Animals received Vidakanachoornam at the dose of 200 mg/kg, p.o.,Group IX: Animals received Vidakanachoornam at the dose of 400 mg/kg p.o.,Group X *Buchanania lanzan* leaf extract at the dose of 100 mg/kg, p.o. Group XI: Animals received *Buchanania lanzan* leaf extract at the dose of 200 mg/kg, p.o.,Group XII: Animals received *Buchanania lanzan* leaf extract at dose of 400mg/kg,p.o.Stress was induced in Mice by restraining animals in well-ventilated horizontal 50-ml conical polypropylene tubes for 12 h during the dark cycle (2000–0800 h) during experimental periods of 14 days, [4] One group of mice was taken as a non-treated naive control group without any restrained conditions and was given full access to food and water (naive-control).One group served as the stress control to which drug was not administered but was subjected to restrained stress, treated groups were subjected to restrain stress as well as drug administration for 14 consecutive days. The animals were not physically compressed and, therefore, did not experience pain Stress procedure is believed to be largely psychological in nature due to the feeling of confinement by the animal [5] Serum samples were prepared for the evaluation of oxidative stress marker .

Estimation of Thiobarbituric Acid Reaction Substances (TBARS) [6]**:**
The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in mouse serum Mouse serum (0.2 ml) was pipetted out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 h at 95^0C and allowed to cool down at room temperature and 1 ml of distilled water and 5 ml of n-butanol-pyridine mixture (15:1 v/v) were added. The tubes were centrifuged at 4000 rpm for 10 min. The absorbance of developed pink colour was measured spectrophotometrically (Shimadzu 1700, Singapore) at 532 nm. A standard calibration curve was prepared using 1-10 nM of .TEP (1,1,3,3-tetraethoxypropane)

Estimation of Blood glutathione [7]
0.2 ml fresh blood was collected from each animal and1.8 ml distilled water was added to it and 3 ml of precipitating solution was added to mixture. The mixture was then allowed to stand for approximately 5 min and then filtered. 2.0 ml of filtrate was added to 8.0 ml of phosphate solution in cuvette and 1.0 ml 5,5-Dithiobis-(2-Nitrobenzoic acid) DTNB reagent was added to cuvette and the optical density (OD) of the yellow-coloured complex formed by the reaction of GSH and DTNB was measured at 412 nm. A standard curve was obtained with standard GSH and absorbance was measured at 412nm

Statistical Analysis: All the results are expressed as mean ± standard deviation (SD) followed by one way ANOVA .The $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Antioxidant assays are performed for variety of inter-related phenomenon, such as adaptogens, stress, rejuvenators etc, as the main cause behind such manifestation of problems is stress. Stress is manifested in the body by virtue of the overload of free radicals, which are generated due to the metabolism of drugs and dietary substances as free radicals. Due to improper nutritional requirements, our body's innate or endogenous anti-oxidant mechanisms are not sometimes sufficient to combat with the invading oxidative stressors or overload of free radicals, leading to the high levels of an enzyme, lipid peroxidase (LPO), which is an indicator of stress. Therefore, assays of anti-oxidants can be a pivot for all of these activities. Comparative antioxidant activity of the three formulations by measuring the TBARS levels and GSH level exhibited that the formulation Vidakanachoornam had maximum antioxidant and adaptogenic potential as it was found to be most significant ***P<0.001 at dose of 400 mg/kg body weight which was comparable to that of ascorbic acid at the same dose. The leaves of *Buchanania lanzan* **P<0.01 also exhibited significant activity but less than that of Vidaknachoornam and the least but significant activity was seen in Muppu *P<0.05 at the equimolar dose. Analysis reflects that Vidakanachoornam, *Buchanania lanzan* and Muppu possess antioxidant and adaptogenic efficacy.

GSH level:
The level of GSH was found to decrease in oxidative stress .Lower level of GSH was seen in animals treated as a model control group. Animals treated as model group showed lower level of GSH as compared with normal control group. Drug treated groups showed significantly (***P<0.001) higher level of GSH at the dose of 400 mg/kg body weight, as compared with animals treated as model control group and the results are found comparable to animals treated with standard reference drug ascorbic acid at the same dose (Table1)

TBARS level:
Thiobarbituric acid reacting substance (TBARS) assay is an indicator of lipid peroxidation and a level of free radicals. The assay is based on the reactions of thiobarbituric acid with malondialdehyde produced during lipid peroxidation. The level of TBARS is found to increase in oxidative stress. Animals treated as model group showed higher level of TBARS as compared with normal control group. Drug treated groups showed significantly (***P<0.001) lower level of TBARS at the dose of 400 mg/kg body weight, as compared with animals treated as model control group and the results are found comparable to animals treated with standard reference drug ascorbic acid at the same dose.(Table2;Fig1)

Table 1: Effect of *Buchanania lanzan* , Muppu and Vidakanachoornam on GSH Level
GSH in Serum of Mice under different dose level

Group	Dose mg/kg	**GSH level (mMol/mg protein**
Group I	Naive Control (10 mL/kg)	31.38 ± 0.76
Group II	Stress Control (10 mL/kg)	14.02 ± 1.44
Group III	Standard Ascorbic 100mg/kg	25.65 ± 1.60***
Group IV	E1 100mg/kg	16.67 ± 0.88*
Group V	E1 200mg/kg	18.38 ± 1.15*
Group VI	E1 400mg/kg	18.35 ± 0.50*
Group VII	E2 100mg/kg	23.17 ± 1.22***
Group VIII	E2 200mg/kg	22.83 ± 0.50***
Group IX	E2 400mg/kg	26.25 ± 0.61***
Group X	E3 100mg/kg	19.24 ± 0.49**
Group XI	E3 200mg/kg	19.90 ± 0.36**
Group XII	E3 400mg/kg	20.00 ± 0.34**

*E1-muppu**
*E2-Vidakanachoornam****
*E3-Buchanania lanzan leaf extract***

Table 2: Effect of *Buchanania lanzan*, Muppu and Vidakanachoornam on TBARS Levels.
(TBARS) in serum of mice under different dose level:

Group	Dose mg/kg	TBARS Level (nMol/mg protein)
Group I	Naive Control (10 mL/kg)	2.40 ± 0.06
Group II	Stress Control (10 mL/kg)	6.00 ± 0.13
Group III	Standard Ascorbic 100mg/kg	3.14 ± 0.05***
Group IV	E1 100 mg/kg	5.78 ± 0.08*
Group V	E1 200 mg/kg	5.74 ± 0.10*
Group VI	E1 400 mg/kg	5.67 ± 0.12*
Group VII	E2 100 mg/kg	3.70 ± 0.02***
Group VIII	E2 200 mg/kg	3.46 ± 0.02***
Group IX	E2 400 mg/kg	3.40 ± 0.02***
Group X	E3 100mg/kg	5.29 ± 0.14**
Group XI	E3 200 mg/kg	5.36 ± 0.19**
Group XII	E3 400 mg/kg	5.41 ± 0.18**

*n=6. *p<0.05, **p<0.01, ***p<0.001 when compared with Naïve control*
*E1-muppu**
*E2-Vidakanachoornam****
*E3-Buchanania lanzan leaf extract***

The result reveals that Vidakanachoornam is showing the maximum adaptogenic effect, followed by plant extract and the least activity is exhibited by Muppu.

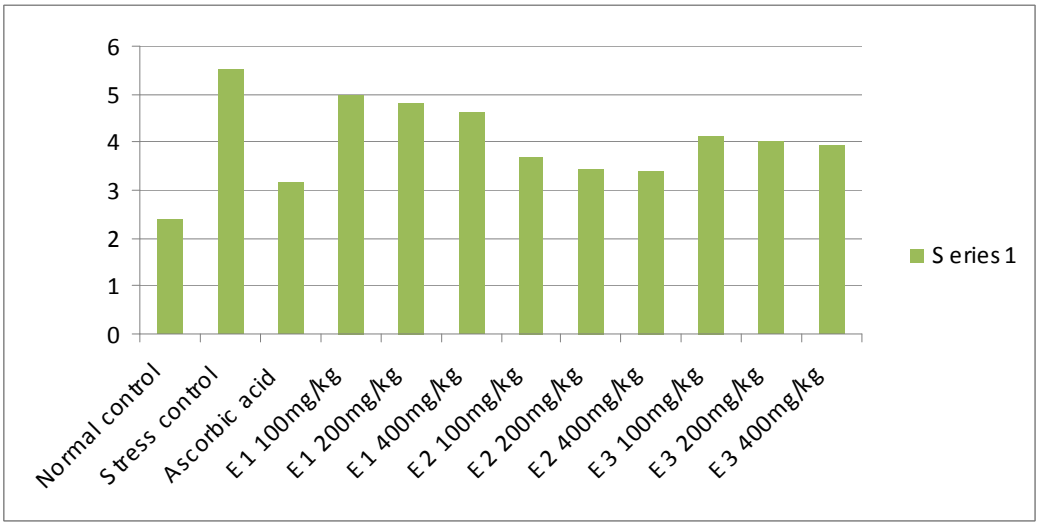

Fig. 1 The histograms represent the effect of the drugs on TBARS levels in naive-control, chronic stress-induced mice (RST-control) and the drug treated mice

The potent antioxidant and adaptogenic activity of Vidakanachoornam could be attributed to due to the presence of *Piper longum* and *Embelica ribes* as ingredients of the ayurvedic formulation Vidakanachoornam . Piperine present in *Piper longum* [8] and embeline present in *Embelica ribes* are reported to be potent antioxidant principles in literature [9] .The extract of *Piper longum* and its major compound, piperine exert antioxidant activity and are protective in many diseases [,8,9,10,11.]*Embelica ribes* present in the Vidakanachoornam is known to posses antioxidant effect as per the literature survey [8]. Phytochemical investigation reveals that the leaves of the plant *Buchanania lanzan* possess polyphenolic compounds such as flavonoids that are known to possess antioxidant activity. Hence the antioxidant property of the extract could be attributed to the antioxidant principles, ie flavonoids [12]

CONCLUSION

The above experimental data provided scientific evidence for the use of the traditional lndian drugs as Adaptogenic and antioxidant agents and also provides rational proof for the universal acceptance and usefulness of the ancient system of medicine

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