

## Pharmacological screening of polyherbal formulation for anti stress activity on Albino rats

Mohd Zubairali Anssari\*, Mohammed Fasiuddin, Syed Salman, Syed Nazer, Mohammed Imran, Mohd Toufeeq, Roshan S and N. L. Mahammed

AZAD College of Pharmacy, Moinabad, R.R. District, Telangana, India-501504.

### Corresponding author\*

Mohd Zubairali Anssari

AZAD College of Pharmacy

Moinabad, R.R. District, Telangana- 501504.

E-mail: [mohammad.pharma2014@gmail.com](mailto:mohammad.pharma2014@gmail.com)

### Abstract

The present study was designed to evaluate adaptogenic activity of *poly herbal formulation* (*Misri, almond*(*Prunus amygdalus*), *Ginger, Fennel Seed* (*Saunf*)) in Wistar albino rats using different experimental models such as Anoxia stress tolerance and swimming endurance stress models. The plant was subjected to preliminary phytochemical screening. The parameters like anoxia stress tolerance and swimming endurance time were recorded. These activities are tested at oral doses of extract at 200 and 400 mg/kg and Geriforte syrup 2ml/kg were used for comparison. Preliminary phytochemical screening revealed the presence of flavonoids, steroids and tannins. Pretreatment with *poly herbal formulation* showed increase in anoxia stress tolerance time and swimming endurance time. The results from the study indicated that Poly herbal formulation possessed significant antistress activity.

**Keywords:** Poly herbal formulation, Phytochemical screening, Antistress activity, Acute toxicity studies, OECD Guidelines 423.

## 1. Introduction

In this modern era, stress has become an integral part of human life.[1] It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress.[2] Stress is considered to be any condition which results in perturbation of the body's homeostasis.[3] If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened.[4] Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous system (CNS), endocrine system, and metabolic system.[5]

Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. 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. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs.[6]

Herbal formulations have been in use for many years not only in Asian countries but also globally for human well-being. The herbal formulations claimed to enhance physical endurance; mental functions and non-specific resistance of the body have been termed as adaptogens.[7] The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Various herbs like *Withania somnifera*, *Embllica officinalis*, *Asparagus racemosus*, *Ocimum sanctum*, *Tribulus terrestris* and *Piper longum* are claimed to have immunomodulatory, adaptogenic, anabolic effects and the ability to improve vital energy.[8] Herbal medicines are known to act synergistically in combination. Polyherbal formulation(PHF) is composed of *Misri, almond*(*Prunus amygdalus*), *Ginger*, *Fennel Seed* (*Saunf*).The main objective of present study is to establish the antistress activity of *Poly Herbal Formulation* and to compare its antistress potential to that of Geriforte syrup.

## 2. Materials and Methods

### 2.1 Experimental Animal

Healthy Wistar albino rats (200-250g) were taken to assess diuretic activity. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of Pharmacy College, (No.1516/PO/a/11/CPCSEA). Animals were housed in polypropylene cages, maintained under standardized condition (12h light/dark cycle, 24°C, 35 to 60%

humidity) and provided free access to standard pellets diet and purified drinking water *ad libitum*. The animals were deprived of food for 24h before experimentation but allowed free access to water throughout.

## 2.2 Standard Drug

Geriforte syrup (from Himalaya pharmaceuticals & co.). Geriforte is an adaptogenic which effectively combats stress and fatigue. It also increases stamina and improves overall performance.

### Key ingredients

**Chyavanaprasha** is enriched with natural ingredients like Indian Gooseberry, Cardamom and Cinnamon, which combat coughs, colds and infections by fortifying the body's natural immunity. The herbs contain excellent antistress and anti-aging properties. It also helps in detoxification. Chyavanaprasha aids in the smooth functioning of the circulatory, nervous and respiratory systems to maintain overall health.

**Winter Cherry** (*Ashvagandha*) is a unique herb with antistress and adaptogenic properties that improves physiological endurance and protects against the effects of stress. It is an antioxidant which scavenges on free radicals in cells that are responsible for premature aging. The herb helps in limiting the formation of these free radicals and subsequently preventing the early aging process.

## 2.3 Test Drug

The composition of test drug *Poly Herbal Formulation* (PHF) (supplied by Natural Remedies Pvt. Ltd, Bangalore), a polyherbal formulation, was as follows:

**Table 1: Composition of test drug Poly Herbal Formulation (PHF)**

Components	Amount present in Medium of Phytochemical		
	PHF	Extract	(% w/w)
<b>Misri</b>	17	Water	(2.1)
<b>Almond</b> ( <i>Prunus amygdalus</i> )	17	Water	(2.9)
<b>Ginger</b>	17	Ethanollic	(4.1)
<b>Fennel Seed</b> ( <b>Saunf</b> )	17	Water	(2.8)

## 2.4 Preliminary Phytochemical Screening

The test drug, *Poly Herbal Formulation* (PHF) was subjected to preliminary qualitative phytochemical investigations and was screened for the presence of secondary metabolites such as steroids, alkaloids, flavonoids and tannins using standard methods.

## 2.5 Acute Toxicity Study

The test was carried out as per OECD guidelines[17]; Wistar albino rats, of either sex, weighing 150-200 g were divided into different groups comprising six animals each.

The **control group** received normal saline (10 ml/kg, p.o.). The **other groups** received 100, 200, 500, 1000, and 2000 mg/kg of the test extract respectively, as well as, extract fractions up to 1000 mg/kg, in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were kept under observation up to 14 days after drug administration to find out mortality, if any.

## 2.6. Induction and Assessment of Stress

### 2.6.1 Swimming Endurance Test

Albino Wistar rats of either sex weighing 150-220 g were selected and divided into 4 groups of six each. **Group I:** Control (Received only vehicle normal saline 0.5%w/v p.o.), **Group II:** Poly herbal formulation (PHF)( 200mg/kg p.o.),**Group III :** Poly herbal formulation (PHF)(400 mg/kg p.o.), **Group IV:** Geriforte syrup (2 ml/kg p.o.).The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37X37X30 cm), filled with water to a height of 25cm. Formulation was given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean swimming time for each group was calculated.[9][10]

### 2.6.2. Anoxia Stress Tolerance Test:

Albino Wistar rats of either sex weighing 150-220 g were selected and divided into 5 groups of six each as:

**Group I:** Control (Received only vehicle CMC 0.5%w/v p.o.),

**Group II:** Poly herbal formulation (PHF)( 200mg/kg p.o.),

**Group III:** Poly herbal formulation (PHF)( 400mg/kg p.o.),

**Group IV:** Geriforte syrup (2 ml/kg p.o.). Animals were treated as shown above for the 3 weeks.

At the end of 1st, 2nd and 3rd week i.e. on 7th, 14th and 21st day 1 h after the treatment stress was induced by placing each animal individually in the hermetic vessel of 1 L capacity to record anoxia tolerance time. The time duration of entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia.[11]

## 2.7 Statistical Analysis

Results are expressed as mean±S.E.M. The differences between experimental groups were compared by one-way Analysis of Variance (ANOVA) (control vs.treatment) followed by Bonferroni's test and were considered statistically significant when  $P < 0.05$ .

## 3. Results

### 3.1 Preliminary Phytochemical Analysis

The preliminary phytochemical studies were performed for testing different phytochemical constituents present in polyherbal formulation. The observations showed the presence of alkaloids, flavonoids, steroids, tannins and phenolics, which were found to be more in methanolic extract.

### 3.2 Acute Toxicity Studies

The poly herbal formulation was found to be safe up to 2000 mg/kg body weight by oral route. After 24 h animals were found well tolerated, there was no mortality and no signs of toxicity. The extracts were found to be safe, so the two dose levels i.e. 200 and 400 mg/kg body weight were selected for the present study.

### 3.3 Adaptogenic (Anti Stress) Activity

#### (i) Anoxia stress tolerance time

The results obtained from the anoxia stress tolerance test were expressed as Mean ±SEM.

**Table 2: Effect of Poly Herbal Formulation on anoxia stress tolerance time in rats**

Treatment groups	Duration of anoxia stress tolerance in minutes		
	First week	Second week	Third week
Control- CMC (0.5% W/V) p.o	29±1.80	29.16±3.49	30.33±2.49
PHF (200mg/kg) p.o	38.66±4.62	41.33±3.42*	44±4.81*
PHF (400mg/kg) p.o	46.5±3.27**	46.33±4.34*	48.66±3.60**
Geriforte syrup(2 ml/kg) p.o	49.16±3.68***	48±3.91**	48.83±2.70**

Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, \*Represents statistical significance vs. control ( $p < 0.05$ )

#### (ii) Swimming Endurance Test

The swimming endurance time was significantly ( $P < 0.05$ ) enhanced on 8<sup>th</sup> day in PHF (200 mg/kg), PHF (400 mg/kg) and Geriforte syrup (2ml/kg) treated groups as compared to the stressed groups.

**Table 3: Effect of Poly Herbal Formulation on swimming endurance time**

Treatment	Swimming endurance time in minutes
control CMC (0.5% W/V) p.o	24.83±2.21
PHF (200mg/kg) p.o	35±1.98*
PHF (400mg/kg) p.o	38.5±2.47**
Geriforte syrup(2 ml/kg) p.o	39.66±3.11**

Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, \*Represents statistical significance vs. control ( $p < 0.05$ )

## 4. Discussion

In the present investigation of *poly herbal formulation* has been evaluated for the antistress (adaptogenic) activity against different types of stresses viz. Anoxia and swimming endurance stress models. Geriforte syrup was used for the comparison. It is reported to possess a non-specific anti-stress activity.[13]

In anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with poly herbal formulation had increased the duration of stress tolerance indicating their adaptogenic/ anti-stress activity (Table 2). This effect may be due to that during stress, the poly herbal formulation was capable of increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress. Adaptogens producing beneficial effects in stress are believed to act by increasing non-specific resistance.[14]

In case of swimming endurance test PHF exhibited significant antistress activity as indicated by increase in swimming endurance time (Table 3). There are reports that plasma levels of adrenaline and noradrenaline are enhanced during stress induced by swimming endurance test. In addition, monoamine oxidase (MAO) levels in the brain are reportedly decreased during stress.[8] The swim endurance test results indicate clearly that the *poly herbal formulation* has the properties whereby it increases the physical endurance as well as the overall performance in rats and possessed significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and MAO.

Literature survey indicates that flavonoids, triterpenes and tannins were reported to possess variety of

pharmacological activities including antistress activity. In the present investigation also preliminary phytochemical screening on PHF gave positive tests for flavonoids, steroids and tannins, this might be the reason for significant adaptogenic property of test extract.[15][16]

## 5. Conclusion

The result from the study showed an increase in duration of anoxia tolerance and swimming endurance time in rats treated with Poly herbal formulation. So the results suggest the adaptogenic activity of the poly herbal formulation, hence it can be categorized as adaptogen. The results are encouraging to pursue further studies on the bioactivity guided fractionation of this poly herbal formulation to isolate and characterize probable bioactive molecule responsible for ant-stress activity.

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