Short Communication

Development of Polyherbal Formulation: Impact of Antioxidants on *In Vivo* Antidepressant Activity in Animal Models

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In Ayurveda, single or multiple herbs are used as medication for various ailments. Depression is often manifesting with various symptoms at the behavioral, psychological and physiological levels. Therefore, the investigation for therapeutic alternative is important. Oxidative stress has shown important biochemical aspects in the depression. This study evaluated effect of natural antioxidant on antidepressant activity of polyherbal formulation on the performance of male mice. Mice were given orally polyherbal formulation without antioxidant and with antioxidant daily for 7 d and then subjected to forced swim test and tail suspension test. After 1 w treatment, both polyherbal formulation significantly reduced immobility time in forced swim test and tail suspension test compared with vehicle treated control group. The immobility time in tail suspension test of polyherbal formulation without antioxidant and with antioxidant was found to be 151.17±4.46 s and 116.33±8.84 s respectively. The immobility time in tail suspension test of polyherbal formulation without antioxidant and with antioxidant was found to be 137.17±5.93 s, 113.50±5.40 s respectively. These results indicate that the antidepressant when given along with antioxidant in mice it gives significant antidepressant effect. The experimental results suggest that the intake of antioxidant may help in reducing the symptoms of depression, *via* supplementation of antioxidant.

Keywords: Antidepressant activity, antioxidant activity, forced swimming test, polyherbal formulation, tail suspension test

Polyherbal formulation has been employed all around the globe owing to its wide range of medicinal and therapeutic value. Drug combinations often give rise to a promising effect in treatment of diseases over a single drug[1]. The idea of drug combination has long been accepted in Western medicine, and it has had a lot of impact over the years. Drug combination treatments of cancer and infectious diseases have given patients new hope in current years[2]. Single or several herbs (polyherbal) are used in Ayurveda for therapy. Single or polyherbal are used in Ayurveda for medication. The concept of polyherbalism was illustrated in the Ayurvedic texts Sarangdhar Samhita to achieve greater therapeutic effectiveness[3]. It is more evident that good therapeutic property can be achieved with a formulation of a single multi-constituent formulation. In polyherbal medicine, medicinal plants with lower doses are strongly recommended to prevent side effects and achieve the necessary pharmacological activity[4]. Polyherbal formulations often eliminate the need to consume several formulations at the same time. Many of these benefits of polyherbal formulation benefited from these positive effects[5]. Many medicinal plants have been directly used as the raw drug and these medicinal plants possess various therapeutic values. These medicinal plants are rich source of unique chemical substances with potent therapeutic effect. Individual active phytoconstituents of medicinal plants are insufficient to achieve the

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desired therapeutic results. When several herbs are combined in a certain ratio, the medicinal effect is enhanced and toxicity is reduced[6]. Depression is a heterogeneous disorder that affects a person's mood, physical health and behavior. It also reflects changes in brain neurotransmitter. Oxidative stress plays an important role in the genesis of depression[7]. Clinical studies show that depressed patients had elevated antioxidant enzyme activity. Major causative factor for major depression is inflammation, autoimmune tissue damage and prolonged psychological stress, which leads to oxidative stress[8]. *Bacopa monnieri* Linn. is an important medicinal plant in Indian traditional Ayurvedic medicines. It is a perennial herb generally known as Brahmi, having family Scrophulariaceae[9]. It is used in traditional medicine to cure a nervous conditions, as a dietary aid, to enhance comprehension, memory and depression and to relieve anxiety and skin disorders in patients; particular applications include the prevention of asthma, insanity, and epilepsy[10]. *Bacopa monnieri* mainly contain flavonoids, glycosides, alkaloids and saponins[11]. *Withania somnifera* Linn. known as Aswagandha or Indian Ginseng is a medicinal herb that has been used for over 3000 years in Ayurvedic and indigenous medicine[12]. From ancient times, it is used as memory booster, anti-stress, adaptogen, nerve tonic and against cognitive deficiencies, insomnia, nausea, contagious disorders, infertility, rheumatoid arthritis and gout in Indian systems of medicine. Its formulations are mostly used in the Ayurvedic and Unani medicines. Sattvic Kapha Rasayana is a Rasayana used to cure neuronal ailments. The mixture of ashwagandha root extract (withanolides rich), and three minerals like zinc chloride, magnesium gluconate, and sodium selenate has potent immunomodulatory potential[13]. *Phyllanthus emblica* Linn. is a known medicinal and edible plant species. Amla is rich source of flavonoids, vitamin C, vitamin E and caroteinods and tannins[14]. Since amla has antioxidant activity, it has been used in traditional medicine to treat a different ailments[15]. The aim of current study was to study effect of antioxidant on antidepressant activity. The objectives of the present work was to formulate, standardize and evaluate antioxidant activity and antidepressant of polyherbal formulation containing antidepressant *Bacopa monniera, Withania somnifera* and antioxidant *Phyllanthus emblica. Bacopa monniera, Withania somnifera* and *Phyllanthus emblica* were procured from herbal store Nashik. Dr. A. N. Aher, Head, Department of Pharmacognosy, N. D. M. V. Ps College of Pharmacy, Nashik did the identification and authentication of plant. The pulverized material processed for hydroalcoholic extraction by maceration. The extracts were dried by vacuum[16]. The animal experiments were performed in accordance with the ethical guidelines as per Committee for Purpose of Control and Supervision of Experiments on Animals, India and approved by the Institutional Animal Ethics Committee (1329/ac/10/CPCSEA). Male Wistar mice (22-26 g) were used for study. The animals were kept in cages at 25° with a 12 h light-dark period and habituated in the laboratory setting for at least 24 h prior to the experiments. Water and a well-balanced diet were available at all times, but no food was provided 12 h before the experiments. Chemicals used in this study were 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, India. Ascorbic acid, tocopherol, obtained from Qualigens Fine Chemicals, N-1-naphthylethylenediamine dihydrochloride, sodium nitroprusside, sodium nitrite, ferrous chloride Sd Fine Chemicals Ltd, India. Imipramine was procured from local pharmacy. Polyherbal Formulation without antioxidant (PF-1) and Polyherbal Formulation with antioxidant (PF-2) was prepared by dispersion methods. Polyherbal suspensions was prepared by crushing extracts in the mortar and pestle to form a smooth paste by adding tween 80 and sodium carboxymethyl cellulose in purified water. Sucrose, methyl paraben, sorbitol and lemon oil were dissolved in purified water and this mixture was added in the smooth paste. Made the final volume of dispersion with purified water[17]. Composition of formulation was given in Table 1. Nitric oxide scavenging activity of polyherbal formulation was performed by sodium nitroprusside-Griess reagent method. In the different concentration of formulation in methanol mixed with 1 mM sodium nitroprusside in phosphate buffer saline solution and incubated at 37° for 150 min. Blank solution was also prepared. After incubation, add 0.5 ml of Griess reagent (1 % sulphanilamide, 2 % o-phosphoric acid add 0.1 % N-(1-naphthyl)-ethylenediamine hydrochloride) and immediately absorbance was taken at 546 nm. Ascorbic acid was used as standard. Percentage inhibition was calculated as per following formula. Half maximal Inhibitory Concentration (IC_{50}) was calculated for each extract[18].

Percentage inhibition=$[(A_{blank}-A_{test})/A_{blank}]\times100$

The hydrogen peroxide scavenging activity was performed as per Al-Owaisi *et al.*[19] method. In phosphate buffer, a 40 mM hydrogen peroxide solution was prepared (pH 7.4). In methanol, different concentrations of polyherbal formulations were prepared. A 0.6 ml H_2O_2 solution was added in the reaction mixture. After 10 min, the absorbance of H_2O_2 was measured at 230 nm against a blank phosphate buffer solution. Percentage inhibition calculated as per above formula and IC_{50} calculated[19]. In the 4 ml of different concentrations of polyherbal formulations, 100 µl of DPPH solution (1.3 mg/ml) was added. 100 µl of DPPH solution in 4 ml methanol was treated as blank. Ascorbic acid was used as standard. After 15 min, the absorbance of each solution was measured at 517 nm[20]. The animals are divided into six groups consist of 6 Albino Swiss mice in every group. All polyherbal formulation was administered by orally, in a dose of 1 and 2 ml/kg/d, standard group were received imipramine in a dose 10 mg/kg/d orally. Control animals received suitable vehicle. The polyherbal formulation or vehicle or standard were administered by oral route (p.o.) 1 h before experimental procedure. All the experimental procedures were started on 4th and 7th d, 1 h after administration of the test or standard. Mice were required to swim in an open cylindrical container (14 cm diameter, 20 cm height) with a water depth of 15 cm and a temperature of 25±1°. After each experiment, the water in the containers was changed[21]. Each mouse was observed for 6 min for immobility. Immobility in forced swim test was described as a mouse's ability to avoid struggling and float motionless in water. Each mouse was considered immobile based on just the motions needed to keep its head above water[22]. Adhesive tape was mounted 10 mm from the tip of the mouse's tail to suspend the mice 50 cm above the floor. During a 6 min period, the overall immobility period was manually measured. Immobility in tail suspension test as the lack of body movements other than those induced by respiration or when they hung inactively and motionless[23]. All experimental results are given as the mean±standard error of the mean. To compare test and control groups one-way analysis or two-way analysis of variance, followed by Dunnett's test was used. A value of $p<0.05$ was considered to be significant. Polyherbal formulations have potential response to treat various diseases. However, due to the over exploitation of the medicinal plants, several of them have become rare. As a result, it's thought that psychological stress is linked to increased oxidant activity and oxidative damage and that long-term exposure to psychological stressors could raise the risk of a variety of diseases[24]. Oxidative stress could be underlying cause for depression or associated behavioral changes. A growing number of people are plagued by different psychiatric disorders, especially depression and anxiety, affecting their daily life and increasing economic burden on the society[25]. PF-1, PF-2 and ascorbic acid were found to be good scavenger of nitric oxide free radical, H_2O_2 and DPPH free radical. PF-2 shown better antioxidant activity as compared to PF-1. IC_{50} was given in Table 2. Depression and anxiety, represents one of the major health problems among other mood disorders worldwide. The animal model studies for neurobiological activities involving central nervous system disorders are encouraging researchers for discovering new therapeutic targets. Forced swim test and tail suspension test are used to investigate depressing conditions in rodents[26]. Major Depressive Disorder is a chronic, recurring and debilitating mental illness that is the most common mood disorder in the United States[27]. In the forced swim test the immobility period was significantly decreased compared to the control group (188.50±2.7 s) by PF-1 and PF-2 (fig. 1). The immobility time of PF-1 was found to be 151.17±4.46 s, 137.33±5.17 s of 1.00 and 2.00 ml/kg/d on 7th d respectively. The immobility time of for PF-2 was found to be 116.33±8.84 and 107.17±4.62 s for the doses. Standard group administered imipramine shown strong activity (110.17±5.53 s). The significant difference observed in immobility time PF-1 and PF-2 on 4th d and 7th d in forced swim test (fig. 1). It indicates that if antidepressant drug given along with antioxidant it gives significant result. In the forced swim test the immobility period was significantly decreased compared to the control group (184.17±5.38 s) by PF-1 and PF-2. The immobility time of PF-1 was found to be 137.17±5.93 s and 117.0±6.22 of 1.00 and 2.00 ml/kg/d on 7th d respectively. The immobility time of for PF-2 was found to be 113.50±5.40 and 96.50±4.62 s. The significant difference observed in immobility time PF-1 and PF-2 on 4th d and 7th d in tail suspension test (fig. 2). PF-2 shows a significant antidepressant effect as compared with PF-1 group in the tail suspension test because it significantly reduced the immobility time (fig. 2). Our result shows

that an antioxidant rich diet effects the depression and anxiety. It is documented that depressive mood is highly related with reduced antioxidant defense. It has been proved that a depressed mood is closely related to a weakened antioxidant defense[28,29]. So intake of antioxidant in diet or medicines will be beneficial in depression or related ailments. Although the precise mechanisms underlying the antioxidant-depression relationship are unknown, the present research is the first to explore that antioxidants have beneficial effects on male mice suffering from depression. In the Coronavirus Disease 19 pandemic, people immune system mainly hampered due to depressive environment and oxidative stress owing to depression also. So, the current research shows that use of antioxidants reduces the genesis of depression. Further studies are needed to determine exact mechanisms. The result indicates that the antidepressant when given along with antioxidant in mice it gives significant antidepressant effect. It can be concluded that oxidative stress may be playing a role in the pathogenesis of depression. The experimental data suggest that the consumption of antioxidant may help in reducing the symptoms of depression, *via* supplementation of antioxidant. In future, advanced research is needed to fully understand the mode of action of antioxidants along with antidepressant.

TABLE 1: FORMULATION COMPOSITION OF POLYHERBAL SUSPENSION

Ingredients (% w/w)	PF-1	PF-2
Bacopa monniera (Hydroalcholic extract)	1.00 g	1.00 g
Withania somnifera (Hydroalcholic extract)	1.00 g	1.00 g
Phyllanthus emblica (Hydroalcholic extract)	-	1.00 g
Tween 80	0.1 g	0.1 g
Sodium CMC	2 g	2 g
Sucrose	10 g	10 g
Sorbitol	05 g	05 g
Methyl parabeen	0.20 g	0.20 g
Lemon oil	0.01 g	0.01 g
Purified water q.s.	100 ml	100 ml

TABLE 2: IC_{50} VALUES OF POLYHERBAL FORMULATIONS FOR ANTIOXIDANT ACTIVITIES

Formulation	IC_{50}		
	Nitric oxide free radical	H_2O_2	DPPH free radical
PF-1	413.15 µl/ml	333.16 µl/ml	399.04 µl/ml
PF-2	138.01 µl/ml	128.21 µl/ml	111.21 µl/ml
Ascorbic acid	24.45 µg/ml	36.7 µg/ml	25.14 µg/ml

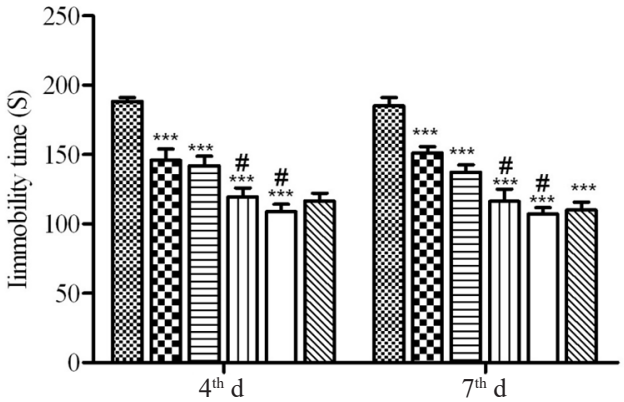

Fig .1: Effect of Polyherbal formulation and imipramine on the time of immobility in forced swim test in the mice. Measurement are represents as mean±standard error of the mean, (n=6), ***p≤0.001 compared to the vehicle-treated group and #p≤0.005 compared with polyherbal formulation without antioxidant (two-way ANOVA followed by the Dunnett's test)
Note: ( ): Control; PF-1 (100); ( ): PF-1 (200); PF-2 (100); ( ): PF-2 (200) and ( ): Imipramine

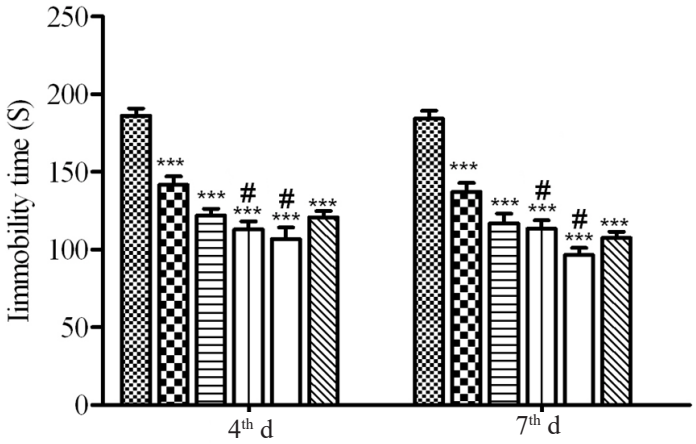

Fig. 2: Effect of polyherbal formulation and imipramine on the time of immobility in tail suspension test in the mice. Measurement are represnts as mean±standard error of the mean; (n=6); ***p≤0.001 compared to the vehicle-treated group and #p≤0.005 compared with polyherbal formulation without antioxidant (two-way ANOVA followed by the Dunnett's test)
Note: ( ): Control; PF-1 (100); ( ): PF-1 (200); PF-2 (100); ( ): PF-2 (200) and ( ): Imipramine

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Conflict of Interest:

All Authors declare that no conflict of interest.

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