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PROTECTION OF SWIMMING-INDUCED OXIDATIVE STRESS IN SOME VITAL ORGANS BY THE TREATMENT OF COMPOSITE EXTRACT OF *WITHANIA SOMNIFERA*, *OCIMUM SANCTUM* AND *ZINGIBER OFFICINALIS* IN MALE RAT

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Abstract

Composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* was administered by gavage at the dose of 40 mg 100 g⁻¹ body weight day⁻¹ to rat orally for 15 days prior to experimentation followed by co-administration of above extract at the same dose for 28 days of swimming to find out the remedial effect of this extract on exhaustive physical exercise-induced oxidative damage. Swimming resulted significant diminution (p<0.05) in the activities of catalase and superoxide dismutase along with elevation (p<0.05) in the levels of thiobarbituric acid reactive substances and conjugated dienes in cardiac, skeletal, hepatic tissues, cerebrum and cerebellum in respect to control. The levels of all these parameters were resettled significantly (p<0.05) towards the extract pretreated cum co-treated swimming group. The antioxidative potency of this composite extract was compared with standard non-enzymatic antioxidant (vitamin-E) in forced swimming state. The above extract has no general toxic effect as reflected here from the study of transaminase activities in liver and kidney. Results lead to conclude that the composite extract of above three plant parts has a therapeutic protective effect on forced swimming-induced oxidative stress in vital organs.

Keywords: Brain tissues, metabolic organs, oxidative stress, phytotherapy, swimming, vitamin-E.

Introduction

Swimming, the exhaustive type of exercise (Jung et al., 2004), has been selected here as a model of physical exercise (Venditti et al., 1996), because muscle trauma caused by other types of physical exercise like prolonged running in treadmill, exercise stimulated electric shock, and plyometric contractions could be avoided. From laboratory investigations, it has been noted that swimming or exhaustive physical exercise results oxidative stress in general (Alessio, 1993; Venditti et al., 1996) by oxygen reperfusion or by consumption of excess oxygen that results tissue damage (Ji, 1995). The production of free radicals increases in parallel with the increase in oxygen consumption during exercise, and the free radical production is directly related to the intensity and/or the duration of exercise. The exercise-induced oxidative stress results impairment in the functional activities of testis and skeletal muscle in rat where oxidative stress is one of the causative factor (Venditti et al., 1996; Burneiko et al., 2004; Misra et al., 2005).

In Indian system of folk medicine, more than one plant in combined way are used for the correction of health disorders and the composite extract of plants in the form of tonic exhibits a better result (Wu et al., 1998). From such knowledge we used here the composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis* for elevation in physical efficiency by correcting the free radical generation related to swimming, a model of exhaustive or strenuous exercise in adult rat (Burneiko et al.,

2004). In this study, the ratio of these three-plant extracts and the specific dose applied has been selected by several pilot experiments as well as in continuation with our previous publication (Misra et al., 2005).

Withania somnifera Dunal (Ashwagandha) is a member of the family Solanaceae and is widely used in Ayurvedic medicine, the traditional medical system of India. Medicinal value of roots of Ashwagandha has already been focused as an antistressor and antioxidant agent (Wagner et al., 1994). Till now, it is used for its variety of health promoting effects and now-a-days is under extensive research for its anti-inflammatory, cardioactive, cholinergic, glutamatergic and GABAergic receptor effects in central nervous system.

Ocimum sanctum Linn (Tulsi) is an erect softy hairy tropical annual aromatic herb or under shrub found throughout India. It belongs to the family Lamiaceae (Labiatae) and is very important for therapeutic potentials since ancient times. The leaves of the plant have been shown to possess good antioxidant as well as antistress potentials (Sethi, 2003) in experimental animals.

Zingiber officinalis Rosc (Ginger) belongs to the family Zingiberaceae, is used for the treatment of several diseases till Vaydic age. It is considered as a popular food spice because of its high content of antioxidants (Kikuzaki and Nakatani, 1993). Ginger is also reported to possess antihepatotoxic and stimulant cum tonic activities (Perry, 1980).

Here, we focused the effect of α -tocopherol on exhaustive swimming-induced oxidative stress in experimental animals as vitamin-E is the least toxic of vitamins (Gerold and Combs, 1992). Out of α , β and γ tocopherols, the α -tocopherol have the greatest vitamin-E activity, which is a potent antioxidant (Hsu et al., 1998). This potent fat soluble antioxidant protects biological membranes against the damaging effects of reactive oxygen species. Not only that, it is a scavenger of peroxy radicals, is probably the most important inhibitor of the free radical chain reaction of lipid peroxidation.

Though many studies have been conducted in relation to oxidative stress imposition by exercise, yet there is no information about the composite effect of roots of *Withania somnifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis* on oxidative stress management in exhaustive swimming-induced adult albino rats. This composite plant extract was used here in continuation to ethnopharmacological report where Trasina, an Ayurvedic herbal formulation, used for oxidative stress management (Bhattacharya, 1997) as well as EuMil which is also another composite extract of four types of plants used for health improvement (Muruganandam et al., 2002). This experiment has been performed to focus the efficacy of above mentioned locally available medicinal plants in composite manner to prevent stress injury in cardiac, skeletal, hepatic and brain tissues due to exhaustive physical exercise in comparison to a standard non-enzymatic antioxidant, i.e. vitamin-E.

Materials and Methods

Plant materials

The roots of *Withania somnifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis* were collected from Gopali, Indian Institute of Technology, Kharagpur, district Paschim Medinipur, West Bengal in the month of May and the materials were identified by the taxonomist of Botany Department, Vidyasagar University, Midnapore. The voucher specimens were deposited in the Department of Botany, Vidyasagar University with specimen numbers Bio-Med/VU/DG/No-3, 4 and 5 respectively.

Preparation of the composite methanolic extract of roots of *Withania somnifera* leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis*

The above-mentioned plant parts were dried in an incubator for 2 days at 40 °C, crushed in electrical grinder and powdered separately. Powder of each plant material at the amount of 50 g was extracted in 250 ml of methanol for 18 hr in a soxhlet apparatus. A deep brown methanolic extract of *Zingiber officinalis*, yellowish brown of *Withania somnifera* and deep green of *Ocimum sanctum* were collected. The extracts were dried at reduced pressure. It was stored at 4 °C and used for next 7 days of the experiment. As per demand, extracts were prepared further throughout the experimental period. When needed, the extracts were suspended in olive oil at the ratio of 1 : 2 : 2 (*Withania somnifera*: *Ocimum sanctum* : *Zingiber officinalis*) and used for the study.

Selection of animal and care

The study was conducted on thirty-six, adult healthy, male albino rats of Wistar strain having the body weight of 120 ± 5 g were selected for this experiment. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed two per cage in a temperature controlled room (22 ± 2 °C) with 12-12 h light-dark cycle (8.00-20.00 h light: 20.00-8.00 hr dark) at a humidity of 50 ± 10 %. They were provided with standard food and water *ad libitum*. The Principles of Laboratory Animal Care, NIH, 1985 were

followed throughout the duration of experiment and instruction given by our institutional ethical committee was followed regarding treatment of the experiment.

Forced swimming programme

The forced swimming of rat was performed in acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with water (34 ± 1 °C) to a depth of 37 cm (Matsumoto et al., 1996). The rats were loaded with a steel washer weighing approximately 4 % of their body weight attached to the tail, which forced the rat to maintain continuous rapid leg movement (Bostrom et al., 1974). The forced swimming technique was modified to some extent here from our previous study in this line (Misra et al., 2005)

Experimental design

Thirty-six rats were divided into six groups as follows:

Group 1: Untreated control (UC) - animals in this group were provided standard food and water without any forceful feeding of olive oil or vitamin-E or composite extract.

Group 2: Placebo control (PC) - received olive oil by gavage (0.5 ml 100 g⁻¹ body weight day⁻¹rat⁻¹) for 15 days prior to start the exercise followed by 28 days of experimental period.

Group 3: Extract pretreated cum co-treated control (EPCC) - received composite extract through oral route as group 2 at the dose of 40 mg in 0.5 ml olive oil 100 g⁻¹body weight day⁻¹ rat⁻¹ for 28 days.

Group 4: Forced swimming (FS) – intermittent swimming 8 h day⁻¹ (30 mins swimming followed by 10 minutes rest) for 28 days as per the design of previous workers (Kayatekin et al., 2002) and some modification of our technique (Misra et al., 2005).

Group 5: Extract pretreated cum co-treated swimming (EPCS) – preconditioning by extract treatment at the same dose as group 3 for 15 days prior to swimming followed by swimming as per the protocol of group 4 and composite extract co-administration through gavage at the above dose throughout 28 days of swimming.

Group 6: Preconditioned followed by swimming and vitamin-E co-administered (PSVC) - rats received vitamin E (α -tocopherol succinate) at the dose of 6 mg 100 g⁻¹body weight day⁻¹ in 0.5 ml olive oil before starting the experiment as well as throughout the experimental period as time frame of group 5.

After completion of 28 days of swimming, all the animals one after another in serial manner were sacrificed within 5 mins of post exercise period. Cardiac muscle, skeletal muscle (gastrocnemius from both hind legs), liver, kidney, cerebrum and cerebellum (same hemisphere in each animal) were collected from individual animal. All the tissues were refrigerated at -20 °C and within 2 hr of refrigeration; the tissues were processed for biochemical studies.

Biochemical assays

Biochemical assay of Catalase (CAT)

The activity of CAT of the above mentioned tissues were measured biochemically (Beers and Sizer, 1952). Liver, cardiac muscle, cerebrum, cerebellum and skeletal muscle tissue portions from each animal were homogenized separately in 0.05 M Tris-HCl buffer solution (pH 7.0) at the tissue concentration of 50 mg ml⁻¹ for the evaluation of CAT activity. These homogenized samples were centrifuged at 10,000 g for 10 mins at 4 °C. In spectrophotometer cuvette, 0.5 ml of H₂O₂ and 2.5 ml of distilled water were mixed and reading of absorbance was noted at 240 nm. The above mentioned tissue supernatants were added separately at a volume of 40 μ l and the subsequent six readings were noted at 30 sec interval. The unit was expressed in terms of mM of H₂O₂ consumption mg⁻¹ of tissue min⁻¹.

Biochemical assay of superoxide dismutase (SOD)

Required amounts of skeletal muscle, cardiac muscle, liver, cerebrum and cerebellum were homogenized in ice-cold 100 mM Tris-cacodylate buffer to give a tissue concentration of 50 mg ml⁻¹ and centrifuged at 10,000 g for 20 minutes at 4 °C. The SOD activity of the supernatant was estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by SOD according to the standard method (Marklund and Marklund, 1974). The buffer was prepared by mixing 50 mM Tris (pH 8.2), 50 mM cacodylic acid (pH 8.2), 1 mM EDTA and 10 mM HCl. In a spectrophotometric cuvette, 2 ml of buffer, 100 μ l of 2 mM pyrogallol and 10 μ l of supernatant were taken and the absorbance was noted in spectrophotometer at 420 nm for 3 mins period. The one unit of the SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50 percent.

Estimation of lipid peroxidation from the concentration of Thiobarbituric acid reactive substances (TBARS) and Conjugated dienes (CD)

The skeletal muscle, cardiac muscle, liver, cerebrum and cerebellar portions were homogenized separately at the tissue concentration of 50 mg ml⁻¹ in 0.1 M ice-cold phosphate buffer (pH 7.4) and the homogenates were centrifuged at 10,000 g at 4 °C for 5 minutes individually. Each supernatant was used for the estimation of TBARS and CD. TBARS, the product formed being due to the peroxidation of lipids, was determined by the reaction of thiobarbituric acid with malondialdehyde. For the measurement of TBARS, the homogenate mixture of 0.5 ml was mixed with 0.5 ml normal saline (0.9 g % NaCl) and 2 ml of TBA-TCA mixture (0.392 g thiobarbituric acid in 75 ml of 0.25 N HCl with 15 g trichloroacetic acid, volume up to 100 ml by 95 % ethanol and boiled at 100 °C for 10 min). This mixture was then cooled at room temperature and centrifuged at 4000 g for 10 mins. The whole supernatant was taken in spectrophotometer cuvette and read at 535 nm (Okhawa *et al.*, 1979).

Quantification of the CD was performed by a standard method (Slater, 1984). The lipids were extracted with chloroform-methanol mixture (2:1), followed by centrifugation at 1000 g for 5 mins. The chloroform layer was evaporated to dryness under a stream of nitrogen. The lipid residue was dissolved in 1.5 ml of cyclohexane and the absorbance was noted at 233 nm to measure the amount of hydroperoxide formed.

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in liver and kidney

For the assessment of metabolic toxicity in connection to treatment of plant extract, we measured GOT and GPT activities in liver and kidney. The above mentioned tissues were homogenized separately at the tissue concentration of 50 mg ml⁻¹ in 0.1 M of ice-cold phosphate buffer (pH 7.4) and the homogenates were centrifuged at 5000 g at 4 °C for 15 mins individually. Each supernatant was used for the estimation of GOT and GPT by standard method (Goel, 1988). Four test tubes were labeled as glutamate oxaloacetate test (OT), glutamate oxaloacetate blank (OB), glutamate pyruvate test (PT) and glutamate pyruvate blank (PB) corresponding to test and blank of GOT and GPT respectively. One ml of GOT substrate (200 mM l⁻¹ DL-aspartate and 2 mM l⁻¹ α -ketoglutarate, pH 7.4) was taken in OT and OB test tubes, and 1 ml of GPT substrate (200 mM l⁻¹ DL-alanine and 2 mM l⁻¹ α -ketoglutarate, pH 7.4) was transferred in PT and PB test tubes. All test tubes were incubated at 37 °C for 5 min in water bath. Sample supernatant at the volume of 0.2 ml was transferred in OT and PT test tubes and these were placed in above water bath. One ml of DNPH was added in PT and PB marked test tubes and then left at room temperature. Incubation of OT and OB marked test tubes were continued for 60 mins after adding of the sample. Then 1 ml of DNPH was added in OB and OP marked test tubes and left at room temperature. The tubes were removed from water bath followed by the addition of 0.2 ml sample in OB and OP marked test tubes. After 20 mins incubation at room temperature, 10 ml of 0.4 N NaOH was added in all the test tubes and mixed by inversion. Absorbance was noted at 505 nm. The change in absorbance was determined by subtracting the blank readings from corresponding test reading. The enzyme activity was expressed in term of unit mg⁻¹ of tissue.

Statistical analysis

Analysis of variance (ANOVA) followed by a multiple two tail t-test with Bonferroni modification was used for statistical analysis of the collected data (Sokal and Rohlf, 1997). Differences were considered significant when $p < 0.05$.

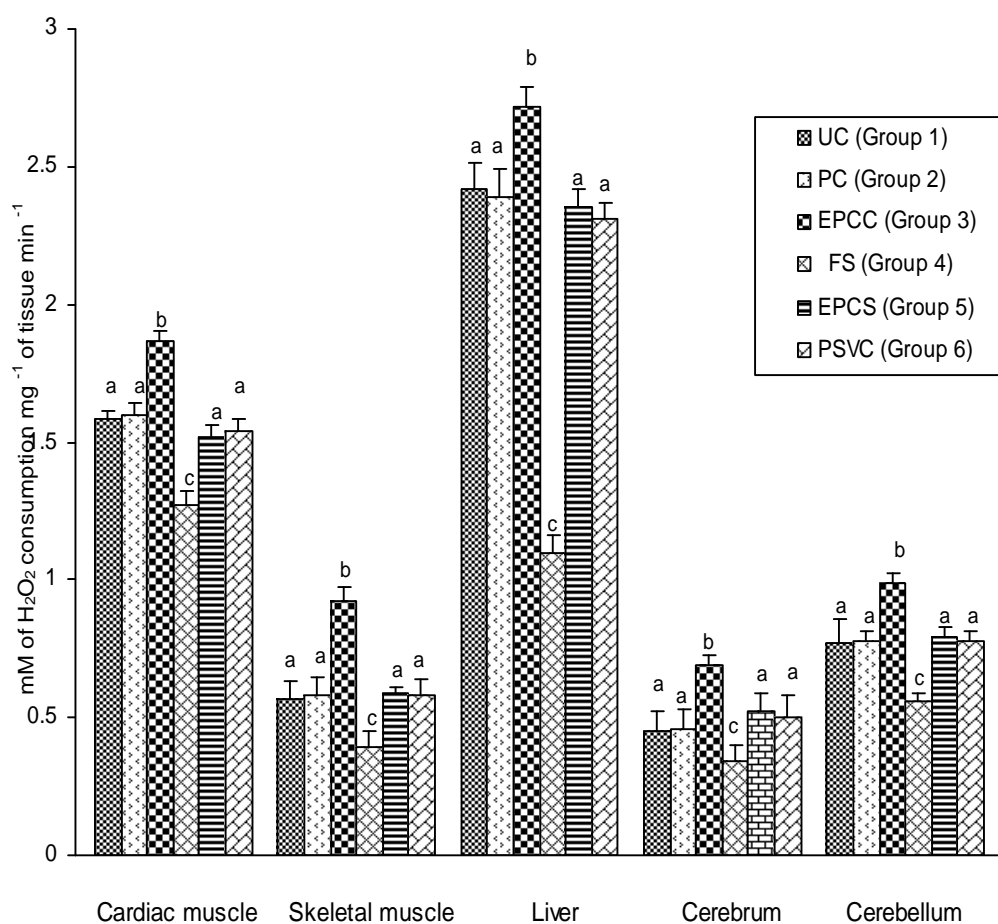
Results

Activities of CAT and SOD in cardiac, skeletal, hepatic and brain tissues were elevated significantly ($p < 0.05$) in extract pretreated cum co-treated control group in comparison to the respective untreated and placebo treated control groups. In forced swimming group, due to strenuous swimming, a significant ($p < 0.05$) diminution was observed in the activities of CAT and SOD in above mentioned tissues when compared to untreated and placebo treated control groups. In extract pretreated cum co-treated swimming group, forced swimming animals were subjected to pretreatment with composite extract followed by co-administration of this extract throughout the exercise period, resulted a resettlement in the activities of CAT and SOD in all of the above tissues to the untreated and placebo treated control groups. There was no significant variation in the activities of CAT and SOD in any of the above tissue when extract pretreated cum co-treated swimming group was compared to preconditioned followed by swimming and vitamin-E co-administered group (Figure 1 and 2).

Quantity of TBARS as well as CD in all of the above-mentioned tissues was elevated in forced swimming group of animals when compared to untreated and placebo treated control groups. These parameters were protected significantly ($p < 0.05$) in extract pretreated cum co-treated swimming group as well as in preconditioned followed by swimming and vitamin-E co-administered group also. Significant ($p < 0.05$) low levels of TBARS and CD in all of the above tissue samples were noted in extract pretreated cum co-treated control group when compare to untreated and placebo treated control groups. No significant difference was noted in these parameters when comparison was made between extract pretreated cum co-treated swimming group and preconditioned followed by swimming and vitamin-E co-administered group (Table 1 and 2).

Due to swimming there was significant ($p < 0.05$) elevation in GOT and GPT activities both in liver and kidney in forced swimming group of animals when comparison was made with untreated and placebo treated control animals (Table 3) but these parameters were resettled to the control level in extract pretreated cum co-treated swimming animals or in preconditioned followed by swimming and vitamin-E co-administered animals.

Fig 1. Protective effect of pretreatment of composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* followed by co-administration of same composite extract for 28 days on catalase activity in cardiac, skeletal, hepatic and brain tissues in swimming-induced oxidative stress in male rat.



Data are expressed as Mean \pm S.E.M. (N=6). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly ($p < 0.05$) in specific tissue sample.

This composite extract has no toxicity induction capacity in metabolic organs, as extract pretreated cum co-treated control animals did not show any significant variation in GOT and GPT activities both in liver and kidney when compared to untreated and placebo treated control animals (Table 3).

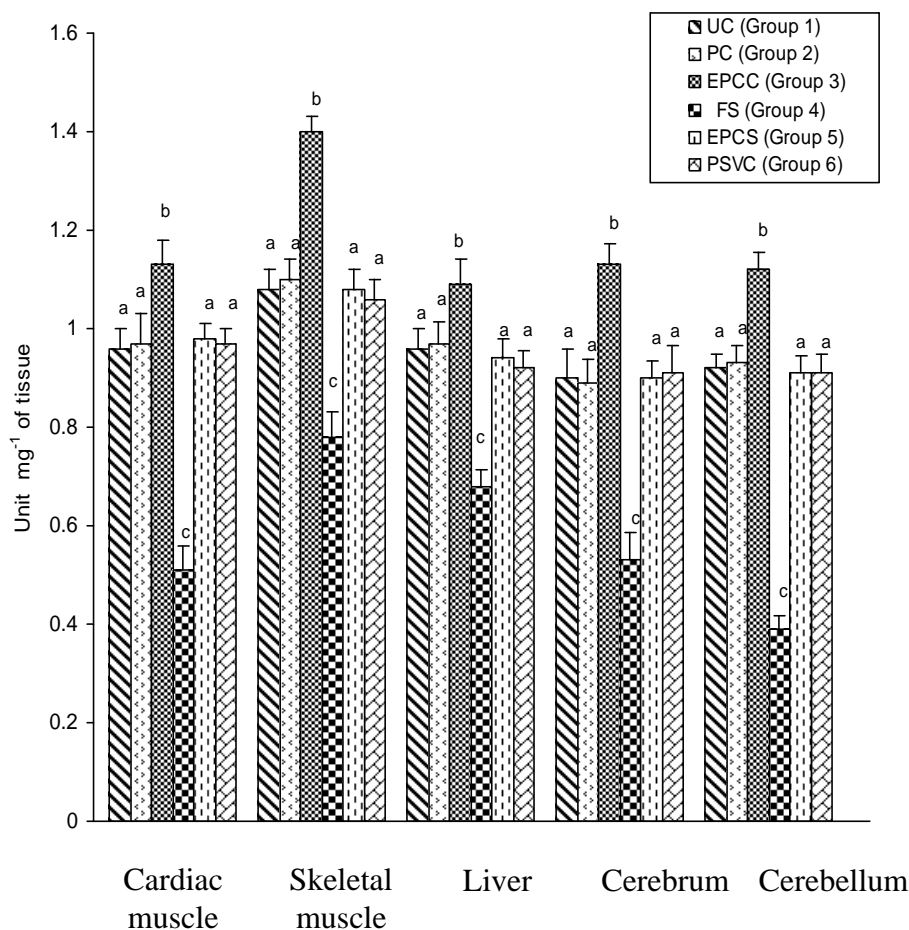
Table 1: Effects of pretreatment of composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* followed by co-administration of same composite extract for 28 days on TBARS levels in cardiac, skeletal, hepatic and brain tissues in swimming-induced oxidative stress in male rat.

Group	TBARS (nM mg ⁻¹ of tissue)				
	Cardiac muscle	Skeletal muscle	Liver	Cerebrum	Cerebellum
UC (Group 1)	42.11±3.12 ^a	57.91±2.97 ^a	52.91±3.01 ^a	14.31±2.87 ^a	7.56±0.41 ^a
PC (Group 2)	41.39±2.87 ^a	58.66±3.10 ^a	52.01±2.89 ^a	14.61±2.76 ^a	7.69±0.67 ^a
EPCC (Group 3)	20.48±2.98 ^b	50.92±2.98 ^b	49.02±3.01 ^a	11.58±2.67 ^b	5.21±0.57 ^b
FS (Group 4)	74.06±3.01 ^c	83.49±2.21 ^c	69.33±2.98 ^b	32.31±2.57 ^c	13.18±0.41 ^c
EPCS (Group5)	39.73±2.98 ^a	58.92±3.10 ^a	52.42±3.01 ^a	18.61±2.89 ^d	7.93±0.53 ^a
PSVC (Group 6)	40.01±2.67 ^a	53.89±3.08 ^a	50.5±2.98 ^a	17.32±2.76 ^d	7.78±0.50 ^a

Each value represents Mean ± S.E.M. (n=6). ANOVA followed by multiple two tail t-test. Data with different superscripts (a,b,c,d) differ from each other significantly (p<0.05) in specific tissue sample.

UC= untreated control, PC= placebo control, EPCC= extract pretreated cum co-treated control, FS= forced swimming, EPCS= extract pretreated cum co-treated swimming, PSVC= preconditioned followed by swimming and vitamin E co-administered.

Fig 2. Corrective effects of pretreatment of composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* followed by co-administration of same composite extract for 28 days on superoxide dismutase activity in cardiac, skeletal, hepatic and brain tissues in swimming-induced oxidative stress in male rat.



Data are expressed as Mean ± S.E.M. (N=6). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly (p<0.05) in specific tissue sample.

Table 2: Effects of exhaustive swimming on CD levels in cardiac, skeletal, hepatic and brain tissues after pretreatment of composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* followed by co-administration of same composite extract for 28 days in male rat.

Group	CD (nM hydroperoxide mg ⁻¹ of tissue)				
	Cardiac muscle	Skeletal muscle	Liver	Cerebrum	Cerebellum
UC (Group 1)	122.21±6.71 ^a	113.76±5.98 ^a	52.42±6.21 ^a	211.14±5.78 ^a	198.09±8.87 ^a
PC (Group 2)	120.01±6.91 ^a	116.58±6.32 ^a	53.84±6.54 ^a	213.16±6.21 ^a	200.08±9.21 ^a
EPCC (Group 3)	104.92±7.11 ^b	92.01±6.43 ^b	40.45±7.01 ^b	187.45±6.45 ^b	174.34±8.98 ^b
FS (Group 4)	179.55±7.22 ^c	162.39±5.90 ^c	110.1±6.76 ^c	270.52±6.32 ^c	264.85±9.09 ^c
EPCS (Group5)	127.48±6.78 ^a	117.17±7.01 ^a	56.26±5.90 ^a	217.25±5.89 ^a	202.51±9.89 ^a
PSVC (Group 6)	124.81±8.10 ^a	116.87±7.09 ^a	55.91±5.89 ^a	220.54±8.21 ^a	205.01±8.91 ^a

Each value represents Mean ± S.E.M. (n=6). ANOVA followed by multiple two tail t-test. Data with different superscripts (a,b,c) differ from each other significantly (p<0.05) in specific tissue sample.

UC= untreated control, PC= placebo control, EPCC= extract pretreated cum co-treated control, FS= forced swimming, EPCS= extract pretreated cum co-treated swimming, PSVC= preconditioned followed by swimming and vitamin E co-administered.

Table 3: Effects of pretreatment of composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum*, and rhizomes of *Zingiber officinalis* followed by co-administration of same composite extract for 28 days on hepatic and renal GOT and GPT activities in swimming- induced oxidative stress condition in male rat.

Group	Liver		Kidney	
	GOT (Unit mg ⁻¹ of tissue)	GPT (Unit mg ⁻¹ of tissue)	GOT (Unit mg ⁻¹ of tissue)	GPT (Unit mg ⁻¹ of tissue)
UC (Group 1)	15.2±0.2 ^a	14.7±0.3 ^a	15.1±0.3 ^a	14.8±0.4 ^a
PC (Group 2)	15.8±0.5 ^a	14.4±0.3 ^a	15.2±0.4 ^a	14.9±0.3 ^a
EPCC (Group 3)	16.1±0.6 ^a	14.8±0.3 ^a	15.4±0.3 ^a	15.0±0.2 ^a
FS (Group 4)	19.7±0.4 ^b	19.6±0.4 ^b	21.1±0.3 ^b	19.8±0.4 ^b
EPCS (Group5)	15.6±0.5 ^a	14.3±0.3 ^a	15.0±0.4 ^a	14.6±0.3 ^a
PSVC (Group 6)	15.1±0.4 ^a	14.5±0.4 ^a	14.9±0.4 ^a	14.7±0.4 ^a

Each value represents Mean ± S.E.M. (n=6). ANOVA followed by multiple two tail t-test. Data with different superscripts (a,b) differ from each other significantly (p<0.05) in specific tissue sample.

UC= untreated control, PC= placebo control, EPCC= extract pretreated cum co-treated control, FS= forced swimming, EPCS= extract pretreated cum co-treated swimming, PSVC= preconditioned followed by swimming and vitamin E co-administered.

Discussion

The present work has been designed to search out the efficacy of the composite extract of locally used medicinal plants for the management of oxidative stress in relation to exhaustive physical exercise by forced swimming in laboratory model animals like rat. Swimming-induced oxidative stress was evaluated by noting the activities of CAT and SOD as well as by the levels of TBARS and CD in cardiac, skeletal, hepatic and brain tissues. Catalase is one of the members in the enzymatic defense system against reactive oxygen species which catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen, and thereby protects cells from oxidative damage (Jena and Patnaik, 1992). Superoxide anion, which is another reactive oxygen species, generates in oxidative stress condition and this is destroyed by SOD, another member of enzymatic defense system of cell. The significant decrease in the activities of CAT and SOD in the above mentioned tissues after forced swimming may be an indication of exercise-induced oxidative threat. In our study, such stress has been protected significantly in extract pretreated cum co-treated swimming group of animals. During oxidative stress, cell responds to reactive oxygen metabolites (ROMs) with SOD. SOD has been detected in a large number of tissues which protects the cell from damage by inhibiting superoxide anions and hydroxyl radical production (Fridovich, 1995). From the results of CAT and SOD, members for scavenging free radicals, it may be stated that the ingredients present in the composite methanolic extract possess antioxidative properties in exhaustive swimming-induced oxidative stress condition. In this experiment, a standard potent non-enzymatic antioxidant (Vitamin-E) was co-treated to the pre-conditioned followed by swimming and vitamin-E co-administered group to compare the antioxidative protective effect of this extract. A significant correction in the oxidative stress condition was noted in vitamin-E co-administered group as well as in the extract pretreated cum co-treated swimming group in comparison to the forced swimming group.

Oxidative stress is characterized by ROS-induced lipid peroxidation, DNA damage and protein degradation. CD and TBARS including MDA, lipid hydroperoxides etc., are the by-products of lipid peroxidation. MDA is secondary product generated during the oxidation of polyunsaturated fatty acids. Significant elevations in the levels of products of free radicals like TBARS and CD in the brain, skeletal muscle, cardiac muscle and liver in forced-swimming group further support the low antioxidant enzyme activity that was corrected significantly after co-administration of the said extract to the forced-swimming animals.

The composite extract of these plant parts resulted a significant elevation in the level of antioxidant status, and its potency is equivalent to a potent established antioxidant known as vitamin-E as shown here. Vitamin-E protects biological membrane against the damaging effects of reactive oxygen species, as this vitamin is effective inhibitor of lipid peroxidation because of its association with membrane lipids. Moreover, vitamin-E itself elevates scavenger enzymes activity (Das and Chowdhury, 1999). Physiological phenomenon in relation to such activity may improve physical performance possibly through an improvement of body's antioxidative potency as well as low rate of production of free radicals in swimming-induced oxidative stress in experimental animals. In this study, our results also support the above view that antioxidant status is elevated in vitamin E co-administered swimming group. Free radical scavenging properties have been evaluated from several classical polyherbal formulations where the rhizomes of *Z. officinalis* or roots of *W. somnifera* or leaves of *O. sanctum* are used as one of the ingredients (Suchalatha et al., 2004).

As there was no significant variation in the concerned parameters between untreated control and placebo control groups, so it may be stated that the observed results are neither due to increased oil intake nor the effect of possible stress response associated with gavage feeding.

The use of polyherbal formulation instead of monoherbal preparation of medicinal plant parts offers much better protection from various angles in exercise-induced oxidative stress animals. This may be due to combined action of the various plant constituents as well as of the isolated active principles rather than by any single component and may be useful as a drug for the prevention of oxidative injuries (Suchalatha et al., 2004). By virtue of their proposed properties and clinical use in Ayurveda, polyherbal formulations like *Trasina* (Bhattacharya 1997), *EuMil* (Muruganandam et al., 2002) and *Arogh* (Suchalatha et al., 2004) etc. perhaps provided potential, therapeutic intervention against oxidative threats, both in health and diseased conditions. The composite extract has been used in this experiment in relation to the traditional use of herbal mixture used by Ayurvedic doctors. How this composite mixture protects the oxidative threat due to exhaustive physical exercise like forceful swimming for long period is not clear but following hypothesis may be stated. The active ingredients present here may elevate the level of antioxidant scavenger enzymes as these plants contain flavonoids, phenolic compounds which are antioxidants, antistressor (Mishra et al., 2000; Prakash and Gupta, 2005) or the ingredients present here may either prevent or destroy the free radical generation in the cells. In addition to physical exercise, psychological stress due to such strenuous physical labour may be one cause for oxidative stress induction and the composite extract may counteract such psychological stress.

Considering all these results it may be concluded that the composite methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis* could be beneficial for the

protection of exhaustive physical exercise like swimming-induced oxidative stress injury on brain tissues and also on the vital tissues like cardiac, skeletal and hepatic which are very much prone to physical exercise-induced oxidative stress. More studies are needed to evaluate the therapeutic value of this extract for the improvement of endurance capacity by minimizing the oxidative threat on vital organs.

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Abbreviations: ANOVA, Analysis of variance; DNPH, Dinitrophenyl hydrazine; EDTA, Ethylene diamine tetra acetic acid; HCl, Hydrochloric acid; H₂O₂, Hydrogen peroxide; NIH, National Institute of Health; OB, Glutamate oxaloacetate blank; OT, Glutamate Oxaloacetate test; p, Probability; PB, Glutamate pyruvate blank; PT, Glutamate pyruvate test; TBA-TCA, Thiobarbituric acid-Trichloroacetic acid; UGC, University Grants Commission.

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