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**Protective Response of Methanolic Extract of *Ocimum sanctum*,  
*Withania somnifera* and *Zingiber officinalis* on Swimming-Induced  
Oxidative Damage on Cardiac, Skeletal and Brain Tissues in Male Rat:  
A Duration Dependent Study**

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**Abstract:** The present study focused the duration dependent protective effects of methanolic extract of root of *Withania somnifera*, leaf of *Ocimum sanctum* and rhizome of *Zingiber officinalis* in composite way on severe exercise-induced oxidative damage in cerebrum, cerebellum, cardiac and skeletal muscles. Results indicate that catalase and superoxide dismutase activities were decreased alongwith increase in the levels of thiobarbituric acid reactive substances and conjugated dienes in aforesaid tissues significantly in forced swimming group which were protected significantly in a graded manner after 14 days supplementation or 28 days co-administration of composite extract and in some cases only after last 7 days of the said extract supplementation. Complete protection was noted when co-administration and swimming periods were same like 28 days time profile but such complete recovery in above parameters was not observed when supplementation was continued for last 7 or 14 days out of 28 days of swimming. The above extract has no general toxic effect reflected here from the study of body weight and transaminase activities in liver and kidney. The results convey the message that above extract has a significant corrective effect on swimming-induced oxidative damage on vital tissues and for which a minimum period of such extract supplementation or co-administration is essential.

**Key words:** Forced swimming, oxidative stress, brain tissues, cardiac and skeletal muscles, antioxidant, phytotherapy

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### INTRODUCTION

Exercise is known to cause oxidative stress by the generation of free radicals from various biological pathways. Out of several types of exercise models, swimming is one of the well-accepted exhaustive types of physical exercise (Jung *et al.*, 2004). It is well known that, during exercise maximal oxygen uptake is elevated 10 to 200-folds above resting level. This elevation in oxygen consumption is an indicator of oxidative stress and it promotes Reactive Oxygen Species (ROS) formation. The mammalian body has evolved a highly fascinating array of endogenous antioxidant defenses against oxidative stress including various antioxidant enzymes. The endogenous antioxidants do not completely protect us against the sum of oxidative stresses threatening our body, which leads to

a net oxidative damage and in the long run contributes to aging and various diseases. Antioxidant enzymes, basically protect the cellular environment from oxidative damage, are also affected by exercise (Seward *et al.*, 1995). Exhaustive exercise is characterized by the disturbance between intracellular pro-oxidant and antioxidant homeostasis. From laboratory investigation, it has been noted that swimming or severe exercise results oxidative stress in general (Alessio, 1993) that causes tissue damage (Ji, 1995). The production of free radicals increases in parallel with the increase in oxygen consumption during exercise and this phenomenon is directly related to the intensity and/or the duration of exercise.

Recently, much attention has been paid to the role of antioxidant system and lipid peroxidation in exercise and

physical training. Many studies (Alessio, 1993; Clarkson, 1995) have reported that acute sub-maximal exercise increases exercise-induced lipid peroxidation. Regular physical training, on the other hand, enriches antioxidant defense system, reduces lipid peroxidation (Akkus, 1995) and eventually reduces exercise-induced oxidative risk. However, it is very difficult to reduce the exercise-induced threat in individual who do not exercise regularly (Sjodin *et al.*, 1990). This exercise-induced oxidative stress results impairment in the functional activities of skeletal muscle also. Experimental evidences indicate that overload training programme is deleterious relative to load less training programme which increase myocardial oxidative stress (Burneiko *et al.*, 2004) as well as impose oxidative damages on cerebral and cerebellar tissues (de Oliveria *et al.*, 2003). Our previous work has been reported in the same line that forced swimming-induced oxidative stress is a causative factor for induction of functional disorders in testis (Misra *et al.*, 2005).

In Indian system of folk medicine, there is a reputation for the use of more than one plant in combined way for the correction of health disorders. In the recent years, it has been well-established that, composite plant extract in the form of tonic exhibits a better result than single plant extract (Borchers *et al.*, 1999). On the basis of such knowledge we used here methanolic extract of roots of *Withania somnifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingiber officinalis* in combined way for elevation in physical efficiency by the management of free radical generation in relation to duration dependent swimming in adult male albino rat. The ratio of these three plant extracts and the specific dose used here has been selected considering its promising result through trial and error and in continuation to our previous publication (Misra *et al.*, 2005).

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

*Zingiber officinalis* Rosc (Ginger), under the family of Zingiberaceae, is such a popular food spice because of high content of antioxidants (Kikuzaki and Nakatani, 1993). Ginger is also reported to possess antihepatotoxic (Hikino *et al.*, 1985) and stimulant cum tonic activities (Perry, 1980).

*Withania somnifera* Dunal (Ashwagandha) is a member of the family Solanaceae and is used in Indian traditional medicine, Ayurveda. Therapeutic importance of different parts of this plant has been established from long past. Till now, this plant is used for its variety of

health promoting effects and now-a-days is under extensive research for its anti-inflammatory, cardio active, cholinergic, glutamatergic and GABAergic receptor effects in central nervous system (Mohan *et al.*, 2004). Medicinal values of roots of ashwagandha have already been focused as an antistressor and antioxidant agent (Wagner *et al.*, 1994).

Till now, several studies have been established depicting the management of oxidative stress imposition in extensive exercising animals. But there is paucity of information about the composite effect of medicinal plant extract on oxidative stress management in exhaustive swimming-induced adult albino rats. No extensive work has yet been done to find out the oxidative damage in brain, cardiac and muscular tissues due to exhaustive swimming and its herbal management. So, in the present study, we aim to observe the effect of forced swimming on oxidative stress induced damages in cardiac and skeletal tissues as well as on brain tissues and its management by extracts of these three plant parts in composite manner for improvement of the endurance capacity of the experimental animals in the long run.

## MATERIALS AND METHODS

**Plant materials:** In this experiment, the roots of *W. somnifera*, leaves of *O. sanctum* and rhizomes of *Z. officinalis* were used. All these medicinal plant parts were collected in the month of May from the Gopali gardening of Indian Institute of Technology, Kharagpur, district Paschim Medinipur, West Bengal. The above mentioned plant parts were identified by the taxonomist of Department of Botany, Vidyasagar University, Midnapore and the voucher specimens were deposited in the same department with their numbers HPCH No. 3, 4 and 5, respectively.

**Preparation of the composite methanolic extract:** The collected plant parts were separately cleaned up in fresh water and then completely dried in an incubator for 2 days at 40°C, powdered separately and 50 g powder of each of which was extracted in 250 mL methanol for 18 h in a soxhlet apparatus. The extracts were dried at reduced pressure, stored at 0-4°C and used for next 7 days of the experiment. The extracts were further prepared during the experimental period as per our requirement. 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:** Thirty-six adult healthy, male Wistar strain albino rats (120±5 g) were selected for the study and were acclimatized to laboratory

condition for 2 weeks prior to experimentation. Animals were cared two per cage in a temperature controlled room ( $22\pm 2^\circ\text{C}$ ) with 12-12 h light-dark cycle (8.00-20.00 h light; 20.00-8.00 h dark) at a humidity of  $50\pm 10\%$ . They were provided with standard chow food and water *ad libitum*. The Principles of Laboratory Animal Care were followed throughout the duration of experiment and instructions given by our institutional ethical committee were followed regarding treatment of the experiment.

**Forced swimming programme:** The forced swimming of rat was performed in acrylic plastic pool ( $90\times 45\times 45$  cm) filled with water ( $34\pm 1^\circ\text{C}$ ) 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 that forced the animal to maintain continuous rapid leg movement (Bostrom *et al.*, 1974). To reduce buoyancy and ensure the imposed workload, the fur of the rats was washed with liquid soap prior to swimming and air bubbles trapped in the fur were removed periodically.

**Experimental design:** Thirty-six adult albino Wistar strain rats were divided into six equal groups. All the animals were forcefully fed either olive oil or composite extract according to the design of experiment to the respective group at 8.00 h through oral ingestion by gavage at fasting state and one hour thereafter food was given to the animals *ad libitum* throughout the experimental period.

**Group I (vehicle treated control):** Rats of this group received olive oil ( $0.5\text{ mL } 100\text{ g}^{-1}\text{ body weight day}^{-1}\text{ rat}^{-1}$ ) for 15 days prior to experimentation for preconditioning followed by 28 days of experimental period through oral route at 8.00 h.

**Group II (extract treated control):** This group of animals were subjected to forceful oral ingestion of methanolic extract of this plant parts at the ratio of 1:2:2 (*Withania somnifera*: *Ocimum sanctum*: *Zingiber officinalis*) and at the dose of  $40\text{ mg } 100\text{ g}^{-1}\text{ body weight day}^{-1}\text{ rat}^{-1}$  in 0.5 mL olive oil for 15 days as preconditioning prior to starting of experiment followed by 28 days of experimental period through oral route at 8.00 h of each day by gavage.

**Group III (forced swimming):** Rats were subjected to swim for  $8\text{ h day}^{-1}$  including rest. The duration of this exercise was fixed for 30 min at a stretch followed by 10 min rest as per experimental design by previous workers

(Kayatekin *et al.*, 2002). The animals were continued to swim as per the above mentioned schedule for 28 days. Olive oil was administered to the rats through gavage as in control group.

**Group IV (pretreatment followed by forced swimming with last 7 days of extract supplementation):** Rats of this group were subjected to preconditioning by oral administration of composite methanolic extract at the same ratio as group II animals for 15 days prior to commencement of forceful swimming. From the next day, the animals were subjected to swim as per the same protocol mentioned in group III and composite extract was administered for the last 7 days (on and from the 22nd day of swimming upto the 28th day) only, just 2 h before starting of exercise.

**Group V (pretreatment followed by forced swimming with last 14 days of extract supplementation):** For the animals of this group, the experimental protocol was maintained as group IV animals but these animals were allowed to forceful ingestion of composite extract during swimming on and from 15th day upto 28th day of experimentation only.

**Group VI (pretreatment followed by forced swimming with extract co-administration for 28 days):** Rats of this group were maintained similarly as in group IV animals. The only difference is that these animals were administered composite methanolic extract for the entire period of swimming (28 days) through oral route at 8.00 h daily.

All the animals of group III, IV, V and VI were accustomed to swim for 3 days ( $30\text{ min day}^{-1}$  basis) prior to 28 days of swimming ( $8\text{ h day}^{-1}$  including rest) as mentioned above.

After completion of 28 days swimming, all the animals one after another in serial manner were sacrificed within 5 min of post-exercise period. Cerebrum, cerebellum, cardiac muscle, skeletal muscle (gastrocnemius from both hind legs), liver and kidney were collected from each animal. All the tissues were refrigerated at  $-20^\circ\text{C}$  and within 2 h of refrigeration; the tissues were processed for biochemical assay.

**Biochemical assay of catalase (CAT):** For the evaluation of CAT activity (Beers and Sizer, 1952) the tissues from each animal were homogenized separately in 0.05 M Tris-HCl buffer solutions (pH 7.0) at the tissue concentration of  $50\text{ mg mL}^{-1}$ . These homogenized were centrifuged at  $10,000\text{ g}$  at  $4^\circ\text{C}$  for 10 min. In

spectrophotometer cuvette, 0.5 mL of H<sub>2</sub>O<sub>2</sub> and 2.5 mL of distilled water were mixed and reading of absorbance was noted at 240 nm. The above mentioned tissue homogenate supernatants were added at a volume of 40 µL and the subsequent six readings were noted at 30 sec interval for each tissue sample.

**Biochemical assay of superoxide dismutase (SOD):** Brain and muscular tissues were homogenized separately in ice-cold 100 mM Tris-cacodylate buffer to give a tissue concentration of 50 mg mL<sup>-1</sup> and centrifuged at 10,000 g for 20 min 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) and 1 mM EDTA and 10 mM HCl. In a spectrophotometer cuvette, 2 mL of buffer, 100 µL of 2 mM pyrogallol and 10 µL of supernatant were taken and the absorbance was noted in spectrophotometer at 420 nm for 3 min period. The one unit of the SOD was defined as the enzyme activity that inhibits the autooxidation of pyrogallol by 50%.

**Quantification of Thiobarbituric Acid Reactive Substances (TBARS) and Conjugated Dienes (CD):** The aforesaid tissues were homogenized separately at the tissue concentration of 50 mg mL<sup>-1</sup> in 0.1 M of ice-cold phosphate buffer (pH 7.4) and the homogenates were centrifuged at 10,000 g at 4°C for 5 min individually. Each supernatant was used for the estimation of TBARS and CD. For the measurement of TBARS, the homogenate mixture of 0.5 mL was mixed with 0.5 mL of 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). The volume of the mixture was made upto 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 min. 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 (2:1) mixture followed by centrifugation at 1000 g for 5 min. 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.

**Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) activities in liver and kidney:** We measured GOT and GPT activities in

liver and kidney for the assessment of metabolic toxicity. Tissue samples collected from the animals were homogenized separately at the tissue concentration of 50 mg mL<sup>-1</sup> in 0.1 M of ice-cold phosphate buffer (pH 7.4) and the homogenates were centrifuged at 10,000 g at 4°C for 5 min individually. The estimation of GOT and GPT activities of each supernatant was followed by standard method (Goel, 1988).

**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 Rohle, 1997). Difference were considered significant when p<0.05.

## RESULTS

**Body weight:** The final body weights between vehicle treated control and extract treated control animals showed no significant difference. Preconditioning of the animals by the composite extract treatment for 15 days prior to swimming followed by forced swimming for 28 days alongwith supplementation of composite extract of the tested plant parts for last 7 days or 14 days of the swimming period resulted a graded recovery in this parameter in contrast to the only forced swimming group. This parameter was reestablished to the matched control level after co-treatment of the said extract throughout the 28 days swimming period (Table 1).

**CAT and SOD activities:** Activities of CAT and SOD in cardiac, skeletal and brain tissues were elevated significantly in extract treated control group in comparison to the vehicle treated control group (Fig. 1 and 2). In forced swimming group, significant reduction in CAT and SOD activities were noted in the aforesaid tissue samples when these data were

Table 1: Effect of exhaustive swimming on body weight and its management by methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* in composite manner in adult rat

| Groups   | Initial body weight (g) | Final body weight (g)  |
|--|-------------------------|------------------------|
| Vehicle treated control (28 days)                            | 120.6±2.4 <sup>a</sup>  | 164.3±3.2 <sup>a</sup> |
| Extract treated control (28 days)                            | 120.3±2.5 <sup>a</sup>  | 168.8±3.4 <sup>a</sup> |
| Forced swimming (28 days)                                    | 121.1±2.4 <sup>a</sup>  | 135.9±3.3 <sup>b</sup> |
| Pretreated cum forced swimming+ last 7 days supplementation  | 120.9±2.3 <sup>a</sup>  | 141.2±3.1 <sup>b</sup> |
| Pretreated cum forced swimming+ last 14 days supplementation | 120.1±2.6 <sup>a</sup>  | 156.2±3.5 <sup>c</sup> |
| Pretreated cum forced swimming+ 28 days co-administration    | 120.8±2.5 <sup>a</sup>  | 166.4±3.2 <sup>a</sup> |

Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test and data with different superscripts (a, b, c) in specific vertical column differ from each other significantly (p<0.05)

Table 2: Effect of forceful swimming on GOT and GPT activities in liver and kidney and its protection by extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* in composite manner in rat

| Groups   | Liver                                |                                      | Kidney                                |                                       |
|--|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
|  | GOT(Unit <sup>-1</sup> mg of tissue) | GPT(Unit mg <sup>-1</sup> of tissue) | GOT (Unit mg <sup>-1</sup> of tissue) | GPT (Unit mg <sup>-1</sup> of tissue) |
| Vehicle treated control (28 days)                            | 15.6±0.4 <sup>a</sup>                | 14.3±0.2 <sup>a</sup>                | 15.0±0.4 <sup>a</sup>                 | 14.8±0.2 <sup>a</sup>                 |
| Extract treated control (28 days)                            | 15.3±0.5 <sup>a</sup>                | 13.8±0.4 <sup>a</sup>                | 14.8±0.3 <sup>a</sup>                 | 14.7±0.4 <sup>a</sup>                 |
| Forced swimming (28 days)                                    | 21.1±0.6 <sup>b</sup>                | 20.9±0.5 <sup>b</sup>                | 22.2±0.4 <sup>b</sup>                 | 19.9±0.5 <sup>b</sup>                 |
| Pretreated cum forced swimming+ last 7 days supplementation  | 19.6±0.4 <sup>b</sup>                | 19.2±0.5 <sup>b</sup>                | 21.9±0.3 <sup>b</sup>                 | 19.0±0.4 <sup>b</sup>                 |
| Pretreated cum forced swimming+ last 14 days supplementation | 17.0±0.4 <sup>c</sup>                | 16.8±0.4 <sup>c</sup>                | 18.61±0.4 <sup>c</sup>                | 16.1±0.4 <sup>c</sup>                 |
| Pretreated cum forced swimming+ 28 days co-administration    | 15.5±0.5 <sup>a</sup>                | 14.4±0.4 <sup>a</sup>                | 15.1±0.4 <sup>a</sup>                 | 14.7±0.4 <sup>a</sup>                 |

Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test and data with different superscripts (a, b, c) in specific vertical column differ from each other significantly (p<0.05)

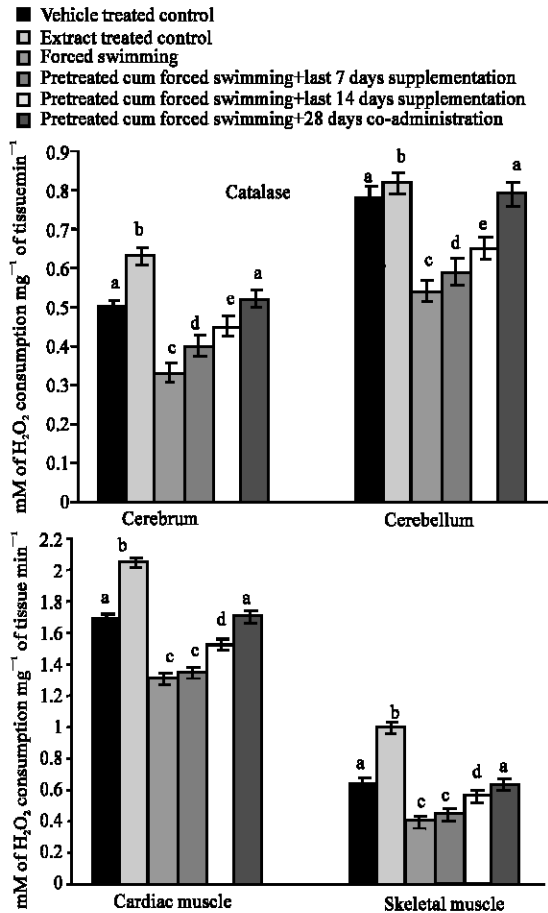


Fig. 1: Effect of pretreatment of composite methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* followed by supplementation of the same extract for 7 or 14 days or co-treatment for 28 days of forced swimming period on catalase activities in cerebrum, cerebellum, cardiac muscle and skeletal muscle in exhaustive swimming-induced oxidative stress in male rat. Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test. Bars with a, b, c, d, e superscripts for a specific tissue sample differ from each other significantly (p<0.05)

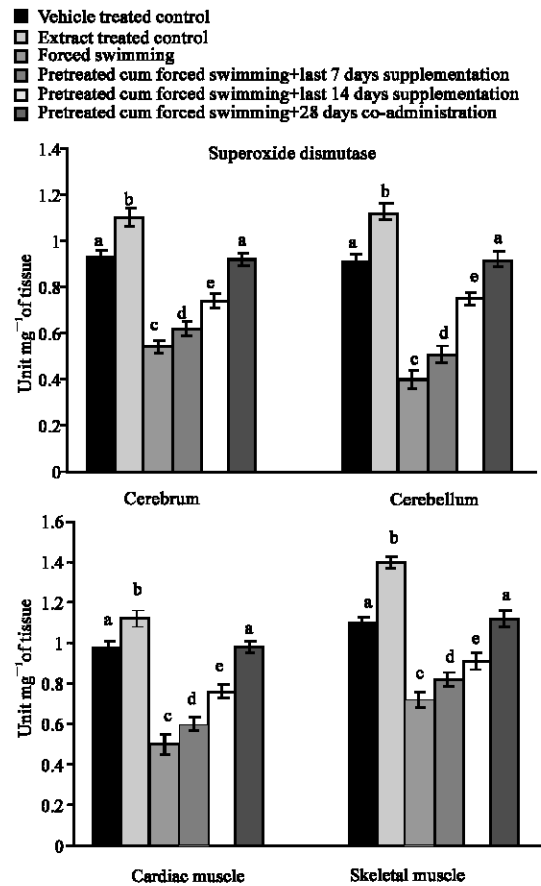


Fig. 2: Effect of exhaustive swimming on superoxide dismutase activities in cerebrum, cerebellum, cardiac muscle and skeletal muscle after pretreatment of composite methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* followed by supplementation or co-administration of same composite extract for 7 or 14 days or 28 days in male rat. Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test. Bars with a, b, c, d, e superscripts for a specific tissue sample differ from each other significantly (p<0.05)

- Vehicle treated control
- Extract treated control
- Forced swimming
- Pretreated cum forced swimming+last 7 days supplementation
- Pretreated cum forced swimming+last 14 days supplementation
- Pretreated cum forced swimming+28 days Co-administration

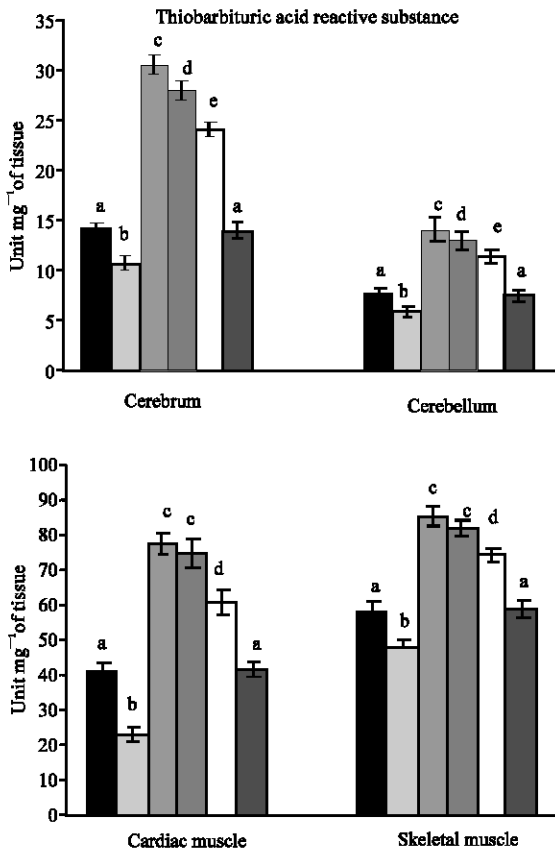


Fig. 3: Effect of pretreatment of composite methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* followed by supplementation of same composite extract for 7 or 14 days or co-treatment for 28 days of forced swimming period on TBARS levels in cerebrum, cerebellum, cardiac muscle and skeletal muscle in exhaustive swimming-induced oxidative stress in male rat. Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test. Bars with a, b, c, d, e superscripts for a specific tissue sample differ from each other significantly (p<0.05)

compared to the vehicle treated control and extract treated control groups. Activities of CAT and SOD in extract pretreated cum supplemented forced swimming groups for the last 7 or 14 days showed a slow recovery in comparison to the only swimming group (group III). Complete resettlement of CAT and SOD activities in all of the above

- Vehicle treated control
- Extract treated control
- Forced swimming
- Pretreated cum forced swimming+last 7 days supplementation
- Pretreated cum forced swimming+last 14 days supplementation
- Pretreated cum forced swimming+last 28 days co-administration

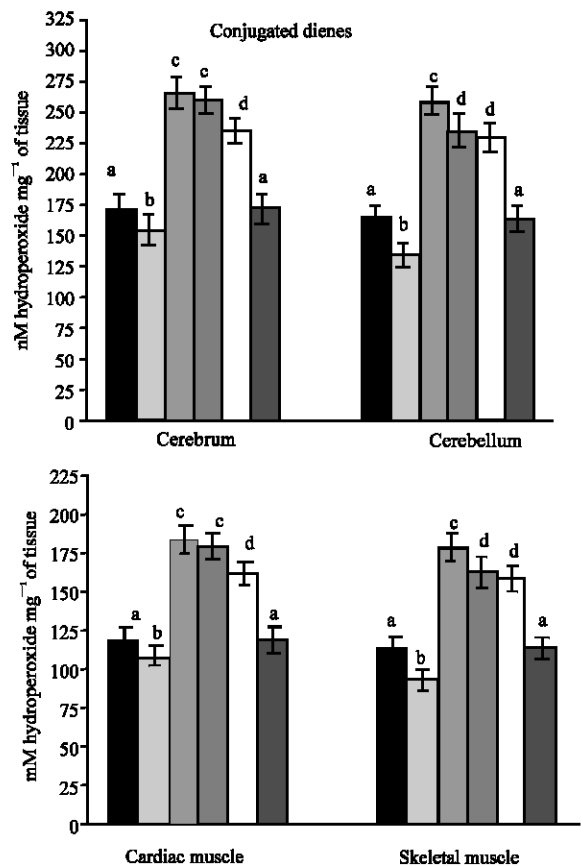


Fig. 4: Effect of exhaustive swimming on CD levels in cerebrum, cerebellum, cardiac muscle and skeletal muscle after pretreatment of composite methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinalis* followed by supplementation or co-administration of same composite extract for 7 or 14 days or 28 days in male rat. Data are expressed as mean±SEM (n = 6). ANOVA followed by multiple two tail t-test. Bars with a, b, c, d superscripts for a specific tissue sample differ from each other significantly (p<0.05)

tissue samples were noticed after co-administration of the composite mixture for the entire period (28 days) of forced swimming (Fig. 1 and 2).

**TBARS and CD levels:** The levels of TBARS and CD in above mentioned tissues were increased significantly in forced swimming group when compared to vehicle treated control and extract treated control groups. Quantity of

TBARS as well as CD in all the above mentioned tissue samples in all of the extract treated swimming groups were decreased in proportion to the duration of the treatment of the composite extract and the said parameters resettled to the control level after 28 days of extract co-administration (Fig. 3 and 4). There was a less significant protection after 14 days supplementation in comparison to the non-treated forced swimming groups of rats (Fig. 3 and 4).

**GOT and GPT activities:** There was no significant variation in liver and kidney GOT and GPT activities between vehicle treated control and extract treated control groups, whereas, significant evaluation in the said parameters was noted in forced swimming group of animals. The activities of GOT and GPT slowly regained towards the control level in extract treated swimming groups after 7 or 14 days supplementation of the composite extract. In 28 days co-administered swimming groups, the GOT and GPT values in liver and kidney were resettled to the matched control group of animals (Table 2).

## DISCUSSION

The present experiments provide a number of observations regarding the duration dependent protective effect of composite methanolic extract of leaves of *Ocimum sanctum*, roots of *Withania somnifera* and rhizomes of *Zingiber officinalis* on forced swimming-induced oxidative derangements on cardiac, skeletal (gastrocnemius) and brain tissues in adult male albino rats.

Exercise promotes health through an improvement of body's antioxidant system (Sevil *et al.*, 2000) but when the production of ROS supercedes the in-house management, an oxidative stress/threat generates that can be protected by the supplementation of antioxidant from external source (Pillai and Pillai, 2002). The supplemented antioxidant alongwith our in-house antioxidative mechanism collectively combat on the oxidative damage either by prompt scavenging of ROS from the tissues or through their low rate of generation in the target organs by improving oxidative burst (Ji, 1999) or by means of some unknown acquired qualities established in the body through exhaustive endurance training.

Body weight is one of the indicators of general toxicity assessment (Nagaraja and Jeganathan, 2003). In this experiment strenuous exercise resulted a significant diminution in final body weights in forced swimming animals as well as in extract supplemented swimming animals for last 7 days or last 14 days, respectively.

Exercise-induced oxidative stress has been overcome here by the supplementation or co-administration of the said extract to the respective groups either partially or completely. This suggests that herbal extract of these plant parts has some remedial effect on oxidative threat that may protect the body weight by preventing general tissue degeneration in connection to oxidative stress threat.

To determine whether the composite methanolic extract have any metabolic toxicity, we measured GOT and GPT activities in liver and kidney because these transaminase enzyme activities are the indicators of metabolic toxicity (Misra *et al.*, 2005). Results of liver and kidney GOT and GPT activities focused that the administered composite extract have no general metabolic toxic effect in resting and extract treated resting animals as well as in exhaustive exercising animals. Prominent elevation in GOT and GPT values in non-trained forced swimming animals indicate that severe exercise imposes metabolic toxicity in these animals and the said adverse toxic effects has been corrected in a duration dependent fashion in respect to the other groups of forced swimming animals through the said extract supplementation or co-administration.

Endogenous antioxidant status in forced swimming animals as well as in composite extract supplemented or co-treated animals were evaluated here by noting the activities of CAT and SOD as these are the important biomarkers for scavenging free radicals (Venkateswaran and Pari, 2003). Exercise-induced oxidative stress is further supported here by the study of antioxidant scavenger enzyme activities. The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radical. The destruction of superoxide radicals is catalyzed by SOD, is an important defense system against oxidative damage. From our experimental results of the aforesaid antioxidant enzyme activities in cardiac, skeletal and brain tissues in extract supplemented or co-administered forced swimming animals it may be stated that the active ingredients present in the methanolic extract of above three plant parts in composite manner possess antioxidative property in strenuous exercising animals.

Oxidative stress is characterized by ROS-induced lipid peroxidation, DNA damage and protein degradation (Summerfield and Tappel, 1983). For the assessment of oxidative stress, the most commonly used tests are the measurement of lipid peroxidation by-products such as CD and TBARS including MDA. MDA is the secondary product generated during the oxidation of polyunsaturated fatty acids. Lipid peroxidation was assessed here by means of quantification of TBARS and



CD levels. The results of elevation of TBARS and CD levels in forced swimming groups further strengthen the low antioxidant enzyme activities in cardiac, skeletal (gastrocnemius) and brain tissues because TBARS and CD are the products of lipid peroxidation. Significant elevation in TBARS and CD levels may also be due to ischaemia-reperfusion phenomenon or as a result of enhanced catecholamine release from which free radicals may also generate via autooxidation or through metal ion or superoxide-catalyzed oxidation process (Dillard *et al.*, 1978). Effective protection in strenuous swimming-induced oxidative stress was observed from the low levels of TBARS and CD in brain tissues as well as cardiac and skeletal muscular tissues that highlighted an improved endurance capacity in 28 days extract co-administered animals. This has probably been possible either from the low level of ROS production or through a rapid dissolution of ROS that has further been strengthened from the elevated activities of important antioxidant defense enzymes CAT and SOD, studied in this experiment.

The concept of Ayurvedic practitioners as well as other workers engaged in the applied field of herbal medicine that composite plant extract provides a much potent role than single plant extract treatment (Borchers *et al.*, 1999), as well as from the outcome of pilot experiments conducted in our laboratory, the present study was formulated using a composite extract of the aforesaid plant parts. The ultimate findings are that the composite extract of these plant parts results significant protection in the level of antioxidant status in cerebrum, cerebellum, cardiac muscle and skeletal muscle (gastrocnemius) after a certain period of co-administration on swimming-induced oxidative stress without causing any general and metabolic toxicity. From this point of view, it may be proposed that further research on this line is essential to find out the active ingredients present in the composite mixture and their specific role through which the therapeutic importance may be evaluated and the outcome of which can be utilized in the athletic community to manage exhaustive endurance exercise-induced oxidative threat in the near future.

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