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Protective Effect of Composite Extract of *Withania somnifera*, *Ocimum sanctum* and *Zingiber officinale* on Swimming-Induced Reproductive Endocrine Dysfunctions in Male Rat

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ABSTRACT

The present study has been designed to find out the effects of forced swimming-induced oxidative stress imposition on primary and secondary sex organs and its protection by plant extract in male Wistar strain rats. This work has been designed to find out the scientific basis of the local use of the composite extract of root of *Withania somnifera*, leaf of *Ocimum sanctum* and rhizome of *Zingiber officinale* by village Ayurvedic doctors to protect the health disorders in connection to strenuous physical exercise, and also to search out the potentiality of above mentioned plant products on swimming-induced oxidative damage. Forced intermittent swimming for 8 hours /day for 28 days resulted a significant elevation in the level of products of free radical i.e., thiobarbituric acid reactive substances and conjugated dienes along with significant diminution in the activities of catalase, superoxide dismutase and glutathione–S–transferase in testis, prostate and seminal vesicle which were protected significantly after co-administration of methanolic extract of said plant parts in composite manner. Testicular steroidogenesis was assessed in this condition by measuring plasma levels of testosterone, which was diminished significantly in swimming group and was protected significantly by the composite extract of the plants parts. The antioxidative potency of this composite extract was compared with potent and standard antioxidant i.e., vitamin-E in forced swimming state. This herbal extract has no toxic effect on metabolic organs that has denoted here by the measurement of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities in liver and kidney. The results suggest that swimming-induced oxidative stress on male sex organs may be protected by using above mentioned medicinal plants extract.

Keywords: *Swimming; Oxidative stress; Medicinal plants; Vitamin-E; Reproductive organs*

In 21st century, people are very much conscious for health promotion following non-chemotherapeutic strategy like diet modification, regular exercise etc. Regular physical exercise like swimming may be the most effective strategy to maintain or promote health status. In our country, there is a long tradition of the use of medicinal plants for health recovery [1, 2]. Moreover, Government of United States of America also established the Centre of Complementary and Alternative Medicine, and defined several ways of such treatment where herbal medicinal treatment shows a promising effect [3]. At a pilot project, we screened several locally available medicinal plants having antioxidative reputation and noted that the composite extract of the three plants selected in this work have promising antioxidant potency which is equal to established antioxidant i.e., vitamin-E.

Forced swimming, a good physical exercise model, is considered as physical stressor also [4]. During physical exercise, oxygen utilization increases 10-15 folds [5] and it is well established that reactive oxygen species (ROS) generation is a direct function of the rate of oxygen utilization [6]. Oxygen reperfusion is another process of ROS imposition due to physical exercise like swimming though there is also a controversy about oxidative stress development due to exercise that focuses on the elevation in antioxidant defense system due to regular exercise [7]. From literature searching, it has been indicated that remarkable dysfunctions are noted in male reproductive system due to intensive exercise [7, 8]. There is a plethora of information about the oxidative stress imposition in sex organs due to chronic swimming [4]. Moreover no information is available

about the herbal management of oxidative damage induced by forced swimming on male reproductive organs. From trial and error, the effective dose of the composite extract and the ratio of the extract of these three plant parts have been determined for maximal management of oxidative injury caused by forceful swimming on male reproductive organs. On that background, the present experimental design has been formulated to find out the level of endogenous antioxidative defensive derangement in male sex organs and its protection by co-administration of composite extract of above mentioned medicinal plants in comparison to potent and established antioxidant i.e., vitamin-E.

Withania somnifera (*W. somnifera*) Dunal (Ashwagandha) is widely used in Ayurvedic medicine, the traditional medical system of India. Its height is 3-4 feet and grows into a bush and is a member of the family Solanaceae. In India, its growth is maximum and at present this plant is cultivated for medicinal purpose. Therapeutic importance of the different parts of this plant has a long history and is mentioned in Charak Sanghita. It is an ingredient in many formulations prescribed for a variety of musculoskeletal condition (e.g., arthritis, rheumatism) and as a general tonic to increase energy, improve overall health and longevity and prevent disease in athletes, the elderly and during pregnancy [9]. It is also used as an antistressor and antioxidant agent [10].

Ocimum sanctum (*O. sanctum*) is considered as sacred plant in the Hindu culture and known as Tulsi or Tulasi in Hindi or Holy Basil in English. It is a tropical annual herb, up to 18 inches tall and grows into a low bush and is a member of the family Lamiaceae (Labiatae). Its therapeutic importance has been mentioned in Charak Sanghita, the ancient textbook of Ayurveda. Leaves of this plant are used in a variety of pathophysiological states like asthma, dysentery, dyspepsia, chronic fever, skin disease, helminthiasis and for ring worms [11]. It is also used as antistressor [12]. It is available throughout India.

Zingiber officinale (*Z. officinale*) (Ginger) has been used for the treatment of several diseases since Baydic age. It is used as antioxidant [13], as well as used in antimotion sickness [14]. It belongs to the family Zingiberaceae. Its common names are calamus, sweet ginger etc. and cultivated throughout India.

MATERIALS AND METHODS

Selection of Animals and Care

The study was conducted on forty healthy, adult, male albino rats of Wistar strain having a body weight of 120 ± 5 g. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed two per cage in a temperature-controlled room (22 ± 2ºC) with 12-12 h dark-light cycle (8.00-20.00 h light: 20.00-8.00 h dark) at a humidity of 50 ± 10 %. They were provided with standard food and water ad libitum. Animal care was provided according to the Guiding Principle for the Care and Use of Animals [15]. Our University Ethics Committee approved the experimental protocol.

Plant Materials

The root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* were collected from Gopali, Indian Institute of Technology, Kharagpur, Paschim Medinipur district of West Bengal in the month of May and the material was identified by taxonomist of Botany Department, Vidyasagar University, Midnapore. The voucher specimens were deposited in the Department of Botany, Vidyasagar University, and the voucher specimen numbers were HPCH No-3, 4, 5, respectively.

Preparation of Methanolic Extract of Root of W. somnifera, Leaf of O. sanctum and Rhizome of Z. officinale

The plant parts were dried in an incubator for 2 days at 40ºC, crushed in an electrical grinder and then powdered separately. 50 g powder of each plant material was extracted in 250 mL of methanol for 18 h in a soxhlet apparatus. The deep brown of *Z. officinale*, yellowish brown of *W. somnifera* and deep green of *O. sanctum* extracts in methanol were collected. The extracts were dried at reduced pressure, stored at (0-4)ºC 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 and used in the study.

Experimental Design

Forty, adult healthy, male albino rats of Wistar strain were divided equally into 5 groups on the basis of the

Table 1. Protective effect of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration of these extract in composite manner on body growth and reproductive organo-somatic indices in swimming- induced oxidative stress condition in rat, and its comparison with vitamin-E treatment. Data are expressed as Mean ± SE (n=8). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly (p <0 .05).

Group	Initial body weight (g)	Final body weight (g)	Elevation in body growth (%)	Testiculo-somatic index (g%)	Seminal vesiculo-somatic index (g%)	Prostato-somatic index (g%)
I	125.4±4.8^{a}	160.9±5.0^{a}	28.31	1.40±0.02^{a}	0.38±0.02^{a}	0.19±0.02^{a}
II	126.2±5.0^{a}	165.0±5.3^{a}	30.74	1.42±0.03^{a}	0.44±0.05^{b}	0.21±0.01^{a}
III	124.3±4.9^{a}	139.0±5.6^{b}	11.83	1.28±0.01^{b}	0.16±0.03^{c}	0.09±0.03^{b}
IV	120.9±5.2^{a}	163.8±4.8^{a}	35.48	1.43±0.02^{a}	0.37±0.02^{a}	0.14±0.01^{c}
V	122.8±5.0^{a}	164.0±4.9^{a}	33.55	1.39±0.04^{a}	0.26±0.04^{d}	0.13±0.02^{c}

Group I – Control
Group II - Control + composite extract treated
Group III - Swimming
Group IV - Pretreatment followed by swimming and composite extract co-administered
Group V - Pretreatment followed by swimming and vitamin-E co-administered

matching of body weights of the animals. The treatment schedule of each group was as follows.

Group I (Control Group). Control rats were kept in rat's cage. Rats of this group received olive oil (0.5 mL/100 g body weight/ day) for 15 days prior to experimentation followed by 28 days of experimental period through oral route at 8.00 h.

Group II (Control + Composite Extract Treated Group). Animals were subjected to forceful oral administration of methanolic extract of these plant parts at the ratio of 1: 2: 2 (*W. somnifera*: *O. sanctum*: *Z. officinale*) at the dose of 40 mg /100 g body weight / day / rat in 0.5 mL olive oil for 15 days prior to starting of experiment followed by next 28 days of experimentation with out swimming. The herbal mixture was administered at 8.00 h of each day by gavage.

Group III (Swimming Group). Rats were subjected to swimming for 8 h/day including rest. The duration of this exercise was fixed for 30 min at a stretch followed by 10 min rest as followed by previous workers [16]. This swimming was continued for 28 days without break. Olive oil was administered through gavage as in group I.

Group IV (Pretreatment Followed By Swimming, and Composite Extract Co-Administered Group). Rats were subjected to preconditioning by oral administration of methanolic extract of these plant parts for 15 days prior to the starting of swimming at the same ratio as of group II. From 16th day, animals were subjected to swimming for 8 h/ day (including rest) at the same protocol like group III for 28 days. Before 2 h of starting the swimming in each day, all the animals of this group were subjected to oral administration of the methanolic extract of these plant parts in composite way at the same dose as per preconditioning period and as of group II. Food was provided to the animals at least 1 h before swimming and the swimming was started from 10.00 h to 18.00 h including rest everyday.

Group V (Pretreatment Followed by Swimming and Vitamin-E Co-Administered Group). Rats were subjected to preconditioning by oral administration of vitamin-E (Alpha- tocopherol succinate) at the dose of 6 mg/100 g body weight/day/rat in 0.5 mL olive oil [17] for 15 days prior to starting of swimming. From 16th day, animals were subjected to swimming for 8 h/day (including rest) at the same protocol like group III for 28 days. Two hours before starting the swimming in each day, all the animals of this group were subjected to oral administration of the vitamin at the same dose as per preconditioning period. Food was provided to the animals at least 1 h before swimming. Including rest period, swimming was continued from 10.00 h to 18.00 h daily throughout the experimentation.

To maintain the same physical stress due to handling of animals and forceful ingestion of extract, all the animals of Group I, II and III were subjected to olive oil treatment by gavage throughout the experimental period at the same time in relation to pre exercise oral treatment of herbal mixture to group IV and vitamin-E to group V.

Forced Swimming Programme

The forced swimming of rats was performed in acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with water to a depth of 37 cm as per design of previous workers [18, 19]. The temperature of the water was maintained at 34 ± 1ºC. The rats were loaded with a steel washer weighing approximately 4% of their body weight attached to the tail. This arrangement forced the rat to maintain continuous rapid leg movement [20]. The fur of the rats was washed with liquid soap prior to swimming and air bubbles trapped in the fur were removed periodically to reduce buoyancy and ensure the imposed workload [21].

After completion of 28 days swimming, all the animals, one after another, were killed within 5 min of post-exercise period. Liver, kidney, testis, seminal vesicle, prostate and blood were collected from each animal. All tissues were refrigerated at –20ºC and within 2h of refrigeration, the tissues were processed for biochemical assay.

Biochemical Assay of Catalase (CAT)

Catalase activity was measured biochemically [22]. For the evaluation of CAT activity testis, seminal vesicle, prostate, liver and skeletal muscle from each animal were homogenized separately in 0.05 M Tris-hydrochloric acid (HCl) buffer solution (pH-7.0) at the

Table 2. Effect of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration in composite manner on TBARS and CD in primary and secondary male sex organs in swimming- induced oxidative stress condition in rat: A comparative study with vitamin –E pretreatment cum co-administration. Data are expressed as Mean ± SE (n=8). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c) in a specific vertical column differ from each other significantly ($p < 0.05$).

Group	TBARS (n M/mg of tissue)			CD (n M hydroperoxide/mg of tissue)		
	Testis	Seminal Vesicle	Prostate	Testis	Seminal Vesicle	Prostate
I	196.79±8.63^{a}	184.52±11.18^{a}	151.62±7.28^{a}	453.32±3.37^{a}	327.89±13.43^{a}	379.73±2.01^{a}
II	166.58±8.42^{b}	163.02±8.17^{b}	123.04±8.23^{b}	380.14±4.33^{b}	290.75±4.21^{b}	300.02±3.02^{b}
III	235.27±11.10^{c}	267.08±11.74^{c}	299.01±9.12^{c}	475.14±6.73^{c}	417.52±3.31^{c}	457.73±4.83^{c}
IV	195.50±10.98^{a}	198.87±9.11^{a}	142.36±9.04^{a}	401.51±8.84^{a}	305.14±8.23^{a}	374.18±3.30^{a}
V	189.34±4.97^{a}	199.70±10.95^{a}	140.32±5.02^{a}	424.09±10.06^{a}	357.47±3.40^{a}	374.67±7.07^{a}

Group I - Control
Group II – Control + composite extract treated
Group III - Swimming
Group IV - Pretreatment followed by swimming and composite extract co-administered
Group V - Pretreatment followed by swimming and vitamin-E co-administered
TBARS- Thiobarbituric acid reactive substances
CD- Conjugated dienes

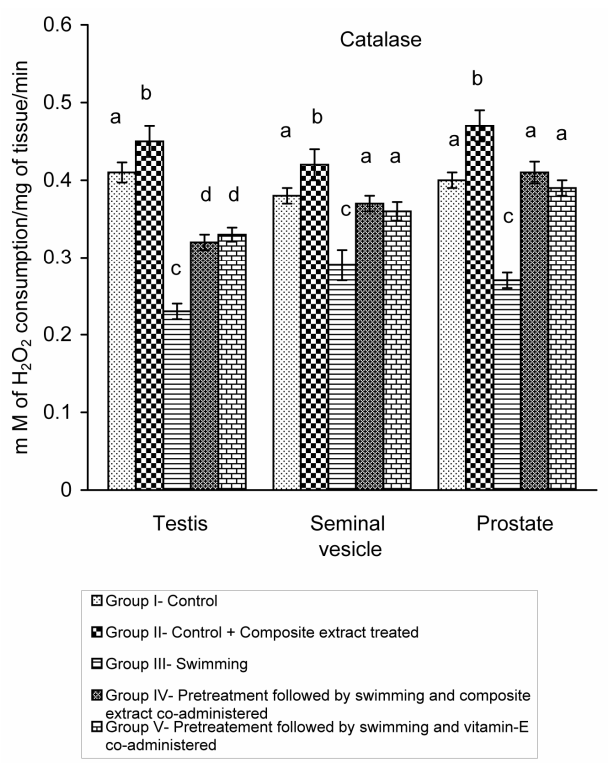

Fig 1. Effect of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration throughout the swimming period on catalase activities in primary and secondary sex organs ,and its comparison with vitamin-E treatment in same schedule in swimming–induced oxidative stress condition in male rat. Data are expressed as Mean±SE (n=8). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b,c,d) for a specific tissue differ from each other significantly ($p < 0.05$).

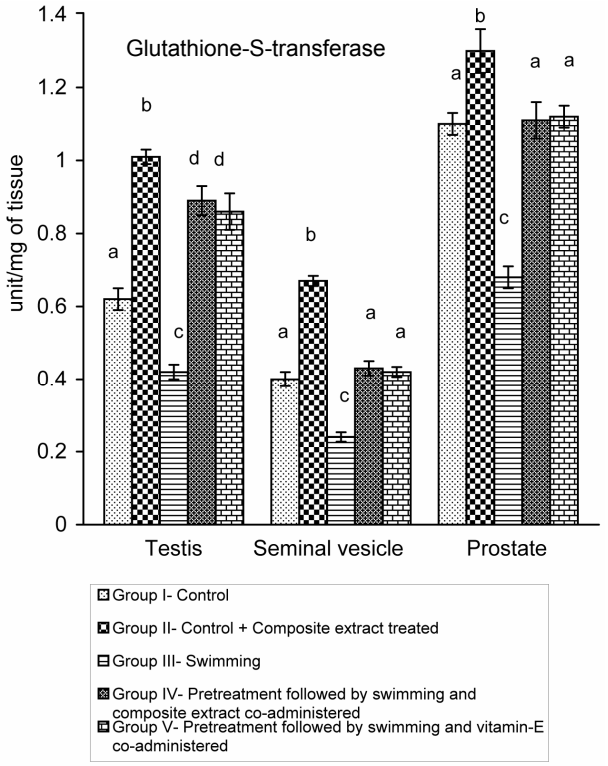

Fig 2. Protective effect of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration throughout the swimming period on glutathione-S-transferase activities in primary and secondary sex organs ,and its comparison with vitamin-E treatment in same schedule in swimming–induced oxidative stress condition in male rat. Data are expressed as Mean±SE (n=8). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b,c,d) for a specific tissue sample differ from each other significantly ($p < 0.05$).

tissue concentration of 50 mg/mL. These homogenates were centrifuged separately at 10,000 g at 4ºC for 10 min. In spectrophotometric cuvette, 0.5 mL of hydrogen peroxide (H_2O_2) and 2.5 mL of distilled water were mixed and reading of absorbance was noted at 240 nm. Tissue supernatants were added at a volume of 40 µl and the subsequent six readings were noted at 30 sec interval.

Biochemical Assay of Superoxide Dismutase (SOD)

Testis, seminal vesicle and prostate were homogenized in ice-cold 100 mM Tris-cacodylate buffer to give a tissue concentration of 50 mg/mL and centrifuged at 10,000 g for 20 min at 4 ^{0}C. The SOD activities of these supernatants were estimated by measuring the percentage inhibition of the pyragallol autooxidation by SOD [23]. The buffer was 50 mM Tris (pH-8.2) containing, 50 mM cacodylic acid (pH-8.2), 1 mM ethylene diamine tetra acetic acid (EDTA) and 10 mM hydrochloric acid (HCl). In a spectrophotometric cuvette, 2 mL of buffer, 100 µl of 2 mM pyragallol and 10 µl of supernatant were poured and the absorbance was noted in a spectrophotometer at 420 nm for 3 min. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyragallol by 50 percent.

Estimation of Glutathione-S-Transferase (GST)

Activities of GST in the tissue samples were also individually measured spectrophotometrically [24] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The assay mixture of 3 mL contained 0.1 mL of 1 mM CDNB in ethanol, 0.1 mL of 1 M reduced glutathione (GSH), 2.7 mL of 100 mM potassium phosphate buffer (pH-6.5) and 0.1 mL of supernatant of the tissue homogenate. The formation of the adduct of CDNB, S-2,4-dinitrophenylglutathione was monitored by measuring the net increase in absorbance at 340 nm against blank. Enzyme activity was calculated using the extinction coefficient of 6.9/M/cm and expressed in unit/mg of tissue.

Estimation of Lipid Peroxidation from the Levels of Thiobarbituric Acid Reactive Substances (TBARS) and Conjugated Dienes (CD)

The testis, seminal vesicle and prostate were homogenized separately at the tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH-7.4) and the homogenates were centrifuged at 10,000 g at 4ºC for 5 min individually. Each supernatant was used for the estimation of TBARS and CD. For the measurement of TBARS, 0.5 mL homogenate was mixed with 0.5 mL of normal saline (0.9 g% NaCl) and 2 mL of

thiobarbituric acid-trichloro acetic acid (TBA-TCA) mixture (0.392g of TBA in 75 mL of 0.25 N HCl with 15 g TCA, the volume of the mixture was made up to 100 mL by 95% ethanol) and boiled at 100ºC for 10 min. This mixture was then cooled at room temperature and centrifuged at 4000 g for 10 min. The whole supernatant was transferred in spectrophotometer cuvette and read at 535 nm [25].

Quantification of the CD was performed by a standard method [26]. 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 to measure the amount of hydro peroxide formed.

Assay of Plasma Testosterone

The collected blood was centrifuged and plasma fraction was separated. Plasma level of testosterone was measured following the immuno-enzymatic method by using enzyme linked immuno sorbent assay (ELISA) reader (Merck, Japan) according to the standard protocol of National Institute of Health and Family Welfare [27]. Here, we followed commercially available competitive solid phase enzyme immunoassay where horseradish peroxidase was used as enzyme-labelled antigen supplied by EQUIPAR, USA, competes with unlabelled antigen for binding with limited number of site of antibody on the micro plates (solid phase). After incubation, bound/ free enzyme labeled antigen separations were performed by simple washing. The substrate of enzyme, H_2O_2 and the chromogen (TMB-3/3'/5/5'-tetra Methyl Benzedine) were added and after the schedule time enzyme reaction was terminated by the addition of stop solution, supplied by EQUIPAR, USA. Testosterone concentration in the sample was calculated based on five standards supplied by the EQUIPAR, USA. The absorbance's of standard and sample were monitored against the blank at 450 nm. The cross-reaction of the testosterone antibody to dihydrotestosterone was 10% and intra-assay precision had a coefficient of variation of 6.2%. As all the samples were run at the same time so there was no inter-assay precision. The assay validated in respect to correctness of the data in our laboratory was 98%.

Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) Activities in Liver and Kidney

For the assessment of metabolic toxicity, we measured GOT and GPT activities in liver and kidney according to the method of Goel [28].

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 [29]. Difference were considered significant when $p < 0.05$.

RESULTS

Body Weight and Somatic Indices of Sex Organs

Body weight was increased at the end of experiment in group I, II, IV and V in respect to their initial body weight (Table 1). In group III, the percentage of elevation in body growth was dramatically less than the other groups due to swimming in non trained rats (Table 1). Testiculo-somatic, seminal vesiculo-somatic and prostato -somatic indices were decreased significantly in group III in comparison to group I and group II. After co-administration of composite extract or vitamin-E in group IV or V respectively these parameters were resettled either to or towards the control level (Table 1).

Catalase Activity

In group II, CAT activities in testis, prostate and seminal vesicle were elevated in respect to group I, IV and V (Fig 1). After swimming (Group III), the activities of this enzyme in above mentioned tissues were decreased significantly in respect to group I (Fig 1). There was a significant protection in this parameter after pretreatment with composite extract followed by its co-administration (Group IV) or vitamin-E pretreatment cum co-administration (Group V) to the animals of the swimming group.

Table 3. Protective effect of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration in composite manner on hepatic and renal GOT and GPT activities in swimming- induced oxidative stress condition in rat, and its comparison with vitamin-E pretreatment cum co-administration. Data are expressed as Mean ± SE (n=8). ANOVA followed by multiple two-tail t-test and data with same superscript (a) in specific vertical column did not different from each other significantly ($p < 0.05$).

Group	Liver		Kidney	
	GOT (unit/mg of tissue)	GPT (unit/mg of tissue)	GOT (unit/mg of tissue)	GPT (unit/mg of tissue)
I	15.8±0.5[a]	14.4±0.3[a]	15.2±0.4[a]	14.9±0.3[a]
II	15.2±0.4[a]	13.9±0.4[a]	14.9±0.3[a]	14.5±0.4[a]
III	16.1±0.6[a]	14.8±0.3[a]	15.4±0.3[a]	15.0±0.2[a]
IV	15.6±0.5[a]	14.3±0.3[a]	15.0±0.4[a]	14.6±0.3[a]
V	15.4±0.4[a]	13.9±0.2[a]	15.1±0.3[a]	14.4±0.3[a]

Group I - Control
Group II – Control + composite extract treated
Group III - Swimming
Group IV - Pretreatment followed by swimming and composite extract co-administered
Group V - Pretreatment followed by swimming and vitamin-E co-administered
GOT= Glutamate oxaloacetate transaminase
GPT= Glutamate pyruvate transaminase

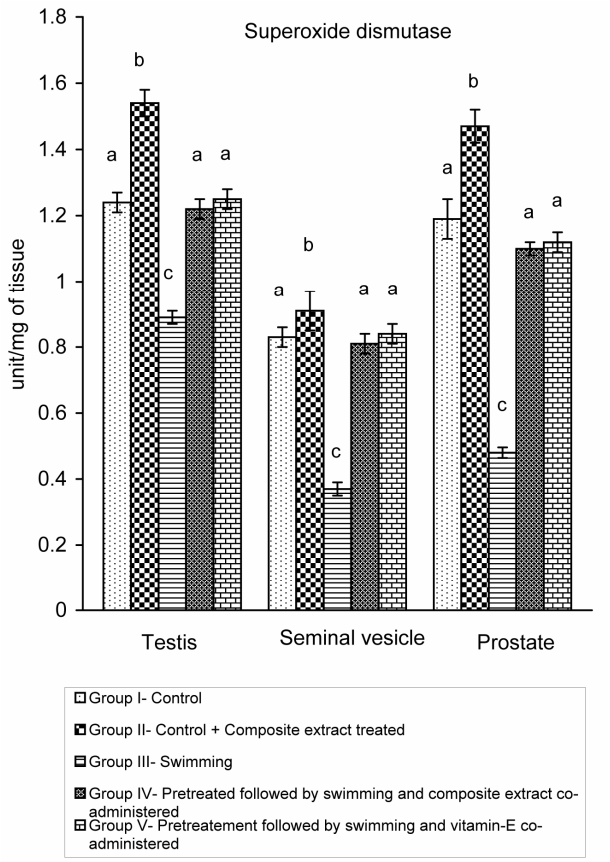

Fig 3. Corrective effects of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration throughout the swimming period on superoxide dismutase activities in primary and secondary sex organs ,and its comparison with vitamin-E treatment in same schedule in swimming–induced oxidative stress condition in male rat. Data are expressed as Mean±SE (n=8). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b,c) differ from each other significantly ($p < 0.05$).

Activities of GST and SOD

Administration of herbal mixtures to non-swimming animals (Group II) resulted a significant elevation in the activities of GST and SOD in testicular, seminal vesicular and prostatic tissues in respect to the animals of group I, IV and V (Fig 2 and Fig 3). The activities of both the above enzymes were decreased significantly in aforesaid tissues in group III when compared to group I (Fig 2 and Fig 3). Pretreatment followed by co-administration of composite extract to the animals of group IV resulted a significant correction of the above enzyme activities in respect to group III and the values were resettled to the control level (Fig 2 and Fig 3). The protective effect of vitamin-E pretreatment followed by co-administration on SOD and GST activities in above tissues showed an insignificant variation between group IV and V.

Quantification of TBARS and CD

Quantity of TBARS and CD both were decreased in testis, seminal vesicle and prostate of group II when compared to group I, IV and V (Table 2). In non trained rats, 28 days swimming resulted a significant elevation in the values of both the parameters in all of the above mentioned tissues when we compared the data in respect to group I (Table 2). In group IV and V, the values of both these parameters in all of the above mentioned tissue samples were significantly decreased than the group III (Table 2) and the values were resettled to the control level (Table 2).

Activities of GOT and GPT

In liver and kidney, the activities of GOT and GPT were not altered significantly among the groups (Table 3).

Plasma Level of Testosterone

After 28 days of swimming (Group III), there was a significant diminution in the plasma level of testosterone in respect to control (Group I). The plasma testosterone level was resettled to the control value in group IV and group V animals after pretreatment cum co-administration of herbal mixture or vitamin-E respectively (Fig 4).

DISCUSSION

Despite the paradox that exercise results in beneficial effects for promotion of good health and prevention of various diseases, there are several reports that strenuous exercise causes oxidative stress imposition in several vital organs by derangement of endogenous antioxidant regulators [30].

Swimming-induced oxidative stress in testis and male secondary sex organs has been established here by noting the low activities of SOD, CAT, GST- important antioxidant enzymes, which is consistent with the observation of others [31]. The decrease in antioxidant enzyme activities due to swimming might be due to their use against the free radicals destruction and or their inhibition by free radical species [32]. It is well established that SOD activity is inhibited by hydrogen peroxide that reduced Cu^{+2} to Cu^{+1} in SOD [23]. The reduced Cu^{+1} can act as promoter of hydroxyl by Haber-Weis reaction [23]. Low-antioxidant enzyme activities further facilitate the increased susceptibility to lipid peroxidation [5]. The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radicals [33]. Reduction of hydrogen peroxide and hydro peroxides to non-toxic products are catalyzed by GST and peroxidase. Another possibility for the low levels in the activities of above mentioned antioxidant enzymes in sex organs may be due to low level of testosterone as testosterone promotes the synthesis of antioxidant enzymes in sex organs [34]. This low plasma level of testosterone due to swimming has been noted here.

Elevations in the levels of products of free radicals like TBARS and CD in sex organs in swimming group again support the low antioxidant enzyme activity that elevate the lipid peroxidation while TBARS and CD are the products of lipid peroxidation. Another possibility for such elevation in TBARS and CD may be due to ischemia- reperfusion phenomenon [35, 36] or due to high rate of catecholamine secretion that generate free radicals either through auto oxidation or through metal ion or superoxide-catalyzed oxidation [37].

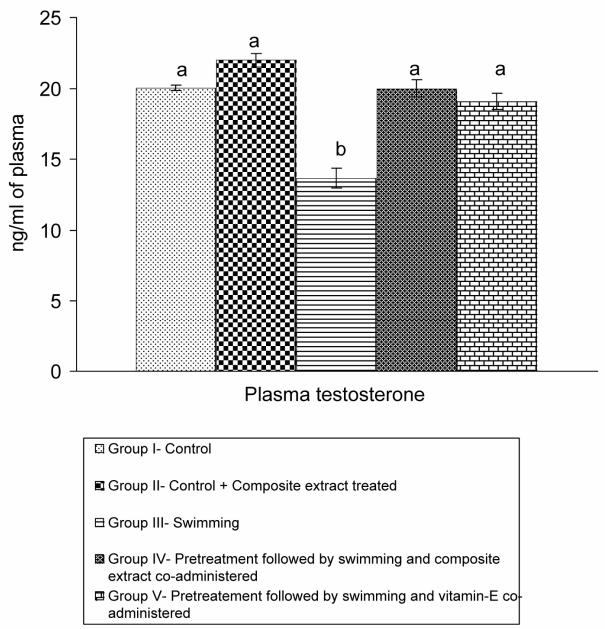

Fig 4. Corrective effects of pretreatment of methanolic extract of root of *W. somnifera*, leaf of *O. sanctum* and rhizome of *Z. officinale* followed by co-administration throughout the swimming period on plasma testosterone level in primary and secondary sex organs, and its comparison with vitamin-E treatment in same schedule in swimming–induced oxidative stress condition in male rat. Data are expressed as Mean±SE (n=8). ANOVA followed by multiple two tail t-test. Bars with different superscripts (a,b) differ from each other significantly ($p < 0.05$).

In this experiment, the composite extract of above mentioned plants has been used in relation to the traditional use of herbal mixture by our village Ayurvedic doctors as well as by other workers who reported that composite plant extract give a better result than single plant extract treatment [1, 38], and by the outcome of pilot experiments conducted in our laboratory. The composite extract of these plants results significant elevation in the level of antioxidant status, and its potency is equivalent to 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 [39] as well as gonadal steroidogenesis [40]. Our results also support the above view that antioxidant status is elevated in vitamin-E co-administered swimming group.

From literature review it has been indicated that *W. somnifera* and *O. sanctum* have some spermicidal effect in vitro [41]. Moreover, *O. sanctum* also inhibits sexual behavior [42]. These discrepancies may be explained from the angle of dose, solvent used for extract preparation and design of the experiment. On the other hand there is also report that *Z. officinale* has testicular stimulating effects [43] which is consistent with our results.

The composite extract pre-exposed cum co-administered group shows a significant protection of the sex organs against swimming –induced oxidative stress by diminution in the level of products of free radicals and elevation in the activities of antioxidant enzymes along with level of plasma testosterone .The actual mechanism for such protection would not be explained clearly but following hypothesis may be proposed. The active ingredient(s) present in this extract may destroy the free radical generation or elevate the level of antioxidant enzymes along with testosterone biosynthesis. Diminution in sex-organ somatic indices after exercise followed by recovery in extract treatment or vitamin-E pretreatment cum co-administered group suggest the effects of this extract on gonadotrophin and testicular steroid synthesis as testicular growth is indicator of gonadotrophin [44] and the growth of accessory sex organs is indicator of plasma testosterone [45]. As the composite extract has no general and metabolic toxic effect reflected here from insignificant variation in body growth and activities of liver and kidney GOT and GPT, so it may be proposed that the results of this work may be disseminated to the sports community for beneficial outcome from physical exercise-induced oxidative stress imposition that significantly interfere the physical performance and endurance.

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