IJPSR (2020), Volume 11, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 May 2019; received in revised form, 26 August 2019; accepted, 03 September 2019; published 01 March 2020

EXERCISE-INDUCED OXIDATIVE STRESS MANAGEMENT BY METHANOLIC EXTRACT OF *ZINGIBER OFFICINALIS* ON SOME VITAL TISSUES IN MALE RAT

Debanka Sekhar Misra [1, 3], Debidas Ghosh [3] and Rajkumar Maiti [* 2, 3]

Department of Physiology [1], Ramananda College, Bishnupur, Bankura - 722122, West Bengal, India.
Department of Physiology [2], Bankura Christian College, Bankura - 722101, West Bengal, India.
Department of Bio-Medical Laboratory Science and Management [3], Vidyasagar University, Midnapore - 721102, West Bengal, India.

Keywords:

Swimming,
Zingiber officinalis,
Antioxidant enzymes,
Lipid peroxidation

Correspondence to Author:
Dr. Rajkumar Maiti

Assistant Professor,
Department of Physiology,
Bankura Christian College, Bankura - 722101, West Bengal, India.

E-mail: rajkumarbcc1976@gmail.com

ABSTRACT: Background: *Zingiber officinalis* known as ginger, under the family Zingiberaceae, is a popular food spice and occupies an important place in Ayurvedic and traditional medicine. Its rhizome is used as antioxidative, carminative and for chronic diseases. **Aims:** Study indicated the curative effects of methanolic extract of ginger on severe exercise-induced oxidative damage in vital organs. **Materials and Methods:** Oxidative stress was established in male rat by forced swimming in an acrylic plastic pool filled with water. The total duration of 8 h per day including rest was allowed for both the treated and untreated forced swimming group of animals. Oxidative stress has been observed by monitoring of SOD, CAT, GST and Px activities along with quantification of TBARS and CD levels in liver, cerebrum, testis, and plasma. Whether the applied dose has toxicity if any, general body weight parameter was assessed. **Results:** Results indicate that SOD, CAT, GST and Px activities were decreased along with increase in levels of TBARS and CD in aforesaid tissues significantly in forced swimming group which were corrected significantly after 15 days pre-treatment followed by 28 days co-treatment of methanolic extract at the concentration of 20 mg/0.5 ml olive oil/100 gm body weight/rat/day in forced swimming group of rats ($p<0.05$). The extract showed non-toxic effect reflected here from the study of percentage gain in body weight. **Conclusion:** This finding reveals that methanolic extract of rhizome of *Zingiber officinalis* exhibited significant protective and curative effects on vital tissues in swimming-induced oxidative stressed rats.

INTRODUCTION: Physical conditioning as well as long term intensive exercise benefits on health promotion and reduction of the risk of non-communicable diseases like cardiovascular disease, cancer, osteoporosis, diabetes mellitus and other chronic ailments along with attenuation of oxidative stress-induced conditions.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).1167-75

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).1167-75

Regular exercise is associated with induced oxidative stress through the production of reactive oxygen species (ROS) including superoxide anion ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$) and peroxyl radicals ($RO_2^{\cdot}$) in excess amount [1-3].

Swimming is an exhaustive type of exercise [4] in which increased production of free radicals take place [5], causing polyunsaturated fatty acid (PUFA) peroxidation in membranes. In *in-vitro* system, erythrocytes are very sensitive to oxidative stress due to the absence of both nuclei as well as mitochondria in their cell [6]. Oxidative stress is considered as an impaired balance between free radical production and the endogenous antioxidant

defense system which mainly leads to accumulation of oxidative damage, activation of stress-sensitive signaling pathways and development of pathologic conditions such as cardiovascular disease, insulin resistance and metabolic disturbances [7, 8]. Due to forced-swimming, oxygen flux increases to active skeletal muscles that initiate enhanced production of ROS and free radicals [9, 10]. Exercise increases the oxygen uptake up to 10 to 20 fold and enhances production of ROS and stress-induced reactive oxygen metabolites which damage biological macromolecules especially DNA, polyunsaturated fatty acids, amino acids and active proteins [9].

In exercised muscles the major sites of ROS generation have been detected in mitochondria, xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase, phospholipase A_2 dependent processes, and some immune cells including macrophages, monocytes, eosinophils and neutrophils [11, 12]. This leads to holistic damage in working muscles when long-term exhaustive exercise is continued without any precautionary measure. Due to exercise increased levels of catecholamines as well as increased release of metmyoglobin from damaged muscles take place. The interactions of metmyoglobin and methemoglobin with peroxides during exercise also have been proposed as mediators of ROS generation [13].

In Indian traditional systems, medicinal plants have been used for the protection of various diseased or stressful conditions like diabetes, hyperlipidemia, anxiety, fatigue, and depression, *etc*. and also in treatment of immunological as well as cardiovascular disorders [14-16]. From such viewpoint, rhizome of *Z. officinalis* (Ginger), under the family Zingiberaceae, is utilized as a popular food spice and occupies an important place in Ayurvedic and Graeco-Arabic system of medicine, where it is commonly used as antioxidative [17], carminative, digestive and to treat chronic rheumatism and gout [18].

In the present study, we aimed to observe the effect of forced swimming on oxidative stress-induced damages in liver, cerebrum, testis and plasma and its management by methanolic extract of aforesaid plant part for improvement of swimming capacity of the experimental rats as remedial measures of imposed stress.

MATERIALS AND METHODS:

Plant Materials: In the month of May rhizome of *Z. officinalis* was collected from Gopali medicinal plant garden, Indian Institute of Technology, Kharagpur, Paschim Medinipur district of West Bengal. The material was identified by Professor Ram Kumar Bhakat, Plant Taxonomist, Department of Botany, Vidyasagar University, and numbered the voucher specimen HPCH No. 5.

Preparation of Extract: The rhizome of *Z. officinalis* was cut into small pieces, dried in an incubator for 2 days at 40 ºC. These were crushed in an electrical grinder and then powdered carefully avoiding contamination and undue moisture. In 250 ml of methanol 50 g dry powder of plant material was extracted for 18 h in a Soxhlet apparatus at 40 ºC. The deep brown extract of *Z. officinalis* in methanol was collected. With the help of coarse sieve filter paper suspension was filtered and evaporated to dryness under reduced pressure at rotary evaporator. From 50 g powder 5 g of extract was obtained and stored at (0-4) ºC. This extract was used for next 7 days of experiment [19].

Selection of Animal and Care: Twenty four adult healthy male Wistar strain albino rats, scientific name *Rattus norvigicus* having bodyweight 120 ± 5 gm were selected for this experiment and were acclimatized to laboratory condition for two weeks prior to experimentation. Animals were caged two per cage in a temperature-controlled room (22 ± 2 ºC) with 12-12 h light-dark cycle (8.00-20.00 h light: 20.00-8.00 h dark) at a humidity of 50 ± 10%. They were provided with standard chowed food and water *ad libitum*. The Principles of Laboratory Animal Care were followed throughout the duration of experiment and instruction given by our Institutional Ethical Committee was followed regarding treatment of the experiment. The Animal Ethical Committee number was VU/IAEC/VI/13.

Forced Swimming Programme: After one hour of serving food to the animals, the forced swimming groups of animals were made ready for swimming. A steel washer weighing approximately 4% of their body weight was rapped to the tail of the forced swimming rats so that the animals may perform rapid leg movement uninterruptedly [20]. The fur of the rats was washed with liquid soap before swimming, and air bubbles trapped in the fur were

removed periodically to reduce buoyancy and ensure the imposed workload.

The forced swimming of rat was performed in an acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with water (34 ± 1 ºC) to a depth of 37 cm [21, 22]. In each day a total duration of 8 h including rest was allowed for both the treated and untreated forced swimming group of animals. 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 [22]. Initially, the first two days of forced swimming was restricted for one hour so that the animals may be accustomed to the swimming environment. During the prolonged period of swimming, the water temperature was adjusted properly. The stool of the animals was cleaned from water by netting in time and freshwater was used when the animals took rest in between the two forced swimming sessions.

Experimental Design: Twenty four adult healthy male Wistar strain albino rats were divided into four equal groups. All the animals except the untreated control group were forcefully fed either olive oil or methanolic extract according to the design of the experiment to the respective group at 8.00 h through oral ingestion by gavage at fasting state. One hour thereafter food was given to the animals *ad libitum* through the experimental period.

Group I: (Untreated Control): Rats of this group were provided only food and water *ad libitum* for 15 days, followed by 28 days throughout the experimental period.

Group II: (Vehicle Treated Control): All the rats in this group were forcefully provided 0.5 ml olive oil/100 g body weight/rat/day for 15 days as preconditioning followed by next 28 days as vehicle treated to match with other experimental groups.

Group III: (Forced Swimming): Another six rats were fed 0.5 ml olive oil/100 g body weight/rat/day through oral gavage for 15 days preconditioning period followed by 28 days duration of forced swimming period.

Group IV: (Forced swimming + *Z. officinalis* Treated): Rest six mature male rats were provided methanol extract of rhizome of *Z. officinalis* at the concentration of 20 mg/0.5 ml olive oil/100 g body weight/rat/day for 15 days as preconditioning state and then for 28 days of forced swimming period at the said dose.

All the animals were provided with food and water *ad libitum* at 9 AM on a regular basis. After one hour of food delivery, at 10 AM forced swimming was performed of all the animals from group III and group IV in separate swimming tubs for 8 h which was designed in the pattern of 30 min forced swimming followed by 10 min rest. All the animals under forced swimming were accustomed to the swimming program for first two days and for which the swimming duration was considered for one hour on those days only.

After completion of the 15 days preconditioning period followed by 28 days of swimming, the rats were killed serially by decapitation within 5 min of completion of the exercise. Light ether anesthetized rats were sacrificed and their blood samples were collected from dorsal aorta using sterilized and heparinized syringe. By centrifugation at 3000g the plasma samples were separated and then frozen at -20 ºC until all the samples have been collected for biochemical study. Body weight and organ weights (liver, testis, and cerebrum) were noted from all the animals, and the samples were refrigerated at -20 ºC.

Biochemical Analysis: The biochemical assay of catalase (CAT) [23], superoxide dismutase (SOD) [24], glutathione-S-transferase (GST) [25], peroxidase (Px) [26] activities along with quantification of thiobarbituric acid reactive substances (TBARS) [27] and conjugated dienes (CD) [28] in the above-mentioned tissues and plasma were measured by standard protocol.

Statistical Analysis: For statistical analysis of the collected data Analysis of Variance (ANOVA) followed by a multiple two-tail t-tests with Bonferroni modification was used [29]. The difference was considered significant when $p<0.05$.

RESULTS:

Body Weight: The final body weights of all groups of animals were increased after 15 days of preconditioning followed by 28 days of experimental schedule in respect to body weight at the initial day of the experiment.

No significant variation in body weights was noted between untreated control and vehicle-treated control animals. In case of forced swimming animals (without any extract treatment) the percentage of elevation in body weight was significantly less than all the other groups due to strenuous swimming in non-trained rats **Table 1**. Whereas, preconditioning followed by treatment of methanolic extract of rhizome of *Z. officinalis* at the dose of 20 mg/0.5 ml/100g body weight/rat/day to the forced swimming animals revealed a significant recovery towards vehicle (olive oil) treated control group as well as untreated control group in the above-mentioned parameter **Table 1**.

TABLE 1: EFFECT OF 15 DAYS PRE-TREATMENT OF RHIZOME OF *Z. OFFICINALIS* IN METHANOL FOLLOWED BY 28 DAYS FORCED SWIMMING ALONG WITH TREATMENT OF ABOVE EXTRACT ON BODY GROWTH IN MALE RAT

Group	Initial body weight (g)	Final body weight (g)	Weight gain (g%)
Untreated control	122.1 ± 4.8[a]	167.3 ± 4.8[a]	37.0
Vehicle treated control (olive oil)	121.1 ± 4.7[a]	165.2 ± 5.1[a]	36.4
Forced swimming (Untreated)	124.5 ± 5.0[a]	130.8 ± 4.9[b]	5.1
Forced swimming + *Z. officinalis* (20 mg)	120.6 ± 4.1[a]	140.9 ± 5.9[c]	16.8

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

Quantification of Thiobarbituric Acid Reactive Substances (TBARS) and Conjugated Dienes (CD) in Liver, Cerebrum, Testis, and Plasma: The quantity of end products of lipid peroxidation *i.e.*, TBARS and CD were not significantly differed between untreated control and olive oil-treated control groups **Table 2**. On the other hand, TBARS and CD levels in liver, cerebrum, testis, and plasma were significantly elevated in forced swimming animals (group III) in comparison to both the untreated as well as vehicle-treated control groups **Table 2**. But, 15 days preconditioning followed by 28 days co-treatment of methanolic extract of rhizome of *Z. officinalis* at the dose of 20 mg/0.5 ml olive oil/100 g body weight/rat/day to the forced swimming animals, a significant diminution in TBARS and CD levels in the above mentioned tissues and plasma samples were noted in respect to untreated forced swimming group (group III) **Table 2**.

TABLE 2: PROTECTIVE EFFECT OF 15 DAYS PRE-TREATMENT OF RHIZOME OF *Z. OFFICINALIS* IN METHANOL FOLLOWED BY TREATMENT OF AFORESAID EXTRACT FOR 28 DAYS ON TBARS AND CD LEVELS IN LIVER, CEREBRUM, TESTIS AND PLASMA IN FORCED SWIMMING-INDUCED OXIDATIVE STRESS CONDITIONED MALE RAT

Group	TBARS (nM/mg of tissue)				CD (nM hydroperoxide/mg of tissue)			
	Liver	Cerebrum	Testis	Plasma	Liver	Cerebrum	Testis	Plasma
Untreated control	115.3 ±4.9[a]	14.1 ±0.21[a]	182.7 ±7.8[a]	42.1 ±0.76[a]	44.1 ±2.4[a]	201.3 ±6.8[a]	403.1 ±7.8[a]	135.6 ±4.6[a]
Vehicle treated control (olive oil)	114.1 ±5.1[a]	14.4 ±0.24[a]	180.1 ±7.7[a]	41.2 ±0.72[a]	44.9 ±3.5[a]	205.2 ±6.1[a]	408.9 ±7.7[a]	137.5 ±4.4[a]
Forced swimming (Untreated)	172.1 ±5.6[b]	31.8 ±0.28[b]	245.8 ±8.0[b]	66.1 ±0.82[b]	68.8 ±3.2[b]	270.5 ±7.9[b]	484.8 ±7.9[b]	197.1 ±5.2[b]
Forced swimming + *Z. officinalis* (20 mg)	149.3 ±5.6[c]	23.5 ±0.27[c]	218.6 ±7.1[c]	53.8 ±0.76[c]	57.6 ±2.6[c]	242.9 ±4.9[c]	450.6 ±7.2[c]	166.2 ±4.7[c]

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

Catalase (CAT) and Superoxide Dismutase (SOD) Activities in Liver, Cerebrum, Testis, and Plasma: Activities of CAT and SOD in liver, cerebrum, testis and plasma were significantly diminished in forced swimming group when the results were compared to the untreated control or vehicle-treated control group **Fig. 1** and **2**. Significant recovery was noted in the activities of above-mentioned enzymes in aforesaid tissues after a fortnights (15 days) preconditioning followed by 28 days co-treatment of methanolic extract of rhizome of *Z. officinalis* at the dose of 20 mg/0.5 ml olive oil/100 g body weight/rat/day in respect to the only forced swimming group of animals. The activities of CAT and SOD were not significantly differed when the results of these parameters were compared between group I and group II **Fig. 1** and **2**.

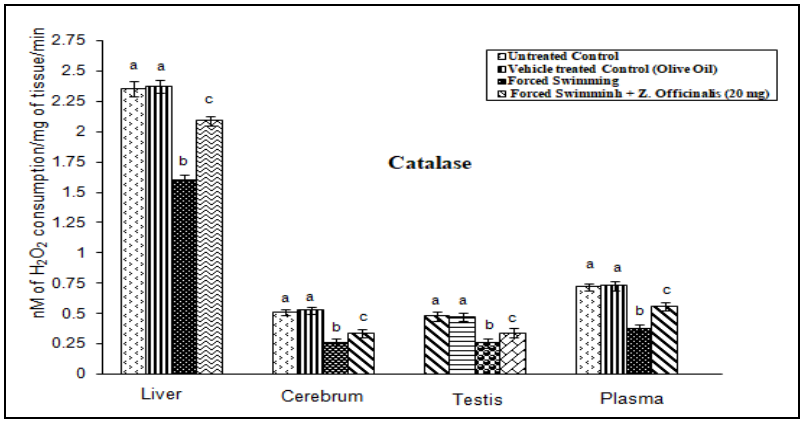

FIG. 1: EFFECT OF PRE-TREATMENT OF METHANOLIC EXTRACT OF RHIZOME OF *Z. OFFICINALIS* FOLLOWED BY TREATMENT OF SAME EXTRACT FOR 28 DAYS DURING FORCED SWIMMING PERIOD ON CATALASE ACTIVITIES IN LIVER, CEREBRUM, TESTIS, AND PLASMA IN EXHAUSTIVE SWIMMING-INDUCED OXIDATIVE STRESS CONDITIONED RAT. Data are expressed as Mean ± SEM (n=6). ANOVA followed by multiple comparisons two-tail t-test. Bars with a,b,c superscripts for specific tissue sample differ from each other significantly ($p<0.05$).

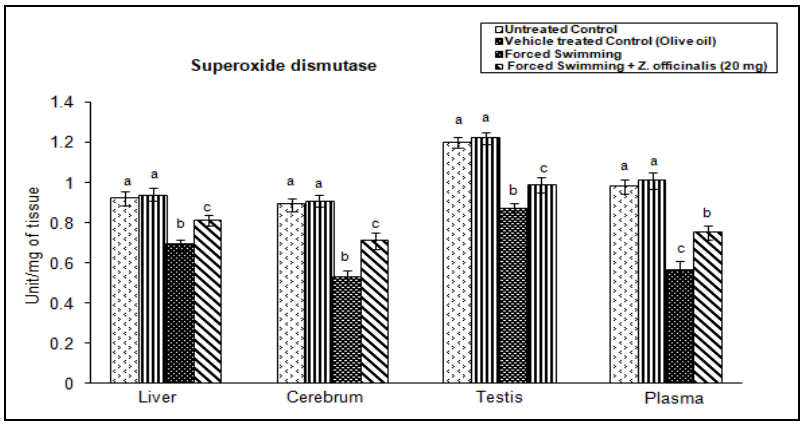

FIG. 2: EFFECT OF STRENUOUS SWIMMING ON SUPEROXIDE DISMUTASE ACTIVITIES IN LIVER, CEREBRUM, TESTIS, AND PLASMA AFTER PRE-TREATMENT OF METHANOLIC EXTRACT OF RHIZOME OF *Z. OFFICINALIS* FOLLOWED BY CO-TREATMENT OF SAME EXTRACT FOR 28 DAYS IN MALE RAT. Data are expressed as Mean ± SEM (n=6). ANOVA followed by multiple comparisons two-tail t-test. Bars with a,b,c superscripts for specific tissue sample differ from each other significantly ($p<0.05$).

Glutathione-S-transferase (GST) and Peroxidase (Px) Activities in Liver, Cerebrum, Testis, and Plasma: There was no significant difference in the activities of GST and Px in liver, cerebrum, testis and plasma samples between the untreated control and olive oil treated control groups **Fig. 3** and **4**.

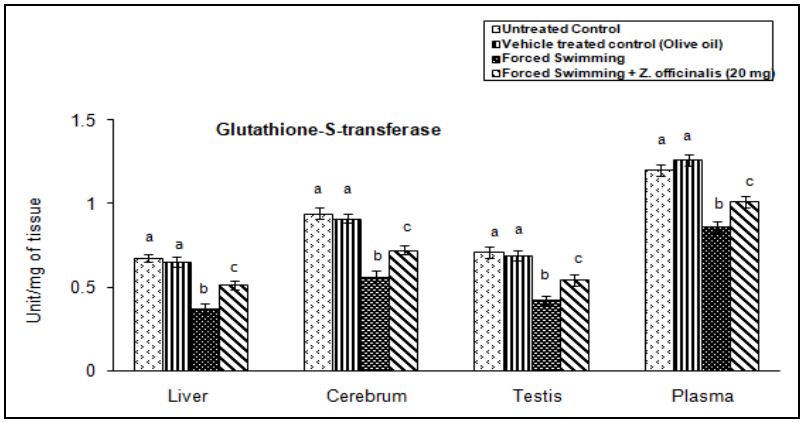

FIG. 3: EFFECT OF EXHAUSTIVE SWIMMING ON GLUTATHIONE-S-TRANSFERASE ACTIVITIES IN LIVER, CEREBRUM, TESTIS, AND PLASMA AFTER PRE-TREATMENT OF METHANOLIC EXTRACT OF *Z. OFFICINALIS* FOLLOWED BY CO-ADMINISTRATION OF SAID EXTRACT FOR 28 DAYS IN MALE RAT. Data are expressed as Mean ± SEM (n=6). ANOVA followed by multiple comparisons two-tail t-test. Bars with a,b,c superscripts for specific tissue sample differ from each other significantly ($p<0.05$).

Rather, a prominent reduction in the activities of the said enzymes was observed in forced swimming group of animals in the above-mentioned tissues and plasma samples compared to the untreated or vehicle-treated control group **Fig. 3** and **4**. After 15 days preconditioning followed by 28 days forced swimming along with treatment of the applied methanolic extract of rhizome of *Z. officinalis* at the dose of 20 mg/0.5 ml olive oil/100g body weight/rat/day to the forced swimming animals, a significant protection was observed in the GST and Px activities in the above mentioned tissues and plasma samples in respect to the extract untreated forced swimming animals (group III).

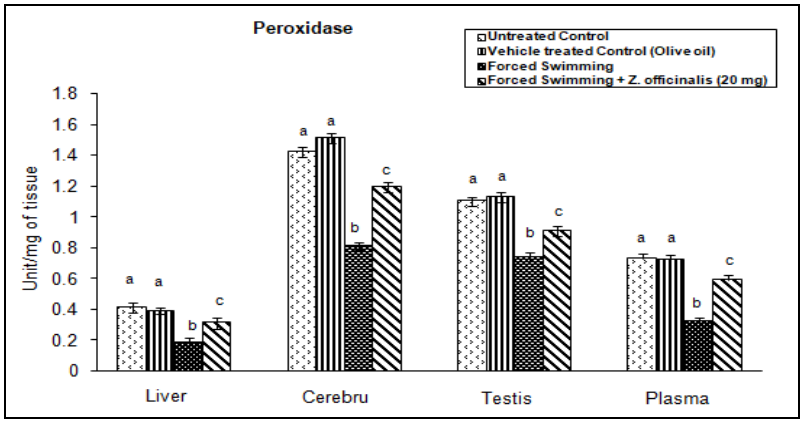

FIG. 4: EFFECT OF PRE-TREATMENT OF METHANOLIC EXTRACT OF RHIZOME OF *Z. OFFICINALIS* FOR 15 DAYS AND AFTER THIS CO-TREATMENT OF ABOVE MENTIONED EXTRACT FOR 28 DAYS ON PEROXIDASE ACTIVITIES IN LIVER, CEREBRUM, TESTIS, AND PLASMA IN FORCED SWIMMING-INDUCED OXIDATIVE STRESS CONDITIONED MALE RAT. Data are expressed as Mean ± SEM (n=6). ANOVA followed by multiple comparisons two-tail t-test. Bars with a,b,c superscripts for specific tissue sample differ from each other significantly ($p<0.05$).

DISCUSSION: At present people are very much conscious for health promotion following a non-chemotherapeutic strategy through maintenance of positive lifestyle like diet modification, regular exercise, *etc*. Regular physical exercise, avoidance of unwanted stress and balanced diet may be the most effective strategies to maintain or promote health status. It also delays aging as well as the onset of several other age-related diseases in our life. Due to regular exercise various beneficial health effects take place of which reduced risk of burning problems such as cardiovascular diseases, osteoporosis and obesity are the important ones.

Swimming is a very good physical exercise and regular swimming for a specific period helps in the overall up-gradation of physiological and physicochemical systems of our body [30]. In contrary, excessive swimming is generally considered as severe or exhaustive type of physical exercise which faces stress especially for oxygen supply, blood supply, and energy supply in a greater quantity to the active tissues. It is a well-known fact that physical training results in favourable cardiovascular changes *via* the autonomic nervous system as the prime mediator. Similarly, forced swimming is considered as physical stressor as proposed by others workers [31]. The present work is mainly designed to find out the antioxidative efficacy of methanolic extract of rhizome of *Z. officinalis* at a minimum dose for the management of forced swimming-induced oxidative damage in several vital organs in male rat.

This experiment was conducted to search out whether the methanolic extract of rhizome of *Z. officinalis* poses any role for the protection of oxidative injury induced by forced swimming. To impose oxidative stress, a long durational forced swimming program with 8 h/day including break was conducted uninterruptedly so as to impose oxidative stress. Such an experimental model has been strengthened by the painstaking research of previous work of others [20]. The experimental result reveals that in extract untreated forced swimming male albino rats, significant reduction in CAT, SOD, GST and Px activities in liver, testis, cerebrum, and plasma were noted after 28 days of forced swimming in comparison to the vehicle-treated control.

The decrease in antioxidant enzyme activity due to swimming in extract untreated animals might be due to their use against the free radicals destruction and or their inhibition by free radical species [32]. Swimming-induced oxidative stress in testis has been established here by noting the low activities of the above mentioned important antioxidant enzymes, which is consistent with the observation of another investigator [33]. This recovery pattern is more significant when the animals were pre-treated followed by co-treated with methanolic extract administration. From laboratory investigations, it has been noted that swimming or exhaustive physical exercise results oxidative stress in general [34] by oxygen reperfusion [35] or by consumption of excess oxygen [36] that results tissue damage [37].

Forced swimming is considered as physical stressor [38] that may affect the decrease in body weight due to long term exhaustive exercise in non-trained laboratory animals [39]. In this experiment, there was a significant diminution in the body weight gain of forced swimming animals but after 15 days pre-treatment followed by 28 days extract co-treatment to the group of animals resulted in a significant elevation in body weight gain in comparison to the forced swimming rat.

Exercise-related hypoxia in different vital organs is partially managed through another possible mechanism known as ischaemic reperfusion phenomenon. After the cessation of exercise, such organs undergo profound 'cardiovascular drift' through which major amount of oxygenated blood is allocated to such organs. It has been estimated that such reoxygenation results well-known burst of ROS production that occurs during ischemia-reperfusion period causing tissue damage [40]. Ischaemic reperfusion injury can occur in several tissues such as heart, brain, kidney, liver, small intestine, gastric mucosa and also in skin [41].

The extent of injury sustained by the heart after partial reversible ischaemic insult is to some extent related to damage caused by free radicals, particularly at the time of reperfusion. Available evidence supports that these free radicals in the reoxygenation phase of injury take part in the accumulation of lipid peroxidation products that have been directly identified by electron spin resonance spectroscopy [42].

Significant elevation in TBARS and CD levels in forced swimming animals may also be due to a result of enhanced catecholamine release from which free radicals may further generate via autooxidation or through metal ion or superoxide-catalyzed oxidation process. Research publications suggest that physical exercise increases catecholamine secretion in the body which releases from sympathetic nerve terminals [43]. Autooxidation of catecholamine to adrenochrome is associated with the formation of superoxide radical and at the same time oxidation of catecholamines which augments the generation of free radical products [44].

After 15 days preconditioning followed by 28 days extract co-treatment in forced swimming animals, the levels of activities of antioxidant enzymes like CAT, SOD, GST, and Px in liver, testis, cerebrum, and plasma were recovered significantly towards the control level. Through this experiment swimming induced oxidative stress protection has been confirmed further by the quantification of TBARS and CD, the end products of lipid peroxidation, in above tissues as there is an inverse relationship in the bodily amounts between antioxidant enzymes and lipid peroxidation end products [45]. The pattern of diminution in the quantities of TBARS and CD in all the above tissues was same as antioxidative enzyme activity resettlement. Oxidative stress protection by plant extract has been supported by other investigators [46] that corroborate the findings of the present experiment in which forced swimming was considered for development of acute stress in male albino rat.

CONCLUSION: From this study, it can be concluded that the methanolic extract of rhizome of *Zingiber officinalis* at the applied dose has a significant protective effect which prevents swimming-induced oxidative damage on vital organs in the male albino rat.

ACKNOWLEDGEMENT: The authors are thankful to Professor Ram Kumar Bhakat, Plant Taxonomist, Department of Botany, Vidyasagar University for authentication of the plant.

CONFLICTS OF INTEREST: The authors declare that no conflict of interest associated with this work.

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How to cite this article:
Misra DS, Ghosh D and Maiti R: Exercise-induced oxidative stress management by methanolic extract of *Zingiber officinalis* on some vital tissues in male rat. Int J Pharm Sci & Res 2020; 11(3): 1167-75. doi: 10.13040/IJPSR.0975-8232.11(3).1167-75.