

# Effects of Grape Seed Extract Supplementation and Physical Activity on Skeletal Muscle of Male Albino Rat

Towseef Ahmad Tantry<sup>1,\*</sup>, Nazir Ahmad Wani<sup>2</sup>

## ABSTRACT

**Objectives:** This study investigated the effect of grape seed extract on swim exercise and oxidative stress in acute and chronically exercised rats. **Methods:** This study was attempted on one month old male albino Wistar rats. Rats were exposed to swim exercise daily for duration of 30 min Day-1. During the experiment estimation of blood parameters, lipid profile, antioxidant enzymes and oxidative stress parameters were evaluated. **Results:** The endurance capacity was increased by 2.7-fold in the supplemented trainees as compared to the non-supplemented swim trainees which showed an increase by 1.9 fold in the end of 9 weeks compared to the first week. Plasma lactate showed a significant reduction by 65% and 77% in swim trained and supplemented trainees compared to the sedentary. The haemoglobin level showed an increase in the swim trained rats by 19% and 28% in the supplemented trainees compared to the sedentary animals. The packed cell volume increased in swim trained rats by 10% and 19% in the supplemented trainees compared to the sedentary animals. Supplemented trained rats showed a reduction in total cholesterol by 13% and 55% in swim trainees with and without supplementation compared to the sedentary rats respectively. The catalase activity exhibited a significant change in supplemented trainees compared to the sedentary by 20% in Soleus and 71% in extensor digitorum longus compared with the respective sedentary muscle and by 75% and 28% compared to the non-supplemented trainees. **Conclusion:** In conclusion, grape seed extract reduces oxidative stress by increasing antioxidant enzymes activity.

**Key words:** Antioxidant, Endurance, Malondialdehyde, Antioxidant, Proanthocyanidin.

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## INTRODUCTION

The continuous production of free radicals in the body because of increase in oxygen consumption due to continuous exercise. These free radicals are harmful for enzymatic as well as non-enzymatic defence mechanisms in the cells.<sup>1,2</sup> The oxygen demand increases during and after the exercise and production of free radicals also increases which showed increased Hb autoxidation, level of catecholamine, hyperthermia and increased lactic acid production.<sup>3</sup> In single bouts of exercise, reactive oxygen species (ROS) level increases which damages nucleic acid proteins and lipids.<sup>1,4,5</sup> However, it is observed in case of regular and non-exhaustive exercise decreases post exercise oxidative damage in body cells and blood.<sup>6</sup> Previous studies have shown that antioxidant defence (enzymatic and non-enzymatic antioxidants) and antioxidant vitamins protects cells from excessive ROS generation.<sup>7</sup> The free radical damage produced in the cells is counter balanced by antioxidants under controlled condition of physiological, nutritional parameters.<sup>3</sup> By increasing dietary components containing nutritional antioxidants, the exercise generated oxidative damage can be controlled.<sup>8</sup> The supplementation of nutrients containing antioxidants

recovers faster from tiredness and controls exercise induced oxidative damage.<sup>9</sup> Previous reports shows that exercise induced damage in rats can be prevented by supplying nutrients containing antioxidants.<sup>10,11</sup> The various pathological changes occur due to body exercise.<sup>12,13</sup> Previous studies have found that regular exercise reduces the oxidative induced diseases.<sup>14-16</sup>

Studies have predicted that grape seed extracts possess proanthocyanidins as flavonoid contents which possess various pharmaceutical activities.<sup>17</sup> Grape seed extract are known to possess more antioxidant potential than Vitamin E and C.<sup>18</sup> Various reports have been conducted on effect of grape seed extract on exercise induced oxidative stress, but there is no report on effect of grape seed extract on exercise induced oxidative stress in acute and chronically exercised rats.<sup>19,20</sup>

Grape (*Vitis vinifera* L.) are known to possess flavonoids as the main components with various biological properties.<sup>21,22</sup> Previous findings have shown grape seed extract treat many diseases due to its antioxidant and anti-inflammatory therapeutic properties.<sup>23</sup> Previous reports have also shown that

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natural grape seed rich proanthocyanidin containing extracts have antioxidant effects on digestive tract by decreasing free radical animal cells.<sup>24,25</sup> The grape seed extract proanthocyanidin is considered as one of the major flavonoid which is used as main nutritional component because of its biological properties.<sup>26,27</sup> Another previous study found that grape seed extract decreases free radical level by increasing antioxidant enzyme defence mechanism and thus prevents exercise induced oxidative damage.<sup>28,29</sup> Despite an abundant study in the field of exercise physiology and exogenous antioxidants, the understanding of the effect of natural antioxidants such as grape seed extract in sedentary and exercise trainees remains largely unclear. Therefore this study has attempted to assess the combined effect of swim exercise and grape seed extract on the skeletal muscle of adult sedentary rat.

## MATERIALS AND METHODS

### Animal care and maintenance

This study was conducted on one month old male albino wistar rats, which were obtained from Raghavendra Enterprises, Bengaluru. The two to three rats were housed per cage at 25±1°C under 12h light and 12h dark conditions. Animals were fed with commercial feed (Amrut Laboratory Animal Feed, Bengaluru) and tap water *ad-libitum*. The present study involving animals were approved by Institutional Animal Ethical Committee (IAEC) Bangalore University, Bengaluru and complied with the guidelines of the Control of the Purpose and Supervision of Experiment on Animals (CPCSEA). All animals were divided into four groups.

1. Group 1. Sedentary (SE-C)
2. Group 2. Supplemented sedentary (SE-C (S))
3. Group 3. Swim trained (SW-T)
4. Group 4. Supplemented swim trained (SW-T (S))

The supplemented groups were fed orally with 70 mg/kg of grape seed proanthocyanidin extract (GSPE). Controls were given supplements of the placebo (distilled water).

### Exercise training

Rats were exposed to swim exercise daily for duration of 30 min day-1. Rats were allowed to swim in a circular plastic tanks (height of tank 77 cm and diameter of 59 cm) containing tap water. The depth of water in the tank was 20 cm. All groups of animals were allowed to swim for 9 weeks, 5 days week-1. During the pre-training period rats swam for 5 min on the first day, 10 min on the second day, 15, 20, 25 and 30 min on consecutive days until they were swimming for 30 min day-1.

### Endurance

During the training period, weekly endurance capacity was recorded. Rats were allowed to swim in water till they got exhausted, i.e. were unable to remain on the surface of water for more than 10 sec was considered as the time of exhaustion and the time was noted. After 24 hr of last bout animals were anesthetized with diethyl ether.

### Blood sampling

After anesthetization, blood was collected weekly from tail for glucose estimation. Blood was collected for blood related parameters by cardiac puncture. Blood was used for estimation of lactic acid, total cholesterol, triglycerides and for high density lipoprotein cholesterol.

### Estimation of packed cell volume

The packed cell volume (PCV) which is the ratio of volume of packed red blood cells to the volume of whole blood was measured by centrifuging the EDTA-treated blood in a microhaematocrit tube at 4000 rpm for 3

min in a haematocrit (RM-12C Micro centrifuge). Haematocrit values are expressed in terms of %.

### Estimation of Haemoglobin

In haemoglobin estimation, blood was mixed with Hb reagent and incubated at room temperature. After incubation absorbance was measured calorimetrically at 540 nm. Haemoglobin was measured by g/dl. Haemoglobin was measured by using Hemocor-D kit.

### Estimation of lipid profile

The estimation of lipid profile includes total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides were measured by COGENT kit (CHOD-PAP method).

### Estimation of blood lactate

The estimation of blood lactate was done by Boller and Summerson method.<sup>30</sup> The deproteinized sample was treated with 20% copper sulphate and diluted with distilled water and calcium hydroxide (Ca(OH)<sub>2</sub>) powder was mixed. After vigorously mixing tubes were centrifuged at 1000 rpm and to a portion of the supernatant 4% copper sulphate was added followed by concentrated sulphuric acid. The samples were boiled and cooled. This was followed by the addition of p-hydroxydiphenyl reagent, samples were incubated at 37°C for 1 hr. Cooled to room temperature and colour developed was measured at 560 nm using lithium lactate as the standard against blank. The blood Lactate was expressed as mg/100ml.

### Estimation of glucose

The glucose measurement was carried out by glucose oxidase peroxidase method by using KRUISE kit. In this method a volume of 1 ml of working enzyme was mixed in serum and incubated for 15 min at 37°C and absorbance was recorded at 505 nm spectrophotometrically.

### Tissue sampling

Animals were decapitated under anaesthesia induced by anaesthetic ether. The samples of soleus and extensor digitorum longus muscles were dissected and excised from both hind limbs and liver, rinsed in ice-cold Tyrode's solution, trimmed of connective tissue and weighed. A 5% homogenate of soleus and extensor digitorum longus muscles was prepared in 50mM phosphate buffer (PBS, pH 7.0). Superoxide dismutase was assayed in the supernatant that was obtained after centrifugation of 5% homogenate at 600X g for 10 min at 4°C. Whereas, sulphhydryl group was assayed in supernatant obtained after centrifugation at 12,000X g for 10 min at 4°C (RV/FM superspin, plastocraft, India).

### Estimation of antioxidant enzymes

The estimation of catalase enzyme was carried out by the method described by Aebi.<sup>31</sup> Briefly, a known volume of the homogenate was mixed with absolute alcohol and incubated at 0°C. Then Triton X-100 was added. A known volume was added in 0.066M hydrogen peroxide and further mixed in phosphate buffer (PBS, pH 7.0) and absorbance was recorded at 240 nm by spectrophotometer. The enzyme activity was determined by extinction coefficient of 43.6 M<sup>-1</sup>cm<sup>-1</sup>. The one unit equals the moles of hydrogen peroxide degraded/min/mg tissue.

### Estimation of superoxide dismutase

The superoxide dismutase activity was estimated by Misra and Fridovich method.<sup>32</sup> A volume of carbonate buffer (pH 10.2), which contains 0.1 mmol ethylenediaminetetraacetic acid (EDTA) followed by 30mmol epinephrine in acetic acid were mixed in tissue extract and absorbance was recorded by spectrophotometer at 480 nm. The superoxide dismutase

enzyme activity was expressed as the quantity of enzyme that inhibits the oxidation of epinephrine by 50%, which is equivalent to one unit and is expressed in terms of units/mg protein.

### Estimation of lipid peroxidation

The Malondialdehyde estimation was carried out by the method as described by Ohkawa.<sup>33</sup> A volume 8.1% of sodium dodecyl sulphate was mixed with tissue homogenate and the reaction mixture was incubated at room temperature. Then the mixture was boiled with 20% acetic acid and 0.6% thiobarbituric acid in water bath. Cool the mixture and add butanol and pyridine in the ratio of 15:1. The mixture was centrifuged at 600 rpm for 5 min and absorbance of separated layer was measured at 532. The Malondialdehyde was expressed in terms of nmol/mg protein.

### Estimation of protein oxidation

The protein oxidation concentration of P-SH in the tissue was estimated by the method of Habeeb.<sup>34</sup> A known volume of buffer containing sodium phosphate (0.08 mol/l), ethylenediaminetetraacetic acid (90.5 mg/ml) and sodium dodecyl sulphate (2%) were mixed to each reaction tube followed by known volume of homogenate containing known quantity of protein. After vortexing, 5,5'-dithiobis-(2-nitrobenzoic acid) was added and vortexed again and incubated at room temperature for colour development. The absorbance was recorded at 412 nm by spectrophotometer. The P-SH concentration was calculated from the net absorbance and a molar absorptivity of 13,600 mol/l/cm

### Estimation of glycogen level

The soleus and extensor digitorum longus muscle and liver of the rat were dissected and washed in ice-cold Tyrode's solution. 25mg of tissue was weighed and homogenized in 2.5ml of 30% potassium hydroxide. Samples were placed in boiling water bath for 10 min. Tissue was disintegrated by shaking the test tube in between a cooled. Absorbance of alcohol was added and centrifuged for 10 min at high speed. Precipitate was dissolved in 1ml of distilled water, followed by the addition of 2.5ml of anthrone reagent, boiled for 10 min and absorbance was measured at 720 nm against the black.

### Estimation of protein

The protein in tissue was estimated by Lowry method.<sup>35</sup> The tissue homogenate was centrifuged at 6000 rpm for 5 min. Then supernatant was taken and alkaline copper sulphate was mixed and incubated at room temperature. Then Folin-phenol solution was added and incubation at room temperature and absorbance was measured at 660 nm. Protein concentration was calibrated from a standard graph with concentrations ranging from 10-50 µg/ml of bovine serum albumin (BSA).

### Statistical analysis

The data for blood and plasma parameters were analysed by ANOVA. The body weight was analysed by student's t-test. Changes in the enzyme activities and oxidative stress indices were subjected to two way analysis of variance (ANOVA). The probability value of  $p < 0.05$  were considered statistically significant.

## RESULTS

The weekly body weights were recorded over a period of 12 weeks of swimming. A progressive increase was noticed with insignificant differences in body mass between the controls, swim trained and swim-trained with GSPE supplemented animals. After 10 weeks of swimming the body mass of SE-C was 250 g, followed by SE-(S) was 220 g whereas SW-T and SW-T (S) were 165 g [Figure 1].

## Endurance

The endurance capacity increased by 2.7 fold in the supplemented trainees compared to the unsupplemented swim trainees which showed an increase by 1.9 fold by the end of 9 weeks compared to the first week [Figure 2].

## Plasma lactate

Plasma lactate showed a significant reduction by 25% and 37% in swim trained and supplemented trainees compared to the sedentary as shown in [Figure 3].

## Organ weight and tissue somatic index

The changes in organ weight and tissue somatic index for the soleus and extensor digitorum longus muscle is shown in the Table 1. The muscle mass for the soleus was  $0.27 \pm 0.002$  for SE-C,  $0.33 \pm 0.003$  for SE-C(S),  $0.39 \pm 0.003$  for SW-T and  $0.41 \pm 0.002$  for SW-T(S) respectively. The muscle mass for the extensor digitorum longus muscle was  $1.11 \pm 0.006$  for SE-C. Whereas as tissue somatic index for the soleus was  $0.11 \pm 0.002$  for SE-C and for extensor digitorum longus muscle was  $0.51 \pm 0.006$  for SE-C respectively [Table 1].

## Blood Analyses

### Blood glucose

Blood glucose level did not show any significant differences in response to training. However, there was an equal decrease by 20% in the supplemented sedentary and supplemented swim trained rats with respect to the unsupplemented sedentary and unsupplemented trainees [Figure 4].

### Haemoglobin

Haemoglobin level showed an increase in the swim trained rats by 19% and by 28% in the supplemented trainees compared to the sedentary animals [Figure 5].

### Packed cell volume

The packed cell volume (PCV) showed an increase in the swim trained rats by 10% and 19% in the supplemented trainees compared to the sedentary animals [Figure 5].

## Blood lipid profile

### Total Cholesterol

Supplemented trained rats showed a reduction in total cholesterol by 13% and 15.55% in swim trainees with and without supplementation compared to the sedentary rats respectively (Figure 6A).

### High density lipoprotein cholesterol

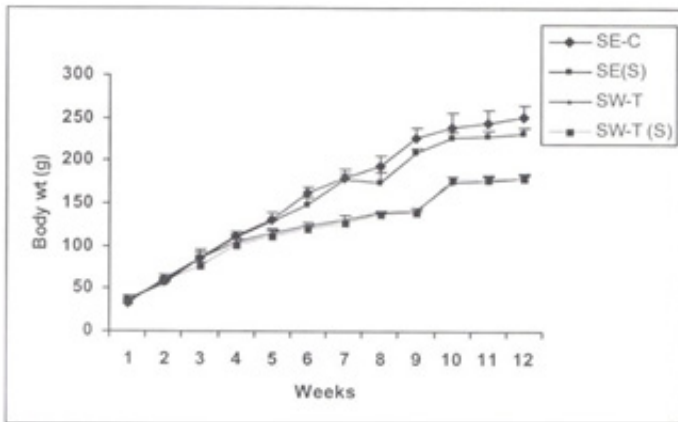
Insignificant change in high density lipoprotein cholesterol level in trained and supplemented animals were seen when compared to the sedentary (Figure 6B).

### Low density lipoprotein cholesterol

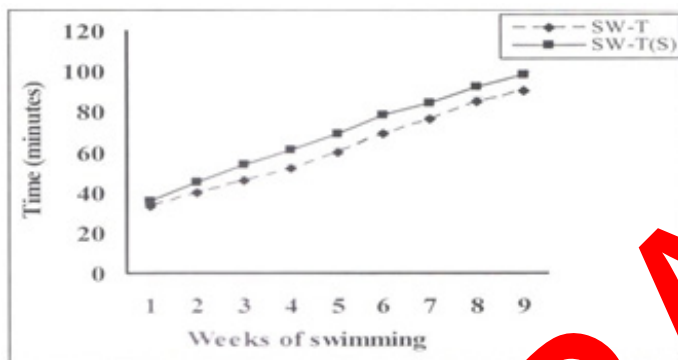
There was a significant decrease in low density lipoprotein cholesterol by 24% and 31% in exercise trained and supplemented trainees respectively (Figure 6C).

### Triglycerides

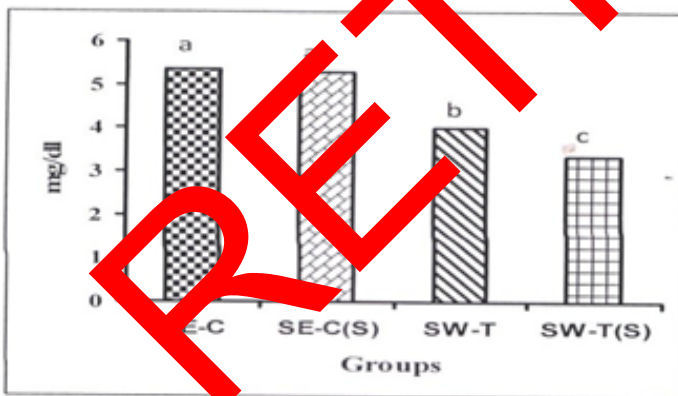
The triglycerides content showed a reduction of 18%, 14% and 22% in sedentary supplemented, swim trained and supplemented trainees when compared to the sedentary (Figure 6D).



**Figure 1:** Weekly body mass changes as a function of exercise and GSPE supplementation. Values are mean  $\pm$  SE of 5 animals. SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented swim trained. Supplement was GSPE.



**Figure 2:** Endurance capacity in the swim trainees. SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Values are mean  $\pm$  SE of 5 animals/group.

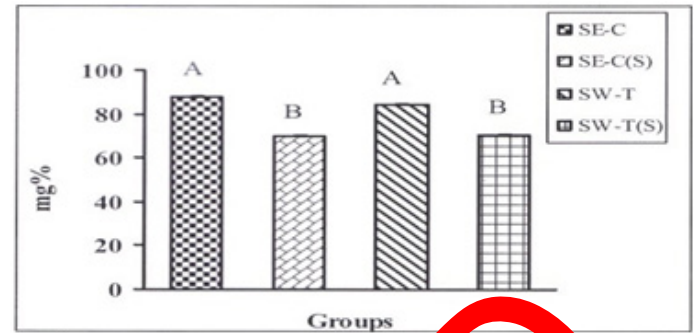


**Figure 3:** Changes in plasma lactate as a function of swim training and GSPE supplementation. SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented swim trained. Values are mean  $\pm$  SE of 5 animals/group.

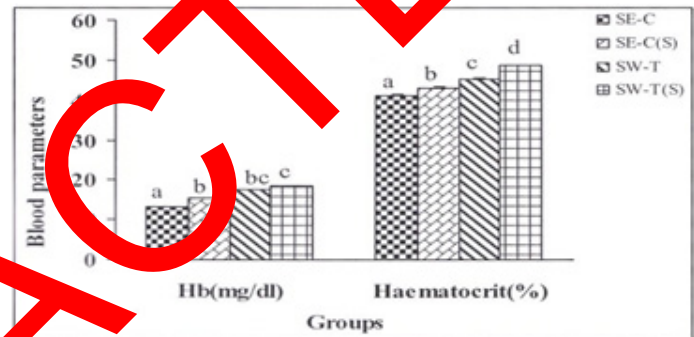
### Antioxidant enzymes

#### Catalase

The catalase activity exhibited a significant change in supplemented trainees compared to the sedentary by 20% in soleus and 71% in extensor



**Figure 4:** Variations in blood glucose as a function of swim training and GSPE supplementation SE-C, sedentary control; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Values are mean  $\pm$  SE of 4 animals/group. Significance was calculated using one-way ANOVA followed by Bonferroni's *post-hoc* test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.



**Figure 5:** Haemoglobin and haematocrit levels in swim trained and GSPE supplemented rats. SE-C, sedentary control; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Values are mean  $\pm$  SE of 4 animals/group. Significance was calculated using one-way ANOVA followed by Bonferroni's *post-hoc* test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.

digitorum longus compared to sedentary muscle and by 7.5% and 28% compared to the unsupplemented trainees (Figure 7).

### Superoxide dismutase

The superoxide dismutase activity was significant increases in swim trained rats by 55% and 100% in soleus and extensor digitorum longus muscles with respect to the sedentary. A further increase was seen in the supplemented trainees by 23% and 25% in soleus and extensor digitorum longus with respect to the unsupplemented trainees (Figure 8).

### Oxidative stress parameters

#### Lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde. Results showed a significant decrease by 26.23% and 22.58% in SOL and EDL respectively of swim trained animals in relation to the sedentary and with decrease of 27% and 19% in the SOL and EDL of supplemented trainees and with respect to the unsupplemented trainees (Figure 9).

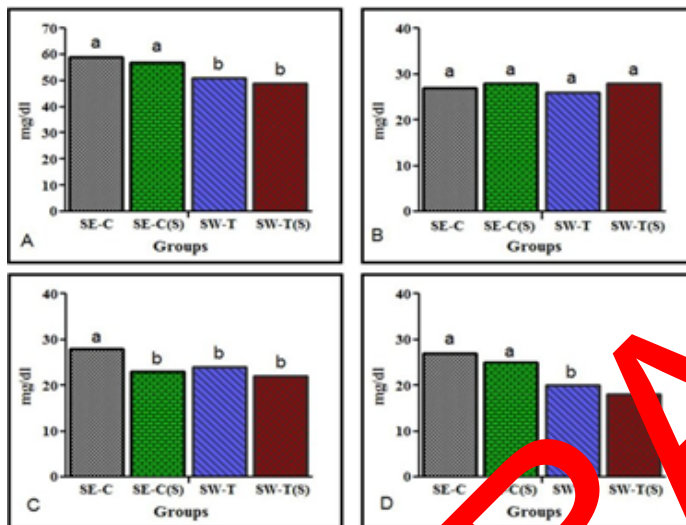
#### Protein oxidation

The P-SH levels were seen to increase in the animals supplemented with GSPE. Significant decrease in P-SH levels by 13.5% was seen in SOL

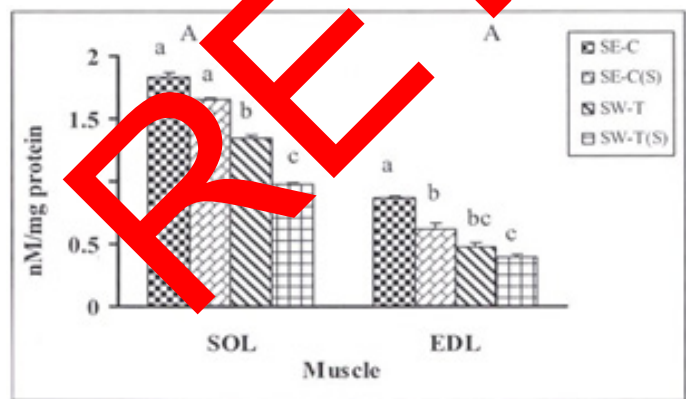
of swim trained rats and an insignificant 6% decrease in the Extensor digitorum longus muscle when compared to the sedentary. P-SH levels decreased by 41% and 43% in the Soleus (SOL) and Extensor digitorum longus (EDL) muscles of supplemented rats with respect to the unsupplemented ones (Figure 10).

### Glycogen level

Glycogen content showed a significant increase by 50% and 70% in the SOL muscle of swim trained and supplemented trainees compared to sedentary rats. There was a significant increase in the glycogen content by 15% and 30% in the EDL muscle of swim trained and supplemented trainees compared to sedentary rats. A significant increase was absorbance in the glycogen content by 15% and 30% in the liver of swim trained and supplemented trainees compared to sedentary rats (Figure 11).



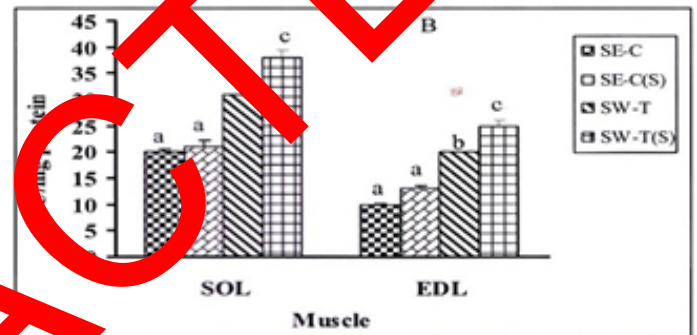
**Figure 6:** Blood lipid profile as a function of swim training and GSPE supplementation. Values are mean ± SE of four animals per group. Significance was calculated using one-factor ANOVA followed by Bonferroni's post-hoc test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.



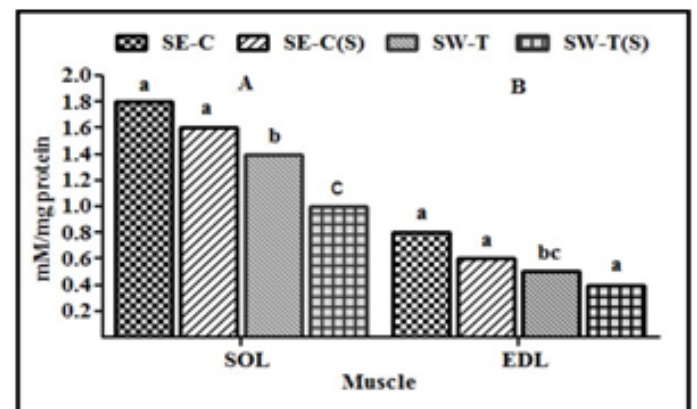
**Figure 7:** Catalase activity in the SOL and EDL of swim trained and GSPE supplemented rats. Values are mean ± SE of 4 animals in each group. GSPE, grape seed proanthocyanidin extract; SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Significance was calculated using two-way ANOVA followed by Bonferroni's post-hoc test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.

### DISCUSSION

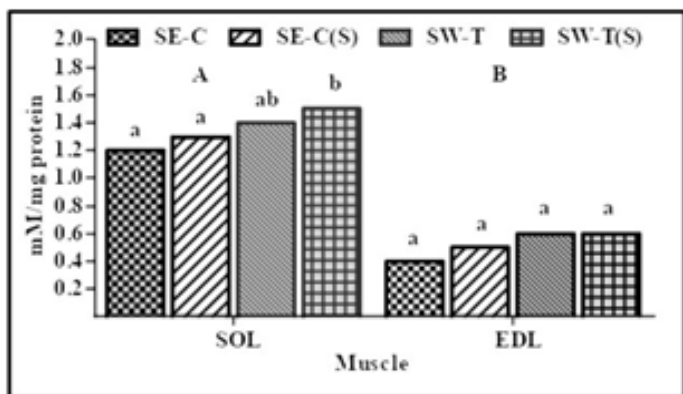
The present findings on the effect of swim training and GSPE on the SOL and EDL muscle are based on the studies conducted on normal male albino Wistar rats. Grape seed extract, one of the bioflavonoid is widely used for its medicinal properties and is commonly available to be taken for antioxidant activities.<sup>36</sup> Our results showed decreased total cholesterol in the plasma of swimmers which is compared with that reported by Richard *et al.*<sup>37</sup> on a 38% decrease compared to their sedentary controls. Our results on lowered serum cholesterol levels in the trained rats after 8 weeks of training than the untrained rats are in accordance with that reported by Edward.<sup>38</sup> Our observations on an higher increase HDL-C accompanied with reduced TG and blood glucose concentrations in the supplemented than in the unsupplemented trainees suggests an anticholesterol and antihyperglycemic effect of GSPE in these rats. The SOD, GSH-Px and catalase antioxidant enzymes are first to defend against free radicals produced during strenuous exercise. The present findings predicts that endurance swim training



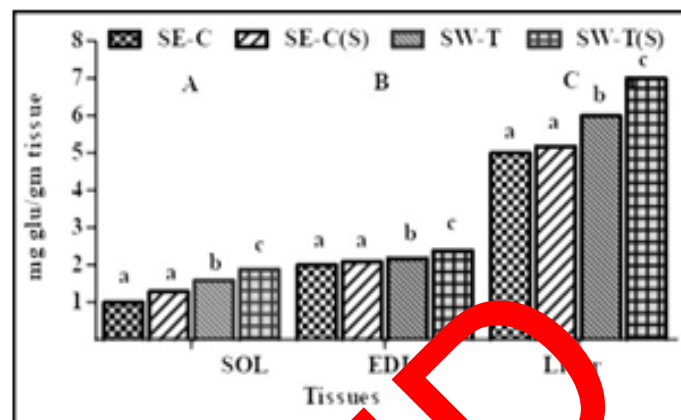
**Figure 8:** Superoxide dismutase activity in the swim trained and GSPE supplemented rats. Values are mean ± SE of 4 animals in each group. GSPE, grape seed proanthocyanidin extract; SE-C, sedentary; SE-C (S), supplemented sedentary; SW-T, swim trained; SW-T (S), supplemented and swim trained. Significance was calculated using two-way ANOVA followed by Bonferroni's post-hoc test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.



**Figure 9:** Lipid peroxidation in the SOL and EDL muscles as a function of swim training and GSPE supplementation rats. Values are mean ± SE of 4 animals in each group. GSPE, grape seed proanthocyanidin extract; SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Significance was calculated using two-way ANOVA followed by Bonferroni's post-hoc test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.



**Figure 10:** Protein sulphhydryl levels in the soleus and extensor digitorum longus muscles of swim trained and GSPE supplemented rats. Values are mean  $\pm$  SE of 4 animals in each group. GSPE, grape seed proanthocyanidin extract; SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Significance was calculated using two-way ANOVA followed by Bonferroni's *post-hoc* test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.



**Figure 11:** Glycogen content in swim trained and GSPE supplemented rats. Values are mean  $\pm$  SE of 4 animals in each group. GSPE, grape seed proanthocyanidin extract; SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Significance was calculated using two-way ANOVA followed by Bonferroni's *post-hoc* test.  $P < 0.05$  was considered significant. Values which share the same letters are insignificant and those that share different letters are significant.

**Table 1: Changes in the body mass, muscle mass and tissue somatic index (TSJ) as a function of swim exercise and grape seed proanthocyanidins extract supplementation.**

S. No	Muscle mass	SE-C	SE-C(S)	SW-T	SW-T(S)
	Final Body	214 $\pm$ 1.9	207 $\pm$ 1.8	190 $\pm$ 2.9*	187 $\pm$ 4.0*
	<b>Muscle mass</b>				
a	Soleus (SOL)	0.23 $\pm$ .002	0.33 $\pm$ .003*	0.39 $\pm$ .003*	0.43 $\pm$ .002*
b	Extensor digitorum longus (EDL)	1.11 $\pm$ .006	1.19 $\pm$ .040	1.2 $\pm$ .040	1.21 $\pm$ .050
	<b>Tissue somatic index</b>				
a	Soleus (SOL)	0.11 $\pm$ .002	0.16 $\pm$ .002*	0.2 $\pm$ .002*	0.23 $\pm$ .002*
b	Extensor digitorum longus (EDL)	0.51 $\pm$ .006	0.57 $\pm$ .006	0.63 $\pm$ .003*	0.64 $\pm$ .002*

Values are mean  $\pm$  SE of 4 animals in each group. SE-C, sedentary; SE-C(S), supplemented sedentary; SW-T, swim trained; SW-T(S), supplemented and swim trained. Significance was analysed by Student's t-test, with respect to the SE-C -  $P < 0.001$ .

enhances antioxidant enzyme activity in the SOL and EDL muscles. The present study has shown increased SOD and CAT activities in the SOL and EDL muscles of swim trained. Similarly increases in SOD and CAT activities post-exercise has been reported.<sup>39</sup> Similar results on upregulation of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase in tissues such as the brain has been reported on supplementation of antioxidants such as Vitamin C and E to swim trainees.<sup>40,41</sup>

The MDA is one of the main marker of lipid peroxidation produced by the breakdown of fatty acids. The present results on significant decreases in lipid peroxidation in the SOL and EDL muscle in the adult swim trained and supplemented trainees may be interpreted in terms of a better antioxidant capacity in the plasma of these groups.<sup>42</sup> The benefits of GSPE in lowering ROS in terms of lipid and protein oxidations in the tissues have been reported by Asha Devi *et al.*<sup>43</sup> for adult as well as middle-aged rats. The current results on protein oxidation have indicated

markedly better increases in the protein sulphhydryl content of the SOL and EDL in the GSPE supplemented animals than in the swim trained one, thereby implicating that the extent of protein oxidation in terms of these groups can be increased by GSPE and GSPE is reported to be non-toxic to rats.<sup>44</sup>

## CONCLUSION

The present study was undertaken to determine, if any, the effects of GSPE and swim exercise training on the skeletal muscle, soleus (oxidative) and extensor digitorum longus (glycolytic) in terms of the antioxidant enzymes, lipid peroxidation, protein oxidation and glycogen. Further, the blood glucose, haemoglobin and haematocrit levels and complete blood lipid were determined. Swimming was accompanied with assessments of endurance capacity and blood lactate levels. Adult male albino rats were used as our experimental animals. The results of the present study indicate that swim trainees are benefited in terms of increased endurance capacity, lowered blood lactate levels, improved HDL-C and reduced total cholesterol and triglycerides. Interestingly, GSPE supplementation when combined with swim training, the responses in the above markers was improved further.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

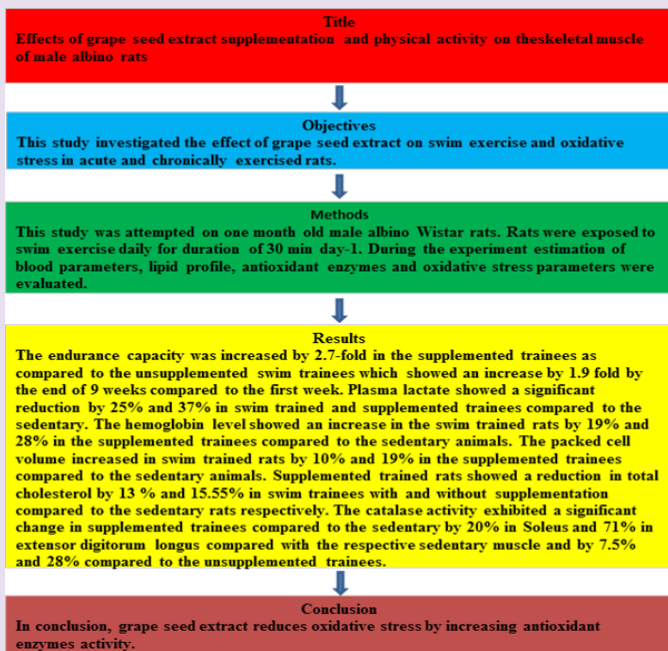
ROS: Reactive oxygen species; IAEC: Institutional Animal Ethical Committee; CPCSEA: Control of the Purpose and Supervision of Experiment on Animals; GSPE: Grape seed proanthocyanidin extract; PCV: Packed cell volume; Hb: Hemoglobin; PBS: Phosphate buffer; EDTA: Ethylenediaminetetraacetic acid; BSA: Bovine serum albumin; ANOVA: Analysis of variance; SOL: Soleus; EDL: Extensor digitorum longus.

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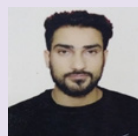
**PICTORIAL ABSTRACT**

**SUMMARY**



In summary, grape seed extract reduces oxidative stress by increasing antioxidant enzymes activity. The present finding predicts that endurance swim training enhances antioxidant enzyme activities in the SOL and EDL muscles. The present study has shown increased SOD and CAT activities in the SOL and EDL muscles of swim trained. the results of the present study indicate that swim trainees are benefited in terms of increased endurance capacity, lowered blood lactate levels, improved HDL-C, and reduced total cholesterol and triglycerides. Interestingly, GSPE supplementation when combined with swim training, the responses in the above markers was improved further.

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