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**Dr. Ajaikumar B. Kunnumakkara**  
Associate Professor

Department of Biosciences and Bioengineering  
Indian Institute of Technology Guwahati  
Guwahati, Assam-781039  
Room # 005, O-Block



**Prof. Dr. Sabu Thomas, Ph.D, DSc, FRS C, CChem.**

Professor & Director of School of Chemical Sciences  
Mahatma Gandhi University, Kerala, India

 <http://scholar.google.com/citations?user=74VyKRYAAA AJ&hl=en>



**Dr. Subhash Gupta**  
Assistant Professor

Department of Biochemistry,  
Institute of Science, Banaras Hindu University  
Varanasi, INDIA



Kolenchery, Cochin, Kerala - 682311

[www.aureabiolabs.com](http://www.aureabiolabs.com)

[info@aureabiolabs.com](mailto:info@aureabiolabs.com)

+91 - 484 305 1500



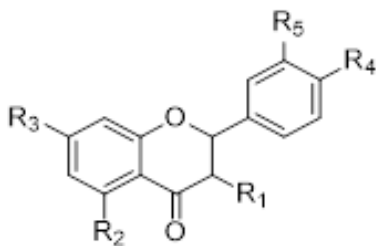
Vitamins, minerals, increased levels of nitric oxide (NO), dopamine and red blood cells (RBC) are required before and during exercise. These are important biological parameters to determine the efficiency of the exercise. Most of the sports people use supplements for body building and increasing muscle strength, stamina and endurance. However, these supplements include high amount of proteins, vitamins, minerals and very often anabolic steroids, which are mostly unsafe and illegal. Therefore, safe and efficacious sports supplements are necessary for body building and increasing muscle strength, stamina and endurance. Accumulating evidence suggest that natural products have high potential in developing sports nutrition. NO mediated vasodilation and high RBC count can increase the oxygen supply to the cells, hence increase the physical endurance. During exercise, the oxidative stress in muscle tissues will increase due to the high production of free radicals and this would cause cell and tissue damage. This oxidative damage can be determined by measuring the levels of LDH and MDA. However, it is now well established that antioxidants play a vital role in protecting tissues from excessive tissue damage during exercise by scavenging the reactive oxygen species (ROS). Therefore, consumption of various sports supplements which contain antioxidants would increase the acute strenuous exercise and chronic exercise training and it is conceivable that dietary supplementation of specific antioxidants would be beneficial. These sports supplements are the functional foods that provide health benefits for the athletes and to improve their performance in sports. This can be achieved by minimizing the impact of the factors that cause fatigue and impair the performance of skilled tasks.

Many natural products are well established antioxidants, inhibit tissue damage and increase the stamina without causing fatigue and other serious side effects and are better than the commercially available chemical supplements. Among the widely used natural products, black

ginger root, pomegranate peel and moringa leaves are known to increase the energy levels in the body and thus prove their benefits as a physical fitness supplement. The mode of actions of these three are as described below:

### **Black Ginger (*Kaempferia parviflora*) Extract**

Black ginger or Thai Ginseng (*Kaempferia parviflora*) is rich in antioxidants due to the presence of polymethoxyflavones and is used as an energy enhancer. Among the methoxy flavones, the major constituents are 5,7-dimethoxyflavone, 5,7,4'-trimethoxyflavone, 5-hydroxy-3,7,4'-trimethoxyflavone and 5-hydroxy-3,7-dimethoxyflavone. The general structure of these are shown in Fig.1.



R<sub>1</sub>, R<sub>4</sub>, R<sub>5</sub> = H, R<sub>2</sub>, R<sub>3</sub> = OMe; 5, 7-dimethoxyflavone,  
 R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> = OMe, R<sub>1</sub>, R<sub>5</sub> = H; 5,7,4'-trimethoxyflavone,  
 R<sub>1</sub>, R<sub>3</sub>, R<sub>4</sub> = OMe, R<sub>2</sub> = OH, R<sub>5</sub> = H; 5-hydroxy-3,7,4'-trimethoxyflavone and  
 R<sub>1</sub>, R<sub>3</sub> = OMe, R<sub>2</sub> = OH, R<sub>4</sub>, R<sub>5</sub> = H; 5-hydroxy-3,7-dimethoxyflavone

**Fig. 1** The chemical structure of major methoxy flavanones from the black ginger extract

Studies have demonstrated that vascular tone is controlled not only by nervous and hormonal influences, but also by local active factors produced by the endothelium by activation of the various nitric oxide synthase (NOS). Nitric oxide (NO), also known as endothelium-derived relaxing factor (EDRF) functions as a cell signaling factor in physiological and pathological processes and contributes to the control of basal and stimulated regional blood flow in humans. Therefore, increase in eNOS would help in these processes. Interestingly, *Kaempferia*

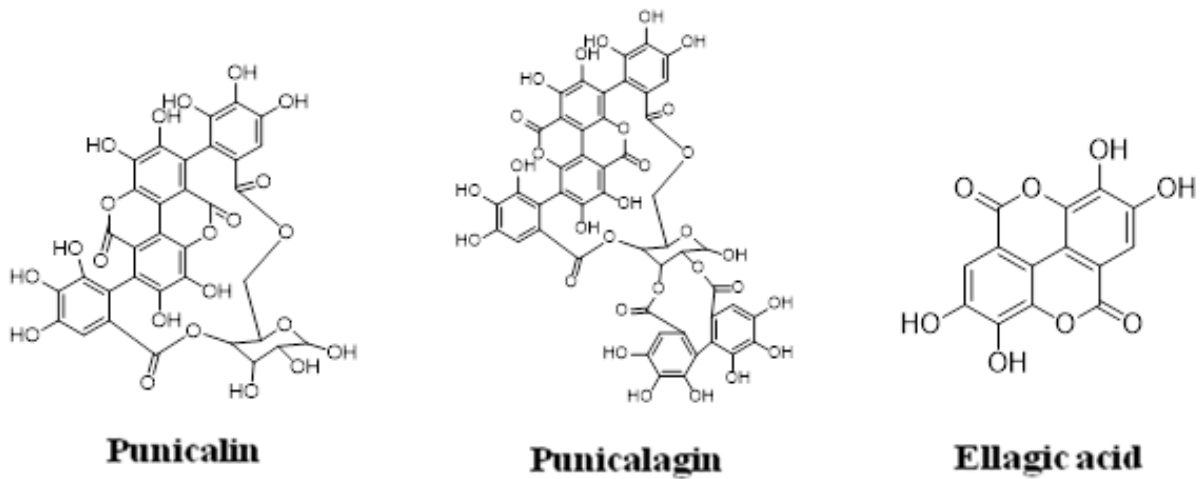


*parviflora* extract (KPE) was shown to dose-dependently increase eNOS mRNA, protein expression and nitrite concentrations in human umbilical vein endothelial cells (HUVEC).

Phosphodiesterases (PDEs) are a group of enzymes that have powerful effects on cellular signaling as they regulate the second messenger, cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP). Studies have shown that 7-methoxyflavones present in KPE significantly inhibits both PDE5 and PDE6. KPE administration also increased oxygen consumption in mice fed on a high-fat diet and was found to enhance physical fitness state in subjects without an exercise habit.

### **Pomegranate (*Punica granatum*) Peel Extract**

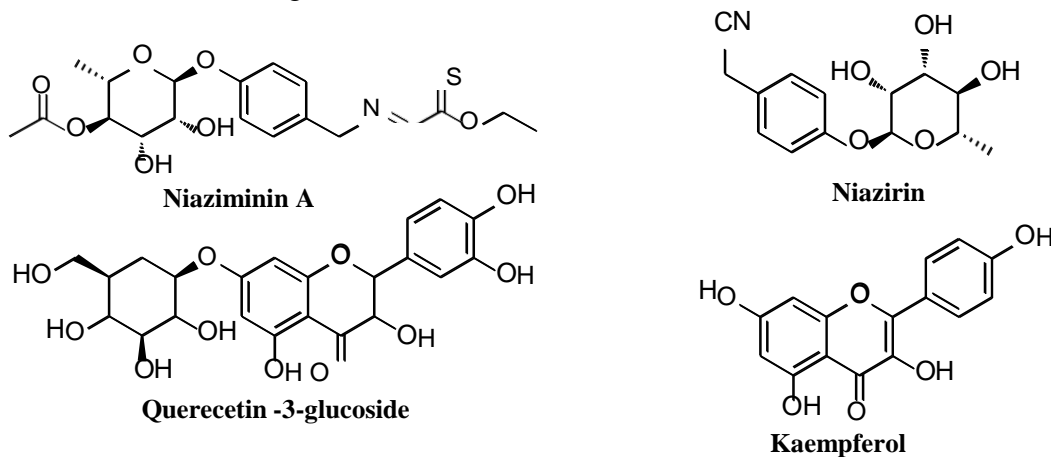
The pomegranate husk or peels are comprised almost 26–30% of the total fruit weight and constitute significantly higher amount of phenolic compounds than in the fruit pulp. The main biologically active compounds present in pomegranate peel are flavonoids (anthocyanins, catechins and other complex flavonoids), hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). The methanolic extract of pomegranate peel is highly antioxidant and anti-mutagenic and was shown to inhibit lipid peroxidation, hydroxyl radical generation and LDL oxidation. Due to its high antioxidant properties, it is also used for the rapid synthesis of silver nanoparticles (AgNPs) in ambient conditions. Chemical structures of some of the active components found in pomegranate peel are depicted below (Fig. 2).



**Fig. 2** Major chemical constituents of the pomegranate peel extract

**Moringa (*Moringa oleifera*) Leaves Extract**

*Moringa Oleifera* leaves are rich in saponins and agents like Niaziminin A, Niazirin, Quercetin-3-glucoside, Chlorogenic acid, Kaempferol. The chemical structure of these agents are shown below in Fig. 3

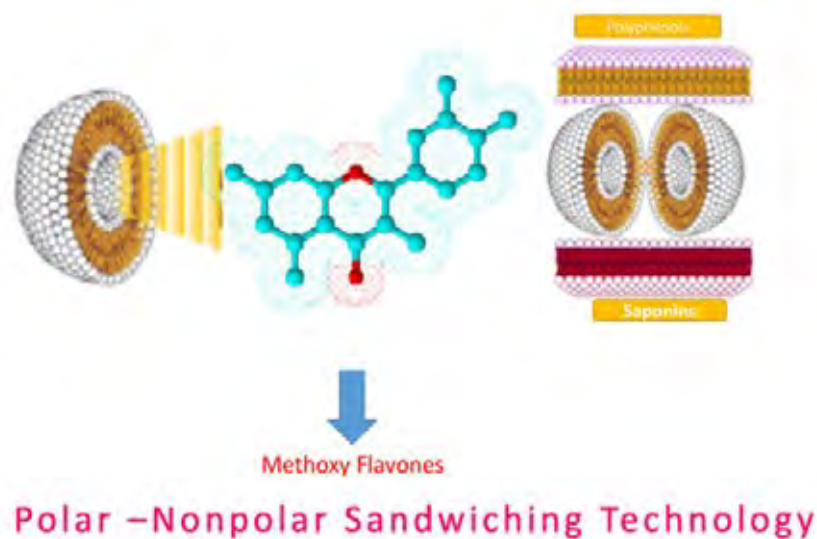


**Fig. 3** Major chemical constituents present in Moringa leaf extract

The consumption of the Moringa leaf extract was shown to increase the hemoglobin level in pregnant women and was able to retain ferritin serum level dismount up to 50%. Aqueous

extract of *M. oleifera* exhibits anxiolytic and antiepileptic properties via GABA mimetic action and can be used for the treatment of epilepsy and anxiety. The moringa leaf extract is highly antioxidant and was shown to suppress both monoamine oxidase type B (MAO-B) and PDE-5 levels in rats, hence could be substituted with conventional protein sources without any deleterious effects on blood parameters. Thus, it could be used as a source of food supplement to improve growth performance.

To reconstitute all the health benefits from these natural sources, we developed a formulation (Fitnox<sup>™</sup>) which consists of the extracts of black ginger, pomegranate peel and moringa leaves, with our very unique PNS – technology. We have previously used the PNS technology to recreate the turmeric matrix with active curcuminoids for the brand – “cureit”. Cureit has been found very effective. To investigate the efficacy of Fitnox<sup>™</sup> for physical endurance, we conducted a clinical trial in 24 healthy adults.





## **Study Design**

This was a randomized, mono centric, double-blind, placebo controlled, parallel-group, clinical trial. The participants were randomized into two groups (250 mg of Fitnox™ and 250 mg starch capsules) in a double-blinded manner at a ratio of 1:1 and received Fitnox™ as per randomization code provided at study site by an authorized person independently. The participants were followed up regularly for all concomitant dosing from the time of screening and the follow up visit was captured and recorded.

The study was continued for three weeks and the participants in both groups were given either 250 mg of sports supplement formulation or placebo (250 mg starch) in capsules once daily. Participants were allowed to consume their regular diet and they visited the clinic on day 0 (In house) which is 2 days after the screening day. Subsequent visit was on day 22 (Visit 2). Physical examination, demographics and vitals recorded on all visits and adverse events and concomitant medication were recorded. Blood samples were taken before and after exercise on the day 0 and 22. Serum chemistry, hematology, lactate levels, heart rate, time to exhaustion, 12 lead-electrocardiogram (ECG) for arrhythmia patterns, ST and RR segments (efficacy assessments), exercise test on treadmill, serum and saliva NO<sub>3</sub> and NO<sub>2</sub> levels at pre and post exercise were performed during screening and final visit.

## **Exercise Procedure**

All the participants were fasted for 10 h before the exercise session with the exception of their morning supplement. During this, heart rate and ratings of perceived exertion were monitored at rest, every 5 min during exercise and 10 min after recovery. The participants were mounted a level motorized treadmill and warmed up for 5 min at a self-selected pace. After the 5-min warm-up, treadmill speed increased slowly until the heart rate reached 80% of predicted



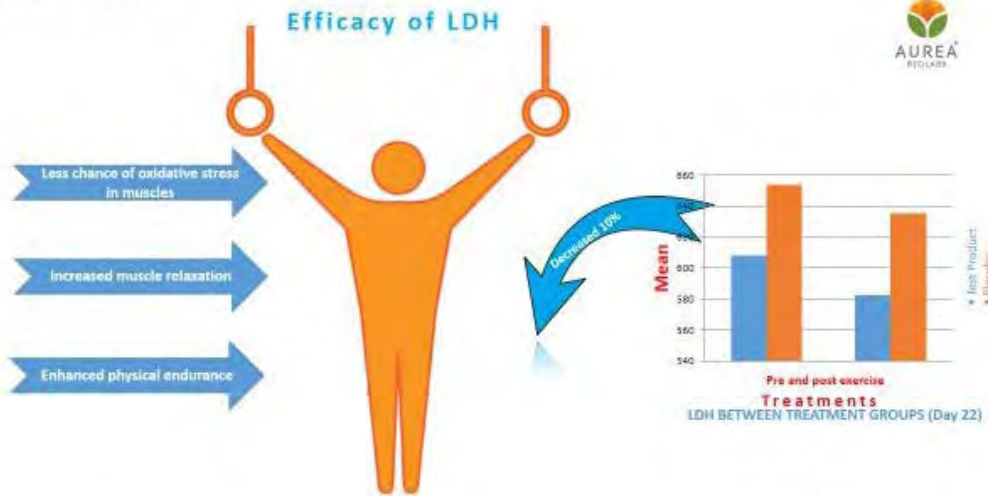


maximal heart rate and the maximum heart rate (100%) was calculated as 220 minus the age of the participant. The participants maintained this pace for 5 min, the treadmill grade was adjusted to -10% and this workload for 30 min, after that they completed a 5 min active cool-down at a self-selected pace and a 5 min seated passive recovery period.

## **Results**

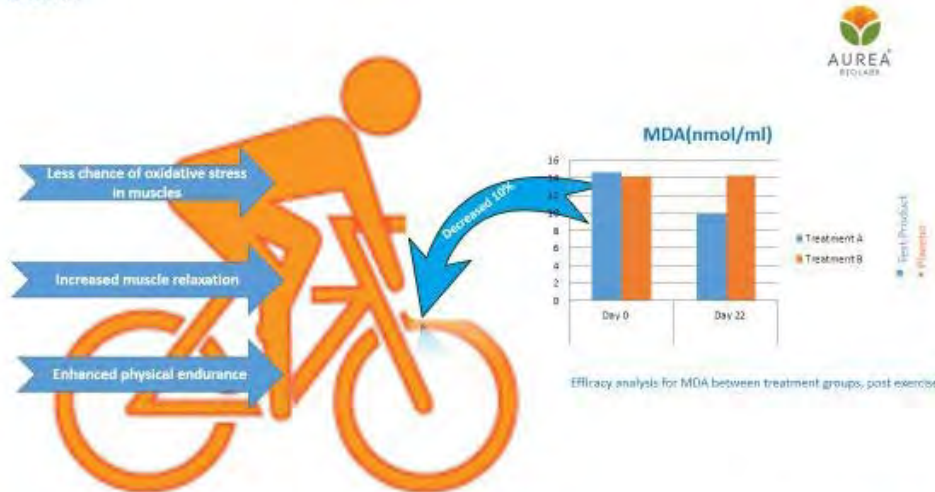
The study assessed the improvement in physical endurance, safety and tolerability of Fitnox™ with the placebo in 24 healthy adults. The primary aim of this study was to determine the LDH levels in pre and post exercise, heart rate, time to exhaustion, 12-lead electrocardiogram for arrhythmia patterns, performance test at 40% and 80% of VO<sub>2</sub> max, serum and saliva NO<sub>3</sub> and NO<sub>2</sub> levels, MDA and LDH levels for oxidative stress at pre and post exercise on day 0 and day 22. The changes in RBC Count and dopamine levels in urine were also analyzed. The secondary aim of this study was the safety of the product assessed by adverse events against placebo. The concentration of nitrate and nitrite in both plasma and saliva on day 0 and 22 in pre and post exercise were given. The plasma nitrate escalated from 6.7 μmol/L (day 0) to 9.7 μmol/L (day 22) before exercise and from 8.8 μmol/L (day 0) to 15.9 μmol/L (day 22) after exercise for the test groups. Likewise, the plasma nitrite concentration was increased from 37.5 to 73.1 (μmol/L) and from 42.5 to 80.3 (μmol/L) for pre and post exercises respectively. Concentrations of the nitrate increased from 6.5 (day 0) to 8.5 μmol/L (day 22) and 8.9 (day 0) to 10.9 μmol/L (day 22) in the saliva before and after exercise, respectively. The saliva nitrite also increased from 37.9 to 46.4 (μmol/L) and 57.1 to 64.5 (μmol/L) in pre and post exercise. The LDH and MDA levels on day 0 and day 22 are shown below.

Bio Marker Studies



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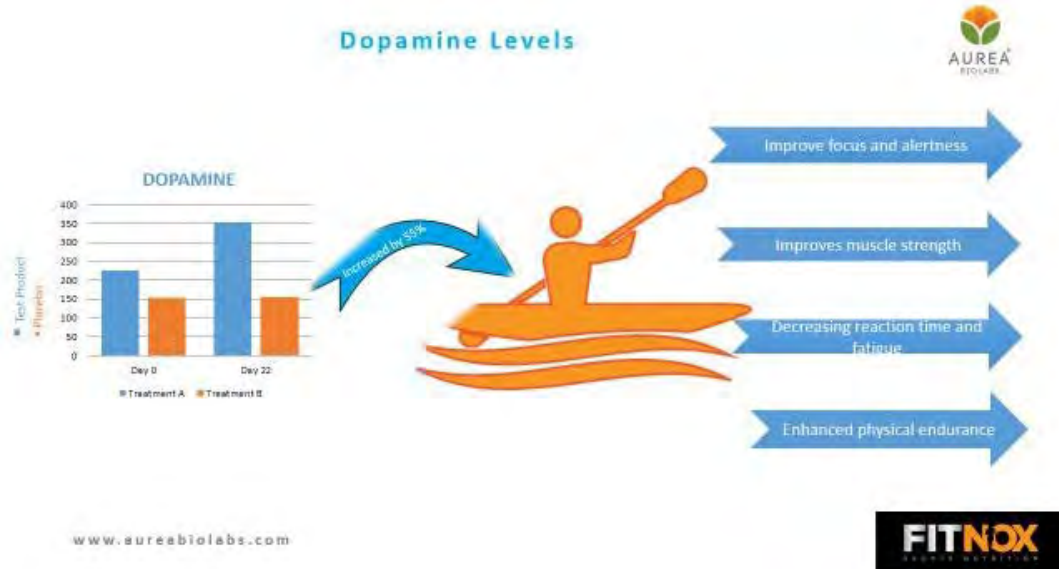
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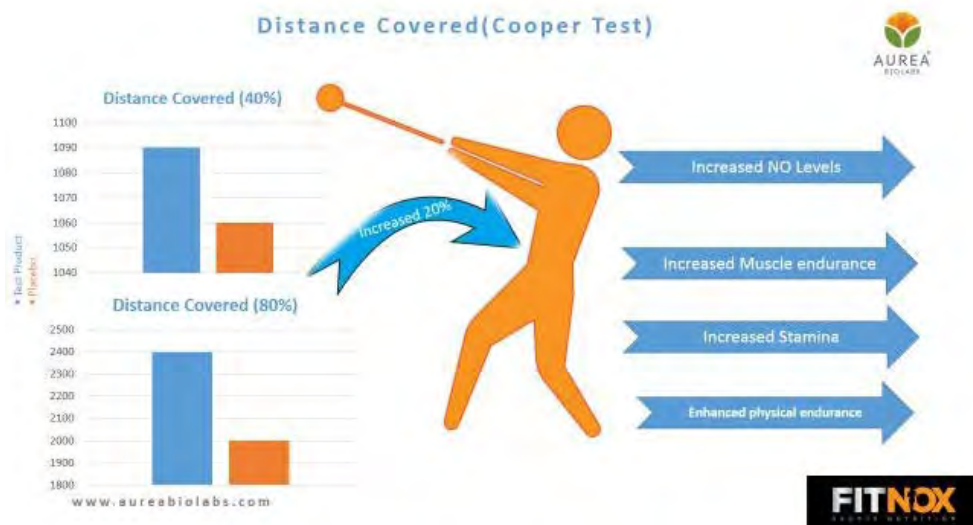
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Serum inflammatory biomarkers LDH and MDA decreased from 673.7 to 578 U/L and from 14.63 to 9.91 nmol/mL, respectively, in Fitnox™ group compared to the placebo group. The dopamine concentration significantly increased from 225.08 µg/24h to 354.25 µg/24h after the administration of the sports nutrition Fitnox™. Heart rate and RBC of the participants after the

administration of Fitnox™ or placebo are given which clearly indicated that there is no significant changes between the treatment groups.



Efficacy analysis for Time to Exhaustion (Between Groups) is shown which showed that the time to exhaustion significantly increased at  $P < 0.05$  in the Fitnox™ group with that of the placebo.





## **Conclusions**

In conclusion, the natural sports supplement formulation, Fitnox™ is shown to increase the nitrate, nitrite, RBC, and dopamine levels in blood before exercise period as well as in post exercise sessions during a period of 22 days. Similarly, the oxidative stress biomarkers such as MDA and LDH also gradually reduced during these periods in both pre and post exercises which shows the high antioxidant capacity of this formulation. The increased dopamine content helped the efficiency in exhaustion tests and these results suggest that Fitnox™ could enhance the physical fitness and stamina of any active person. There were no unusual or abnormal physical changes noticed in the participants. Therefore, Fitnox™ is very useful for sports persons who participate in acute, chronic and strenuous exercises that require physical endurance in order to perform quick exercise without tiredness.

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**Aurea Biolabs (P) Limited**

Cochin, Kerala, India

☎ : +91 484 305 1500 / 276 0497

☎ : +91 484 276 0689 / 237 0981

🌐 : [www.aureabiolabs.com](http://www.aureabiolabs.com)

✉ : [info@areabiolabs.com](mailto:info@areabiolabs.com)