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Pharmacological Investigation of *Amaranthus paniculatus* Seeds Extract

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Abstract-

The focus while study is on development of *Amaranthus paniculatus* seeds as a Herbal sports medicines. As *Amaranthus paniculatus* seeds have high energy value hence hypothesized here, 'it can be use as Herbal Sports Medicine'. The Wistar Albino Rats were used during study and accordingly experimental models were designed. *A. paniculatus* seeds water extract prepared then two groups of animals were set for the study; Test-1 (T1), Test-2 (T2) and respectively 250mg/kg, 500mg/kg of extract were administered orally. Change in the weight, swimming capacity, change in plasma creatinine, TG, glucose and certain electrolyte level were observed on animals. The results show that's, there were not significant changes in weight gain (3.667±2.894g) for 500mg/kg of extract. There are significant differences in the swimming time to exhaustion between control group (101.3± 3.403 sec) and each treatment group (177.2±9.547sec and 213.2±5.896sec respectively for T1 and T2). In order to clarify its mechanism plasma biochemical parameters were measured in the forced swimming treated Rats. In present study 500mg/kg of extract shows significantly difference in treatment group when compared to control group for TG (70.83±7.769mg/dL), calcium (5.976 ± 0.1371mg/dL) and chloride (104.1±2.693mg/dL). And in plasma Creatinine, Glucose and Electrolytes like magnesium, phosphorus, potassium, sodium doesn't having any significant differences. As seeds rich in Amino Acids- ornithine, arginine, alanine, glutamic acid, tyrosine, n-butyric acid, methionine, leucine and isoleucine that might be responsible for change in studied pharmacological activities.

Key Words: *A. paniculatus*, Swimming capacity, Biochemical's, Electrolytes, Amino Acids.

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Introduction

In Ayurveda the seeds (beeja) are given a superior importance. Many drugs are used in the systems are seeds, they used to posses general qualities like smigdha (unctuous) due to fatty materials in it and many are used as balya (tonic) due to the high protein contents. Many seeds are used in the food, like Green gram, Horse gram, Barley etc. for nutritive supplements (Kokate *et al.*, 1990).

Amaranthus paniculatus (Amarnthaceae) is the world's most nitrous grain. In India, Amaranthus grain also called the Royal crop or RAMA's grain. The seeds of Rajgira are nutritious and its leaves are an important source of proteins and vitamins and minerals like Calcium, iron. It was reported that Amaranthus seeds are an alternative natural source of squalene. (Bhattacharjee Paramita *et al*.2012) Amaranthus seeds has high energy value: 407 kcal. (Rajyalakshmi *et al.*,1994). The seeds contain 0.66% phospholipids. Major constituents of these phospholipids are: phosphoatidylethanolamine,

phosphatidylcholine and phosphatidylinositol; presence of small quantities of sphingomyelin and lysolecithin is also reported. The seed contain a high amount of protein 13.1% which is comparable to that of casein (Wealth of India 1986).

Our previous findings showed that, *Amaranthus paniculatus* seeds shows presence of glycoside, carbohydrate, protein, amino acids and fixed oils. More details TLC result shows presence of n-butyric acid, alanine, tyrosine, valine. And The HPTLC results shows presence of ornithine, arginine, alanine, glutamic acid, tyrosine, n-butyric acid, methionine, leucine, isoleucine (Parmar BKand Sheth NR., 2020). As it's well known proteins form from amino acids and protein is a good source of energy especially in sports persons (Ushir YV et al., 2018). Presently sports persons are using synthetic drugs which are prohibited by WADA (World anti doping agency). So, focus while study is on development of Herbal sports medicines. For that it's important that drugs which has a high nutritive value and which can enhance the performance of sports persons. As *Amaranthus paniculatus* seeds have high energy value hence hypothesized here, 'it can be use as Herbal Sports Medicine'. This present study is intended to prove, the above hypothesis by studying experimental model and various biochemical parameters.

Materials and Methods

Procurement of Seeds

Seeds of *Amaranthus paniculatus* were obtained from a local market in Rajkot City. Seeds were cleaned with water, then make

dried, and powdered it. The plant material was taxonomically identified and authenticated by Dr. Sunita Gerg, NISCAIR, New Delhi, India, and the voucher specimen (SU/DPS/HERB/2013/57) was retained in the Department of pharmaceutical sciences, Rajkot for further references.

Chemicals and Instruments

UV Spectrophotometer, the levels of creatinine, glucose, triglyceride and electrolytes (sodium, potassium, chloride, calcium, phosphorus, magnesium) were analyzed with commercial kits (Sigma-Aldrich Chem.).

Preparation of Extracts

By Cold maceration water extract prepared as, weigh about 5gm of powdered drug in a 250ml conical flask, and poured 100ml of distilled water. Set aside for 24hrs, shaking frequently. Filtered it. Evaporate to dryness on water bath, then cool and collected. Both Ethanol and water extract kept in desiccators for further use. These test samples were dissolved in distilled water when they were administered orally to the rats

Animals

Wistar Alnino Rats of either sex weighing (200+25gm) were used in the present study. They were housed in cages (20cm x 32cm x 14cm) under automatically controlled condition of temperature. They were given free accesses to water and commercial diet. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IACE) as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPSCA).

Experimental Designs

After an adaptation period for a week, the 18 rats were randomly divided in three groups, a control group and two Test groups, of three each. The rat in the treatment group TEST -1 were orally administered 250mg/kg, extract of *Amaranthus paniculatus.* Treatment group TEST – 2 were orally administered 500mg/kg of extract. While the control group received same volume of distilled water. The Rats were made to swim for 15 min., on alternate day for 10 days. On tenth day Rats fasted overnight for blood sampling

Measurement of the Forced Swimming Capacity

The forced swimming capacity test was employed in our study to evaluate the effects of plant extracts on exercise durability of Rats. It is commonly accepted that swimming in an experimental exercise model (Orlans, 1987; Lapvetelainen et al., 1997). The forced swimming capacity of Rats was measured with measured with acrylic plastic pool (90 X 45 X 45cm) filled with water to a depth of 35 cm (Matsumoto et al., 1996; Kamakura et al.,

2001). The temperature of the water was maintained at 34±1ºC. The swimming time to exhaustion was used as the index of the forced swimming capacity. The rats were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7-s period. (Kyungah J., et al., 2004)

Analysis of Blood Biochemical Parameters

After anesthetization with ether, whole blood samples were collected in heparinized tubes. Blood was collected from the retro orbital plexus of Rats. Plasma was prepared by centrifugation at 4ºC for 10 min. and stored at -70º C in a deep freezer.

Estimation of Creatinine

100ul plasma and Reagent-3 added in standard and extract separately and to it 1000ul working creatinine reagent added. The contents were mixed and incubated for 30 sec, at room temperature and absorbance of sample (AT) and standard (AS) against reagent blank was measured at 505nm. Creatinine is reacts with Picric acid in an alkaline medium to form an orange colored complex. The amount of complex formed is directly proportional to the creatinine concentration.

The total Creatinine calculated by formula, Plasma Creatinine (mg/dl) = AT2 –AT1/AS2 – AS1 X 2. (AT1: Initial O.D. of Test, AT2: Final O.D. of Test, AS1 : Initial O.D. of Standard, AS2 : Final O.D. of Standard).

Estimation of Triglycerides

10ul plasma and Reagent-2 added in standard and extract separately and to it 1000ul Reagent 1 added. The contents were mixed and incubated for 10 min. at room temperature and absorbance of sample (AT) and standard (AS) against reagent-1 as a blank was measured at 505nm. Triglycerides calculated by formula, Triglycerides (mg/dl) = Absorbance of Test/Absorbance of std. x 200.

Estimation of Glucose

10ul plasma and Reagent-3 added in standard and extract separately and to it 1000ul working glucose reagent added. The contents were mixed and incubated for 10 min. at room temperature and absorbance of sample (AT) and standard (AS) against working reagent as a blank was measured at 505nm. The glucose in plasma or serum calculated by formula, Serum/Plasma glucose (mg/dl) = Absorbance of Test/Absorbance of std x 100

Measurement of Electrolytes

Calcium

The method used is based on the metallochromogen Arsenazo III which has a higher affinity for calcium ions. Arsenazo combines with calcium ions at pH 6.75 to form highly colored chromospheres. 25ul standard and extract added in 1000ul reagent, the contents were mixed and incubated for 10 min. at room temperature and absorbance of sample

(AT) and standard (AS) taken against reagent as a blank then measured at 630 nm. The calcium calculated by formula, Calcium (mg/dl) =AT/AS X Conc. of Std.

Magnesium

At alkaline pH magnesium reacts with xylidyl blue and produces a chelating red colored compound. The red increasing color is proportional to magnesium concentration.

10ul standard and extract added in 1000ul reagent, the contents were mixed and incubated for 10 min. at room temperature and absorbance of sample (AT) and standard (AS) taken against reagent as a blank then measured at 505 nm. The magnesium calculated by formula, Magnesium (mg/dl) =AT/AS X Conc. of Std.

Chloride

Chloride ions react with mercurous thiocynate to form mercury perchlorate and thiocyanate. Thiocyanate forms a red complex with ferric ions in the presence of nitric acid. 10ul standard and extract added in 1000ul reagent, the contents were mixed and incubated for 10 min. at room temperature and absorbance of sample (AT) and standard (AS) taken against reagent as a blank then measured at 480 nm. The Chloride calculated by formula, Chloride in Plasma (mEq/L) =AT/AS X Conc. of Std.

Phosphorus

Inorganic phosphate reacts in acid environment with molybdic acid to form an unreduced phosphomolybdic acid complex, which absorbs light at 340nm. The absorbance is directly proportional to the phosphorus conc. in the sample. 20ul standard and extract added in 1000ul reagent, the contents were mixed and incubated for 5 min. at room temperature and absorbance of sample (AT) and standard (AS) taken against reagent as a blank then measured at 340 nm.

The phosphorus calculated by formula, Phosphorus in Plasma (mg/dl) =AT/AS X Conc. of Std.

Potassium

Potassium ions in a protein free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphynylboron. The turbidity produced is proportional to the potassium concentration. 20ul standard and extract added in 1000ul reagent, the contents were mixed and incubated for 5 min. at room temperature and absorbance of sample (AT) and standard (AS) taken against reagent as a blank then measured at 630 nm.

The Potassium calculated by formula, Potassium in Plasma (mg/dl) =AT/AS X Conc. of Std.

Sodium

This method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly as the concentration of sodium in the test specimen. 10ul standard and extract added in 1000ul color reagent, the contents were mixed and incubated for 5 min. at room temperature and absorbance of sample (AT) and standard (AS) taken against reagent as a blank then measured at 630 nm. The sodium calculated by formula, Sodium in Plasma (mg/dl) =AT/AS X Conc. of Std.

Statistical Analysis

All the data were expressed as mean±SEM from six observations. The data obtained was analyzed using the one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test for determining the level of significance and $p < 0.05$ will be considered statistically significant.

Results and Discussion

Effect of *Amaranthus paniculatus* Extract on Bodyweight and Weight Gain.

Body weight was recorded before feeding extract (initial) and after feeding extract for 10 days (final), and weight gain was computed (Table 1). There was no significant difference between the control group and each treatment group. So drug has positive effect without increasing in weight. It can be helpful to sports person to maintain their bodyweight. There was no significant difference between the control group and each treatment group. In the present study, Amaranthus paniculatus extract had no significant effect on body weight compared to the control group.

Table 1: Effect on Bodyweight and Weight Gain.

Body Weight (g)	Control Gr.	T1	T2
Initial	253.3 ± 8.819	265.0 ± 10.25	271.7 ± 11.95
Final	254.3 ± 11.70	270.3 ± 10.54	274.7 ± 11.40
Weight Gains	4.333 ± 2.216	5.333 ± 2.459	3.667 ± 2.894

Effect of *Amaranthus paniculatus* Extract on Forced swimming Capacity

The results of forced swimming capacities are shown in Table 2. There are significant differences in the swimming time to exhaustion between the control group and each treatment group ($p<0.05$). There are significant differences in the swimming time to exhaustion between the control group and each treatment group.

Table 2: Effect on Forced Swimming Capacity

Control	T1	T2
101.3± 3.403	***177.2±9.547	***213.2±5.896

The swimming time is longer in Test 1 group when compare to Control group and also swimming time is longer in Test 2 than the control group (Fig. 2).

Effect of *Amaranthus Paniculatus* Seeds Extract on Plasma/Serum Biochemical Parameters

Creatinine, is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatine is primarily synthesized in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine, glycine, and methionine) by S-Adenosyl-L-Methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high energy compound phospho-creatine. During the reaction Creatine:phosphocreatine, catalyzed by Creatine Kinase, spontaneous conversion to creatinine may occur. We hypothesized that, if Sports persons are done more exercise, than more creatine will convert into creatinine. In Fig. 3a shows effect of *Amaranthus Paniculatus* seeds extract on plasma creatinine level after swimming exercise. There were no significant difference found in plasma creatinine level between control group and each of the other treatment group.

Triglyceride (TG), is an ester derived from glycerol and three fatty acids. Calories ingested in a meal and not used immediately by tissues are converted to TG and transported to fat cells to be stored. Hormones regulate the release of TG from fat tissues. So they meet the body's needs for energy between meals. There is evidence that free fatty acids and TG fatty acids can provide energy for muscular contraction. Jones and Havel (1967) documented increased clearance of plasma TG as well as increased oxidations of free fatty acids by skeletal muscle in normal rat during prolong exercise. In this study, plasma TG levels were significantly lower in both treatment groups than in the control group ($p<0.05$), this result suggests that plant extract of *Amaranthus paniculatus* could increase fat utilization of Rats during swimming. Fig. 3b shows significant difference in plasma TG level in Rats after swimming exercise.

Glucose, there were not significant differences in plasma glucose level in Rats. Fig. 3c shows plasma Glucose level in Rats after swimming exercise. It was estimated that higher plasma glucose level of control group was due to shorter swimming time than that of the both treatment group. Generally it is interpreted that the elevation of TG and decreases Glucose level shows a consumption of energy.

Table 3: Effect of *Amaranthus Paniculatus* Extracts on Plasma Biochemical Parameters

Parameters (mg/dL)	**Control**	**T 1**	**T 2**
Creatinine	0.4512±0.02190	0.4792 ± 0.1156	0.4765±0.006270
Triglyceride	191.1 ± 33.94	140.9 ± 27.82	70.83 ± 7.769**
Glucose	92.51 ± 9.508	78.43 ± 21.15	72.46 ± 9.461
Sodium	3177 ± 75.79	2983 ± 107.6	3284 ± 86.34
Potassium	655.4 ± 48.02	698.7 ± 33.75	731.1 ± 71.28
Calcium	5.773 ± 0.3979	5.815 ± 0.1861	5.976 ± 0.1371
Chloride	83.80 ± 4.871	88.83 ± 4.244	104.1 ± 2.693**
Magnesium	1.113±0.00445	1.116±0.00236	1.114± 0.003683
Phosphorus	7.408 ± 0.6081	6.615 ± 0.7298	5.541 ± 0.2288

Measurement of Electrolytes

An electrolyte is a compound that ionizes when dissolved in suitable ionizing solvents such as water. This includes most soluble salts, acids, and bases. Some gases, such as hydrogen chloride, under conditions of high temperature or low pressure can also function as electrolytes. Electrolyte solutions can also result from the dissolution of some biological (e.g. DNA, polypeptides) and synthetic polymers (e.g. polystyrene sulfonate), termed polyelectrolytes, which contain charged functional groups. In physiology, the primary ions of electrolytes are sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), hydrogen phosphate (HPO_4^{2-}), and hydrogen carbonate (HCO_3^-).

All known higher life forms require a subtle and complex electrolyte balance between the intracellular and extracellular environment. In particular, the maintenance of precise osmotic gradients of electrolytes is important. Such gradients affect and regulate the hydration of the body as well as blood pH, and are critical for nerve and muscle function. Various mechanisms exist in living species that keep the concentrations of different electrolytes under tight control.

Both muscle tissue and neurons are considered electric tissues of the body. Muscles and neurons are activated by electrolyte activity between the extracellular fluid or interstitial fluid, and intracellular fluid. Electrolytes may enter or leave the cell membrane through specialized protein structures embedded in the plasma membrane called ion channels. For example, muscle contraction is dependent upon the presence of calcium (Ca^{2+}), sodium (Na^+),

and potassium (K^+). Without sufficient levels of these key electrolytes, muscle weakness or severe muscle contractions may occur.

Electrolyte balance is maintained by oral, or in emergencies, intravenous (IV) intake of electrolyte-containing substances, and is regulated by hormones, generally with the kidneys flushing out excess levels. In humans, electrolyte homeostasis is regulated by hormones such as antidiuretic hormone, aldosterone and parathyroid hormone. Serious electrolyte disturbances, such as dehydration and overhydration, may lead to cardiac and neurological complications and, unless they are rapidly resolved, will result in a medical emergency. It was stated that the most important effect of regular exercise was on blood biochemistry and effect of regular exercise on blood cells, lipids and electrolytes were different (Koc et al 2010).

In our study we finding that Calcium level in plasma were increased (Fig. 2f). Baltaci et al., (1998) stated that significant increases were recorded due to exercise at different severities and durations. Finding of Baltaci et al., do support our findings. When we look at our finding, we can see that there is not any significant difference in Magnesium plasma level (Fig. 2h). An analysis of studies to determine the effect of exercise on magnesium level reveals different findings. Studies have shown that magnesium level increases in studies that the high amounts of magnesium sent-away from the body through perspiration and urination during exercise might result in magnesium deficiency (Lukaski and Nielsen, 2002). Newhouse et al., determined that there were decreases in magnesium level after long distance runs (Newhouse and Finstand, 2000). Sodium plasma level (Fig. 2d) was decreased and Potassium plasma level (Fig. 2e) was increased. But both Sodium and Potassium plasma level was not statistically significant. Koc et al (2010) determined in the study conducted in order to make a comparison between blood electrolyte levels of athletes and sedentary university students that Sodium and Potassium level was higher in athletes and this difference was significant, which does not support our findings. In our study there were significantly increased in Chlorine plasma level (Fig. 2g). And also Phosphorus level (Fig. 2i) is decreased in plasma, but it was not significantly decreased.

Finally we can summarize here results as; there were not significant changes in body weight and weight gain. There are significant differences in the swimming time to exhaustion between control group and each treatment group. In order to clarify its mechanism, blood biochemical parameters were measured in the forced swimming treated Rats.

In present study TG and chloride found significantly difference in treatment group when compared to control group. And in plasma Creatinine, Glucose and Electrolytes like Calcium, magnesium phosphorus, potassium, sodium having not any significant differences.

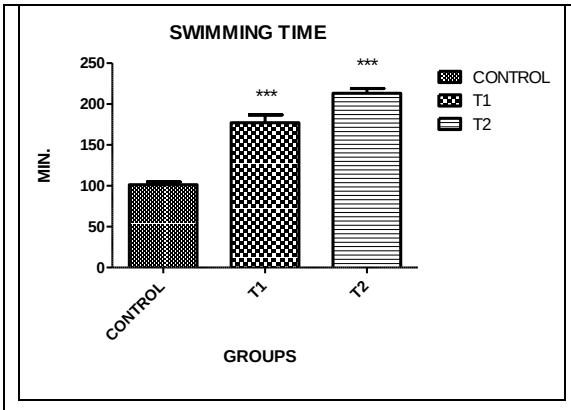

Fig. 1: Swimming Time of Rats

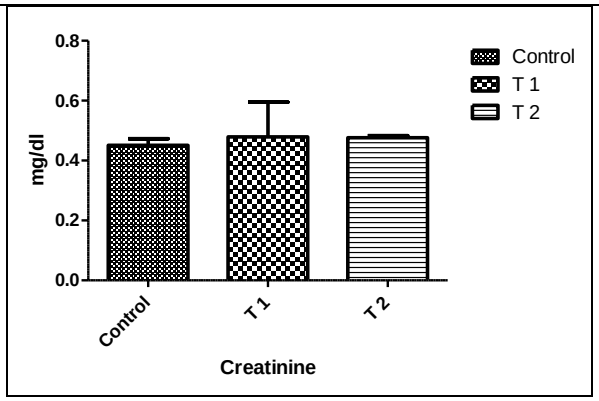

Fig 2a: Estimation of Creatinine

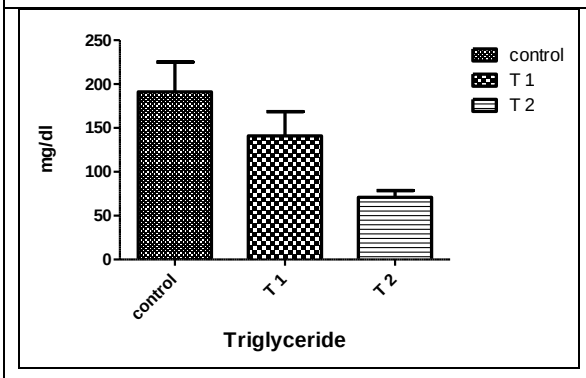

Fig 2b: Estimation of Triglyceride

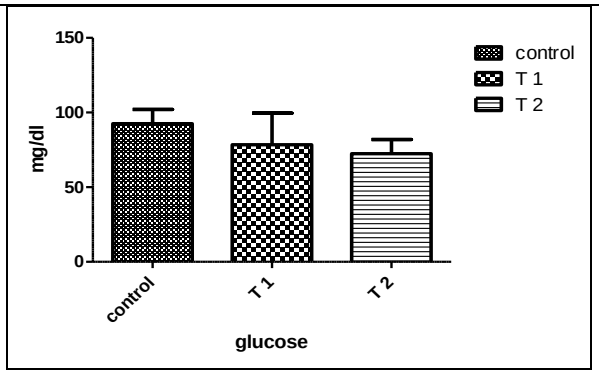

Fig 2c: Estimation of Glucose

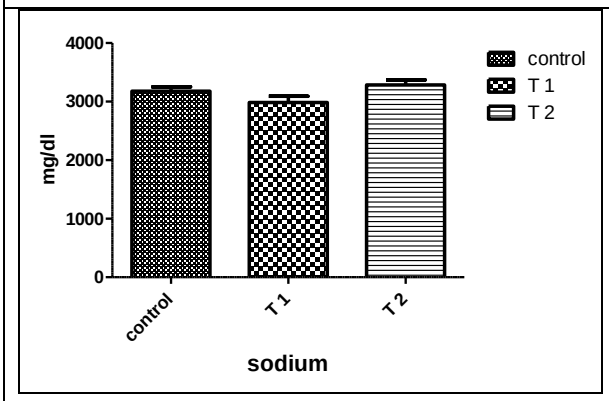

Fig 2d: Estimation of Sodium

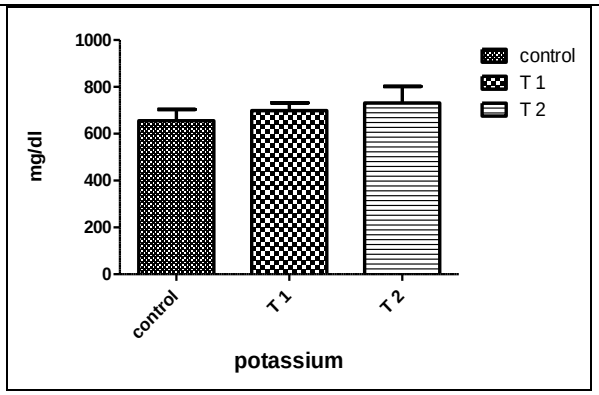

Fig 2e: Estimation of Potassium

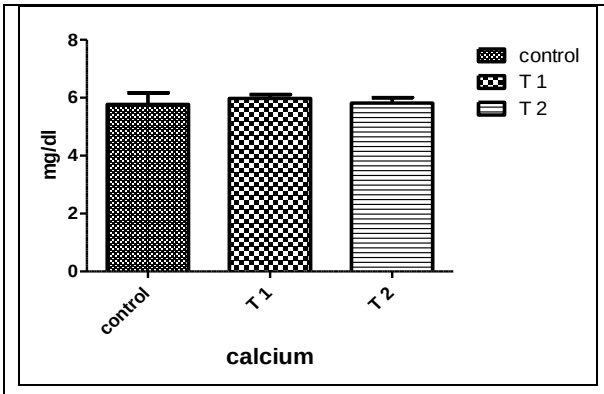

Fig 2f: Estimation of Calcium

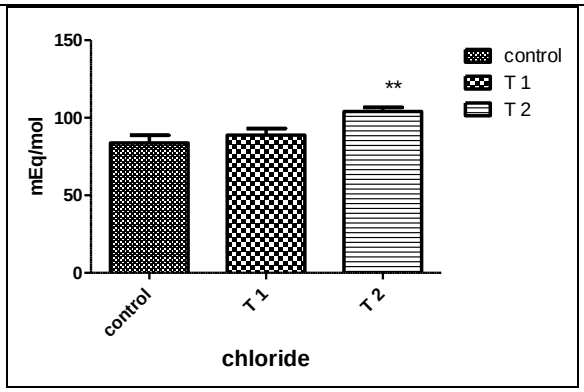

Fig 2g: Estimation of Chloride

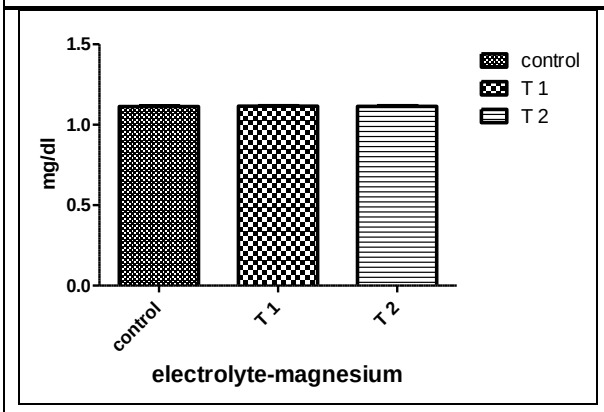

Fig 2h: Estimation of Magnesium

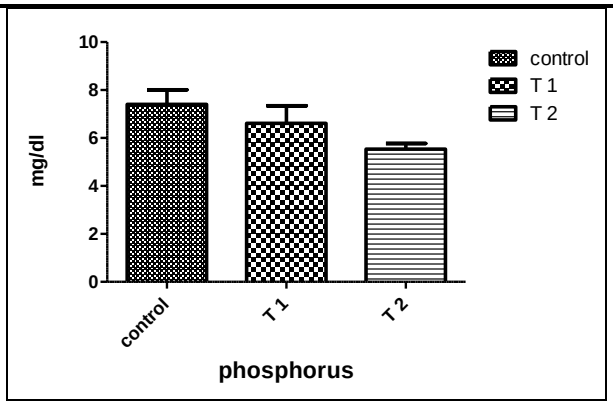

Fig 2i: Estimation of Phosphorus

(Values are expressed in mg/dl in plasma volume as mean ± SEM followed by Dunnet's multiple comparison test *p<0.05, **p<0.05, ***p<0.001 is number of animals=6. Comparisons are made against Control group, Test 1 and Test 2.)

Conclusion

From the present investigation, it was concluded that there might be possibility of presence of ornithine, arginine, alanine, glutamic acid, tyrosine, n-butyric acid, methionine, leucine, isoleucine. And as it has high energy value, it can provide more energy with help of forced swimming test it was proved that it can extend the duration of swimming time in Rats. *Amaranthus panicuatus* seeds when provided at 250mg/kg, 500mg/kg per day, may favorably influence selected markers of exercise. More work is needed to extend these findings, in particular using a larger sample of subjects and the inclusion of additional markers of exercise and performance.

Conflict of interest

The authors declare no conflicts of interest.

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