

Evaluation of nutritional and sub-acute toxicological study of plant based supplement of *Achyranthes aspera*

Nudrat Fatima^{1*}, Nabeela G Dar², Hina Imran¹, Tehmina Sohail¹, Uzma Asghar², Zahra Yaqeen¹, Shazia Syed³ and Khalid Jamil²

¹Pharmaceutical Research Centre PCSIR Labs Complex, Karachi, Pakistan

²Food and Marine Resources Research Centre, PCSIR Labs Complex, Karachi, Pakistan

³Department of Chemistry, University of Karachi

Abstract: The present study was conducted for the nutritional, microbiological and toxicological evaluation of test compound having main ingredient *Achyranthes aspera*. Nutritional value assessment, microbiological analysis and toxicological studies were conducted according to the standard reported methods which exhibited that *A. aspera* contains moisture 4.05%, proteins 20.54%, fats 0.903%, ash 20.25%, carbohydrates 54.26% and energy 294 Kcal. Vitamin profile was found to be B¹ 0.27mg/100g, B² 0.28mg/100g, B³ 0.58mg/100g, B⁶ 0.27mg/100g and B⁹ 39µg/100g. The content of sodium, calcium, magnesium, potassium, chloride and phosphorus was found to be 1119.67, 5385.23, 5446.08, 1343.6, 675880.73 and 1447.5mg/kg respectively and trace metals i.e. iron, copper, zinc, manganese and aluminum were detected as 283.05, 8.062, 48.37, 16.12 and 9.853 mg/kg respectively. The microbiological result indicated that the compound qualifies the international standards of microbial limit and was found free from *Salmonella* species. The toxicological study was conducted to find safe use of *Achyranthes aspera* compound in human as a nutritive supplement in blood disorders. The toxicity studies exhibited that the test compound has a good effect on general health as an increase in body weights of animals of test group was noticed as compared to that of control group. Blood parameters before and after the study were monitored which confirms our hypothesis by showing an increase in hemoglobin from 9.133 to 10.96, RBC count from 3.11 to 3.6, WBC count from 5.68 to 5.73 and platelets from 245 to 319.

Keywords: *Achyranthes aspera*, toxicity studies, nutritional value, microbiological analysis, blood parameters.

INTRODUCTION

The importance of diet and nutrition in human health and disease is well established. Nutrients are required in preventing diseases. It is true that, nutrition is an essential part of every individual whether they are children, teenagers, adults or aged people. Nutrition refers to the nourishing of our body, in order to keep it healthy and proper functioning. It is a human responsibility to provide the body with all its necessary food, vitamins, and minerals to continue the daily life processes. A person can live healthily by using the proper nutrient composition in his food and if it is deprived then we have to take some nutritive supplements for compensation. It is reported that plants are good source for vital macro and micronutrients (Shendkar *et al.*, 2011).

Achyranthes aspera Linn. (Amaranthaceae) commonly known as Chirchita (Sumeet *et al.*, 2008; Srivastav *et al.*, 2011) is an important medicinal plant. It is widespread in the world as found in Asia, Africa, Australia and America and claimed as possesses numerous pharmacological activities (Srivastav *et al.*, 2011; Paul *et al.*, 2010; Sutar *et al.*, 2008). The whole plant, the root, the seeds were used and had various chemical constituents including alkaloids, flavonoids, saponins, steroids and terpenoids. Other important constituents like oleanic acid, protein, iron,

calcium, phosphorous, Vitamin C are also reported in *A. aspera* (Sumeet *et al.*, 2008).

Traditionally it was used for many ailments. Despite of its importance, few reports exist in the literature regarding the chemistry or antioxidant activities. The aim of the present study was to evaluate *Achyranthes aspera* for its safe use in human as a nutritive supplement in blood and bone marrow disorders. For this purpose, acute oral toxicity and sub-chronic toxicity studies were carried out in laboratory animals (Swiss albino mice and albino Wistar rats of either sex).

MATERIAL AND METHODS

Chemicals

All the chemicals used were of analytical grade Merck (Germany) and Sigma (USA).

Test material

Achyranthes aspera (Chirchita) was purchased from local market of Karachi and identified by taxonomist PCSIR Labs Complex Karachi (voucher no. AA-11-242011) Ash of the whole *A. aspera* (active ingredient), washed extruded cereals (diluent) and turmeric (preservative) was ground and mixed together with milk and sugar (taste developers) which was treated as test compound in a dose of 31.84mg/kg body weight/day.

*Corresponding author: e-mail: drnudrat_ekq@yahoo.com

Instrument

Buchi Distillation Unit K-314, Protein Buchi Digestion Unit K-424, Soxhlet Fat Extraction assembly, Oven (Memmert), Furnace (Gallenkemp) HPLC (Perkin Elmer, Model 200, USA), Atomic Absorption Spectrophotometer (Hitachi model Z-5000).

Nutritional value analysis

Nutritional value analysis was carried out according to the standard reported methods. Proximate analysis i.e. the percentage of moisture, fat, protein, carbohydrate, ash and energy was calculated according to the standard method (Delwiche, 2005). Mineral profile was also analyzed among which sodium, calcium, magnesium, potassium, chloride, phosphorus, iron, copper, zinc, manganese and aluminum were found by the method stated in AOAC (Mississawyes Ontario, 2007). Vitamin B profile was carried out in which B¹, B², B³, B⁶ and B⁹ were estimated (Kirk and Sawyer, 1991).

Table 1: Effect on weights of animals after sub-chronic toxicity studies

| S. No | Group | Weight in g |
|-------|-----------------------|--------------|
| 1 | Group1 Baseline | 21.60±1.8104 |
| 2 | Group1After one month | 22.12±1.6652 |
| 3 | Group2 Baseline | 21.79±1.6196 |
| 4 | Group2After one month | 21.80±1.7870 |

F value = 0.157; p-value=0.925, All data is presented in Mean ± S.D.

Microbiological analysis

The test material was microbiologically analyzed that either the material can be used as nutritional supplement or not. The sample qualified the basic microbiological tests required for a compound to be used as a nutritional supplement. The standard method was used as reported (Larry and James, 2001).

Toxicity Studies

Animal selection

Before proceeding for toxicity studies animals i.e. Swiss albino mice (20-30g) and Wistar strain rats (165-170g) reared at animal house of PCSIR Labs Complex Karachi, were selected and housed separately and kept under strict observation for a period of 3 weeks with free access to food and water. Any animal showing sluggish movement or any sign of illness was rejected. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Ethical Committee PCSIR for conducting the study (Ref. No. 45/ET/11).

Acute oral toxicity test

Acute toxicity study of *Achranthes aspara* was carried out in albino rats (Wistar strain). Eighteen albino rats of either sex were selected and divided into three groups, (n=06). Animals of group 1 and 2 received the test drug in a dose

of 0.153g and 0.271g respectively while the animals of group 3 i.e. control group received normal feed with excipients only, in the same quantity according to standard method of acute oral toxicity test (Loomis, 1978). The cages of animals were marked with their respective doses and were observed strictly for the first six hours and then for the period of 72 hours. Daily observation on general health, growth, gross physical and behavioral activities and also the mortality if any was noted and recorded.

Table 2: Nutritional value analysis of nutritional supplement

| S. No. | Parameters | Average |
|--------|----------------|--------------------------|
| 1. | Moisture | 4.050±0.050g/100g |
| 2. | Protein | 20.540±0.120 g/100g |
| 3. | Fat | 0.903±0.002g/100g |
| 4. | Ash. | 20.250±0.030g/100g |
| 5. | Carbohydrates | 54.260±0.490g/100g |
| 6. | Sodium | 1119.670±3.140mg/kg |
| 7. | Calcium | 5385.230±18.410 mg/kg |
| 8. | Magnesium | 5446.080±7.990mg/kg |
| 9. | Potassium | 1343.600±106.530mg/kg |
| 10. | Chloride | 675880.730±7014.090mg/kg |
| 11. | Phosphorus | 1447.500±10.500mg/kg |
| 12. | Iron | 283.050±14.140mg/kg |
| 13. | Copper | 8.062±0.480mg/kg |
| 14. | Zinc | 48.370±1.540mg/kg |
| 15. | Manganese | 16.120±0.840mg/kg |
| 16. | Aluminum | 9.853 ±0.140mg/kg |
| 17. | B ¹ | 0.270±0.040mg/100g |
| 18. | B ² | 0.280±0.050mg/100g |
| 19. | B ³ | 0.580±0.030mg/100g |
| 20. | B ⁶ | 0.270±0.040mg/100g |
| 21. | B ⁹ | 39.000±9.000mg /100g |
| 22. | Energy | 294.000±10.500kcal |

All data is presented in Mean ± S.D.

Table 3: Microbiological Analysis

| S. No | Microbiological Parameters | Result |
|-------|---------------------------------|------------------|
| 1 | Estimated Aerobic plate control | < 10 cfu/g |
| 2 | MPN Coliforms | < 3 /g |
| 3 | MPN Faecal Coliforms | < 3 /g |
| 4 | Estimated MYC | < 10 cfu/g |
| 5 | Salmonella SPP | Not detected/25g |

Sub-chronic toxicity studies

Animals, i.e. Swiss albino mice of either sex weighing between 19-25g were divided into two groups, each group comprised of 10 animals (5 male and 5 female). Group 1 received feed containing test drug in a dose of 31.84mg/kg body weight/day while Group 2 received normal diet added with excipients used in the supplement.

The animals were provided water *ad libitum*. Cages were marked and observed strictly. During observation the parameters like gross physical and behavioral changes, morbidity and mortality were noted. Animal body weights were noted at baseline and after completion of one month study period (Loomis, 1978; Yaqeen *et al.*, 2011).

Blood collection

Eye: A capillary pipette containing anticoagulant (EDTA for cell counting, heparin for flow cytometry) was inserted in the lateral can thus and blood collected from the retro orbital sinus. A total of 1ml of blood was removed from this site. After collection, the pipette was removed and bleeding stopped when the eye returned to a normal position (Nemzek *et al.*, 2001).

STATISTICAL ANALYSIS

The values were calculated as mean \pm SD. All the data was analyzed statistically by student *t* test. The *p* values at ≤ 0.05 level were considered significant (Steel and Torrie, 1996).

RESULT

The present study was conducted for the nutritional, microbiological and toxicological evaluation of *Achyranthes aspera*. The nutritional analysis exhibited that *A. aspera* increases the weights of experimental animals after feeding the test compound for a period of one month (table 1). The plant material (*A. aspera*) contains moisture 4.05%, proteins 20.54%, fats 0.903%, ash 20.25%, carbohydrates 54.26% and energy 294 Kcal. Vitamin profile was found to be B¹ 0.27mg/100g, B² 0.28 mg/100g, B³ 0.58mg/100g, B⁶ 0.27mg/100g and folic acid (B⁹) 39 μ g/100g. The mineral content was observed as Na 1119.67mg/kg, Ca 5385.23mg/kg, Mg 5446.08mg/kg, K 1343.6mg/kg, Cl 675880.73mg/kg, P 1447.5mg/kg and trace metals were in the ratio of Fe 283.05mg/kg, Cu 8.062mg/kg, Zn 48.37 mg/kg, Mn 16.12mg/kg, Al 9.853mg/kg (table 2). The microbiological result indicated that the plant qualifies the international standards of microbial limit and was found free from *Salmonella* species (table 3). The toxicological study was conducted for the safe use of *Achyranthes aspera* in human as a nutritive supplement in blood disorders. The acute oral toxicity and sub-chronic toxicity studies exhibited that the test compound has a good effect on general health and an increase in body weights of animals of test group as compared to the weights of animals of control group animals. Blood parameters before and after the study were monitored which confirms our hypothesis by showing an increase in hemoglobin from 11.04 \pm 0.11 to 12.24 \pm 0.38, RBC count from 6.6 \pm 0.07 to 7.14 \pm 0.46, WBC count from 6.32 \pm 0.49 to 6.93 \pm 0.73 and platelets from 294.2 \pm 104.47 to 331.2 \pm 89.89 (table 4).

DISCUSSION

The present study shows that test compound has a good effect on general health and qualifies the basic microbiological tests required for a compound to be used as nutritional supplement and blood parameters. The weights of test group animals increased from 21.6 \pm 1.8104g to 22.12 \pm 1.6652g as compared to that of the control group animals i.e. from 21.79 \pm 1.6196g to 21.8 \pm 1.7870g (table 1). Nutrition value analysis exhibited that all essential macronutrients such as proteins, fats and carbohydrates and also the micronutrients like the vitamins and minerals are present in the compound (table 2). The function of the macronutrients is to provide energy and strength to the body while the micronutrients play an important role in the working of the cells of our body (Nemzek *et al.*, 2001). Adequate and proportionate intake of carbohydrates, proteins and fats is very essential to stay away from infections, blood and bone marrow disorders diseases. The proteins do the task of building the tissues of the muscles and also maintaining and repairing them. By consuming protein rich foods, you can meet the need for amino acids, which are necessary for proper functioning of the body. People taking low protein diet develop a protein gap of alarming proportion. This shortage can be replaced by plant based high protein nutritional supplements. Trace elements and minerals are physiologically important particularly in body metabolism (Ahmad *et al.*, 2007). The microbiological test was performed which again showed positive results (table 3). Good nutrition is essential for the physical as well as the mental growth of the children. It has been observed that the children who receive undernourished food, which is devoid of the nutrition components mentioned above, suffer from many diseases and disorders and often experience stunted growth.

Increase in Hemoglobin concentration, RBC count, and other blood parameters (table 4) give a clear cut picture that the feeding of test compound for long time has good effect on body.

The test drug also assist and help to maintain the proper nourishment of the tissues, it was found to affect the body weight of animals both in males and females of test group as compared to control groups. Literature survey supports to effectiveness of the test compound as an energetic tonic with many other benefits for protecting and maintaining better life expectancy. It is reported that the test compound possesses hepato-protective, nephro-protective and bronchio-protective activities. The plant is also reported for significant anti-oxidant and hypolipidemic activities (Sumeet *et al.*, 2008; Srivastav, 2011). This could make the test compound a nutraceutical of choice for the persons losing their health for reasons unknown. The plant is easily available in Pakistan and is cost effective in comparison to other expensive nutraceuticals.

Table 4: Effect of test nutritional supplement on blood parameters

| S. No | Blood Parameters | Control group | Test group | t-test | p-value |
|-------|------------------|---------------|--------------|--------|-----------|
| 1 | Hemoglobin | 11.04±0.11 | 12.24±0.38 | 6.790 | P< 0.0001 |
| 3 | RBC | 6.60±0.07 | 7.14±0.46 | 2.590 | P<0.05 |
| 4 | WBC | 6.32±0.49 | 6.93±0.73 | 1.550 | N.S |
| 5 | Platelets | 294.20±104.47 | 331.20±89.89 | 0.600 | N.S |
| 6 | MCV | 59.80±5.93 | 66.80±3.96 | 2.190 | N.S |
| 7 | MCH | 17.80±0.84 | 18.20±0.84 | 0.756 | N.S |
| 8 | MCHC | 32.40±2.30 | 40.00±3.67 | 3.920 | P<0.005 |
| 9 | Neutrophil | 26.60±1.52 | 28.00±1.87 | 1.300 | N.S |
| 10 | Lymphocyte | 66.80±10.89 | 72.80±6.83 | 1.040 | N.S |
| 11 | Monocyte | 1.40±0.55 | 1.80±0.84 | 0.894 | N.S |
| 12 | Eosinophil | 1.00±0.00 | 1.20±0.45 | 1.000 | N.S |
| 13 | Basophil | 0.00±0.00 | 0.00±0.00 | 0.000 | N.S |

All data is presented in Mean ± S.D.

CONCLUSION

It can be concluded promptly that the test compound is safe in therapeutic doses for prolong use, improves hematological indices and general health. The sub-chronic toxicity studies show that the test drug has good effect on body weights and has no harmful effect on vital organs which was noted during autopsy of test animals. Hence it is beneficial and safe for use in human as a nutritive supplement.

REFERENCES

Ahmad T, Atta S, Ihsanullah, Aurang Zeb, Nagra SA and Perveen S (2007). Characteristics of silybummarianum as a potential source of dietary oil and protein. *Pak. J. of Sci. and Ind. Res.*, **50**: 36-40.

Delwiche S (2005). Cereal Foods. *In: Official methods of analysis of AOAC International*, W. Horwitz. 18th ed. Association of official Analytical Chemists, Washington D.C., USA, pp.1-61.

Kirk RS, Sawyer R. Cereals and Flours (1991). *In: Pearson's composition and analysis of foods*. 9th ed. Longman Scientific and Technical Harlow Essex, U.K. and New York, pp.301-25.

Larry Maturin and James T. Peeler, January 2001. BAM: Aerobic Plate Count, Bacteriological Analytical Manual Chapter 3.

<http://www.fda.gov/Food/FoodScienceResearch/Laboratory Methods/ucm063346.htm>

Loomis TA (1978). *Essentials of Toxicology*. 3rd ed. Lea & Febiger, Philadelphia, pp.198-233.

Mississawyes Ontario (2007). Agilent Technologies. LC and LC/MAS. *In: the essential chromatography and*

spectroscopy catalog. 2007-2008; Agilent Technologies, p.656.

Nemzek JA, Bolgos GL, Williams BA and Remick DG (2001). Differences in normal values for murine white blood cell counts and other hematological parameters based on sampling site. *Inflammation Res.*, **50**: 523-527.

Paul D, De D, Ali KM, Chatterjee K, Nand DK and Ghosh D (2010). *Contraception*, **81**: 355-361.

Shendkar CD, Chandrachood PS, Pawar AB, Lavate SM, and Deshpande NR (2011). Quantitative estimation of macro, micronutrients and trace elements by X-ray fluorescence spectroscopy (XRF) from *Achyranthesaspera* Linn. *International J. Chem. Tech. Res.*, **3**:610-613.

Srivastav S, Singh P, Mishra G, Jha KK and Khosa RL (2011). *Achyranthesaspera*-An important medicinal plant: A review. *J. Nat. Prod. Plant Resour.*, **1**:1-14.

Steel RGD and Torrie JII (1996). *Principles and procedure of Statistics. A biometric approach* 2nd ed., McGraw-Hill, New York, Pp.6-15.

Sumeet D, Raghvendra D and Mehta K (2008). *Achyranthesaspera* Linn. (Chirchira): A Magic Herb in Folk Medicine. *Ethnobotanical Leaflets*, **12**: 670-676.

Sutar NG, Sutar UN, Sharma YP, Shaikh IK and Kshirsagar SS (2008). Phytochemical investigation and pharmacological screening of leaves of *Achyranthes aspera* L. as analgesic and antipyretic. *Biosci. Biotec. Res. Asia*, **5**: 841-844.

Yaqeen Z, Fatima N, Imran H, Rahman A, Rehman Z, Sohail T and Syed S (2011). Efficacy studies of polyherbal vitamin supplement in experimental animals. *Int. J. Biol. And Biotech.*, **8**: 195-201.