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ORIGINAL ARTICLE

Pumpkin seed effects on haemoglobin level on rabbit animal.

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ABSTRACT… Objective: To evaluate pumpkin seeds effects on improving low haemoglobin level in rabbits. **Study Design:** Experimental study. **Setting:** Pharmacology Department PUMHSW, Nawabshah. **Period:** January 2020 to March 2020. **Material & Methods:** Thirty adult health rabbits were chosen for the study by keeping inclusion and exclusion criteria as standard. Pumpkin seeds powder measured quantity 250 mg and 500 mg was given to different groups for 60 days as once a day respectively. The samples of blood were taken at standard equal. At zero time, first reading marked as day 0. Thereafter further samples were taken twice monthly for 60 days. Complete Blood Count was analyzed with Automatic Hemoglobin Analyser. Data was assessed statistically in groups as means through correlated pairs t test and thru SPSS version 21.0. For all comparisons, upto 0.05 P value was considered significant. **Result**: The mean Haemoglobin values on day 0 were found non-significant statistically within study groups while comparing with control. Whereas, there a growing increase from day 15 to day 60 was observed in the Haemoglobin mean values in group B and C on all successive reading taken twice monthly. All the interpretation creates highly significance statistically when as compared with control. It shows the pumpkin seed's haematopoietic effect in anaemia cases due to iron deficiency. **Conclusions:** Seeds of pumpkin can be substituted with common methods as a good plant nutritional source for to improve haemoglobin in anaemia due to iron deficiency.

Key words: Auto-analyzer, Haemoglobin, Haematopoietic, Pumpkin Seed.

INTRODUCTION

Human diseases are increasing all over the world which has led to the need to search for alternative medicine also known as natural medicine.[1] For thousands of days medicinal plants are used like important therapeutic agents to alleviate diseases afflicting the human race.[2,4] Medicinal plant different parts may be utilized in the manufacturing of valuable medicines.[3] The pumpkin plant is cultivated in the world's different areas. Seeds and pulp are traditionally utilized as medicine.[5] The plant can be grown and harvested after 2-3 months of plantation. Pumpkin seeds can be stored after harvest for up to six months.[6] Nutritional value of the pumpkin seeds is high. 100 grams gives energy up to 559 calories. It contains carbohydrates 10.71 grams, proteins 30.23 grams and fats 49.05 grams.[7.8]

Anemia is a disorder characterized by a low level of RBCs, and hemoglobin.[9] These patients are at more risk for hospitalization with increased morbidity and mortality.[10] Anemia during pregnancy is a universal problem[11] predisposing to adverse pregnancy consequences.[12] It can be prevented by adopting timely measures for preventable causes of anaemia development.[13]

Anemia is caused by multiple factors such as nutritional, inherited, socio-economic, dermatograph, malabsorption, infectious, chronic diseases.[14] Iron deficiency is the commonest nutritional deficiency which affects approximately 700 million people all over the world. About 56% pregnant women in under develop globe are anaemic. In the Indo-Pakistan region it is 88%. South Asian and African countries belongs to areas of severe iron deficiency and have 40%

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prevalence.[15] IDA in Pakistan is predominant in children below the age of 5 years and young pregnant women.[16] The factors predispose are poor nutrition, poor eating habits such as pica, parasitic infections such as malaria and multiple pregnancy.[17] IDA might be diagnosed via means of complete blood count and iron status. Anemia is usually hypochromic and microcytic in nature.[18]

Keeping in view the hazardous effects of low haemoglobin level, present study was planned for the evaluation of pumpkin seeds effect on improving low haemoglobin level on rabbits.

MATERIAL & METHODS

This Experimental pilot study performed on rabbits at animal house situated in Peoples University of Medical and Health Sciences in Nawabshah after getting ethical committee approval (No.PUMHS/SBA/PVC/150) during the period from January 2020 to March 2020, Thirty (30) adult healthy rabbits were recruited for study after keeping inclusion and exclusion criteria as standard with age 16–24 months and weight 1.5 - 2.5 kg of the same genus and species (Oryctolagus Cuniculus Species, Order Lagomorpha). Sick rabbits, baby rabbits, pregnant and underweight rabbits were excluded from the study.

After selection, thirty (30) rabbits divided randomly into groups A, B and C. In each group 10 rabbits kept. Group A kept on fresh hay and water marked as a control group. While Group B and C given 250 mg and 500 mg pumpkin seeds powder correspondingly once a day along with fresh hay and water. For easy identification, were distinct appropriately with an identification number.

Fresh pumpkin seeds after purchase were ground to powder finely with home electric grinder. The powder packed in the plastic wrap after being measured on an electrometer and the packages stored on room temperature. The calculated amount of pumpkin powder of 250 mg and 500 mg fed one time daily for 60 days to study groups correspondingly. The rabbits feed in a sterile atmosphere. The seed powder initially mixed with 10 cc tap water and then dispensed directly into the rabbit's mouth with the help of a feeding syringe and it was checked to ensure that it was properly empty. Rabbits were weighed on a weighing machine (Compact, HYTEK) intermittently while taking sample.

BLOOD SAMPLE COLLECTION SCHEDULE

Blood sampling was done at a standardized level. First sample was marked as day 0 (at time zero). Thereafter samples were drawn every two weeks on the 15th, 30th, 45th and 60th days. Blood sample was taken from rabbit's ear by venipuncture and thereby transferred to tube contains the anticoagulant (EDTA) for CBC test. These samples analyzed at DR Lab of PUMHSW Nawabshah.

For Complete Blood Count (CBC), Automatic Hemoglobin analyzer (Nihan Kohden Mek-6420 k, Japan) used. These parameters can also be analyzed by the methods applied for assessment in a common laboratory.

For all observations, Data are expressed as mean ± SEM and evaluated statistically by group by means of correlated pairs t test using SPSS version 21.0. The data is accessible in the form of tables, graphs. For all comparisons, upto 0.05 P value was considered significant.

RESULTS

At the start of the study, all the rabbits were healthy. None of them had any disease. No side effects were seen in study groups B and C during the study, maintained on pumpkin seed supplementation at 250 mg and 500 mg per day correspondingly.

On day 0, hemoglobin, erythrocyte and HCT mean values were high as compared to the B and C groups. These provide a basis for a better evaluation of the effect of pumpkin seed supplementation or treatment in those with low or deficient hemoglobin indices (Table-I).

On day 0, Haemoglobin mean values were not statistically significant in the study groups compared to the control group.

Parameter	Group A (Control) (n 10)	Group B (n 10)	Group C (n 10)
Haemoglobin (%)	11.08±0.14	10.47±0.31	10.93±0.17
RBCs (million/cmm)	5.52±0.11	5.12±0.15	5.19±0.18
HCT (%)	33.48±0.54	32.08±0.82	33.26±0.50
MCV (fl)	60.38±1.19	62.84±1.02	63.92±1.42
MCH (pg)	20.16±0.25	20.48±0.14	21.0±0.50
MCHC (g/dl)	32.89±0.32	32.66±0.55	32.82±0.19

Table-I. Evaluation of parameters on day 0 of control among groups B and C (Baseline) (n=30)

Day	Group A (n 10)	Group B (n 10)	Group C (n 10)
0	11.08 0.14	10.47 0.31^{NS}	10.93 0.17^{NS}
15	11.29 0.31	12.60 0.31***	12.37 0.14
30	11.67 0.11	13.17 0.22***	12.46 0.14
45	12.22 0.13	13.45 0.17***	13.17 0.21
60	12.19 0.13	13.76 0.21***	13.48 0.20

Table-II. Association of haemoglobin in groups B and C from day 0 to day 60 as compared to control (n=30)

Whereas from day 15 to day 60 there was a collective increase in mean hemoglobin values in the two study groups in all successive readings observed every two weeks. All these observations established to be highly statistically significant when compared with the control. It highlights the hematopoietic effect of pumpkin seed on iron deficiency anemia cases (Table-II). Figure-1 reflects the graphical illustration of the these interpretation.

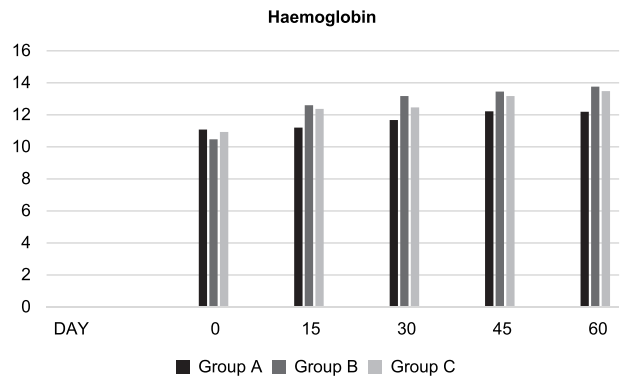

Figure-1. Association of haemoglobin of groups B and C from day 0 to day 60 as compared to control

DISCUSSION

Plants remain the main source of medicines. For protective and medicinal purposes these are used in the world.[19] Its chemical components affect the body's systems.[20] Proteins from plants can be a source as alternative to animal proteins.[21] Plant seeds are also good source of edible oils.[22] Due to high expenses of animal protein, researchers are now researching for the new sources.

A study conducted make comparison of consumption of 30 grams per day of iron fortified cereal yielding 7.1 mg and kernels of pumpkin seed yielding 4.0 mg iron correspondingly used for 28 days. After the consumption period, the level of iron in the blood increased indicating an improvement in the iron status. However, it may be recommended in a restricted dose.

The presence of white blood cells, platelet count and weight, as well as hemoglobin indices were also observed. Initially on day 0, hemoglobin in group A was higher than in group B and C. It might be because of physiological differences. It be a positive point, with lower hemoglobin there is a greater chance of absorbing and utilizing the nutrients found in pumpkin seed.

On consecutive reading on day 15, 30, 45 and 60, there was a very significant collective boost in hemoglobin level in groups B and C as compare to the control group. A slight non-significant increase in hemoglobin was also observed in the control, because they also were on hay feed, which contains at some levels iron and various nutrients. When comparing group B and C which were kept on pumpkin seeds at a dose of 250 mg and 500 mg respectively, a non-significant difference in hemoglobin level was observed. Therefore, despite the significant increase in hemoglobin level in both groups, the required results could be obtained at a low dose (250 mg/day). These comments are consistent with

the study of Malgwi et al (2014)[5], Yongabi et al (2014)[23] who observed an increase in hemoglobin level in rats.

That conclusion is in conflict with Lawal et al (2015)[24] study, who conducted a study on Rats by keeping them at two different doses. He noticed a significant variation in their hemoglobin level. It could be due to the surrounding environment that can influence the study results.

CONCLUSION

Pumpkin seed is a good nutritional plant source and can be replaced by traditional methods used to get better hemoglobin in cases of iron deficiency anemia.

RECOMMENDATIONS

Studies in humans are recommended to evaluate the proven benefit of pumpkin seed in human at various levels. It is economical and patient acceptance will be on high due to the lack of side effects over the optimum dosage. It also prevents the loss of precious seeds after using pumpkin.

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2	Ghulam Mustafa Dahri	Data analysis, Discussion.	
3	Ali Gul Tunio	Data analysis.	
4	Gunesh Kumar	Analysis, Introduction.	
5	Habibullah Shaikh	Introduction.	
6	Ghulam Sarwar Shaikh	References.	