Original Research Article

Body weight, hematological and biochemical parametersof wistar albino rats fed with chia seed concentrate

Abstract

Chia seeds are among commonly used natural remedies to control obesity, diabetes mellitus and cardiovascular diseases. A research was conducted to evaluate the effects of chia seeds on Body weights, hematological parameters, blood glucose, and lipid profilesin Wistar rats.In the current study,Wistar rats were fed on chia seed powder mixed with broiler chicken feedmash at concentrations of 0, 5,10,15 and 20%. Body weights, hematological parameters, blood glucose, and lipid profiles were monitored for 28 days.Results indicate that for the treated groups, there was significant increase in white blood cell (WBC) count and lymphocytes in a dose dependent manner (R^2=0.87; p=0.032).Plasma glucose and lipid profiles also decreased significantly in an dose dependentmanner, whereby, for glucose, group 4 rats-decreased from 138.5 ± 0.8 to 80.3 ± 12.9 mg/dLfrom day 0 to day 28 of treatment. The results show no significant difference on weight gain in chia treated groups compared to control group. There were no significant variations in the other hematological parameters.In conclusion, findings in this study illustrated thatchia seeds can be the good candidate for controlling blood glucose, lipid profiles and improving hematological parameters and alsobody weightin animals.

Keywords: Chia seeds, blood glucose, lipid profile, rats

Introduction

Recent reports from the WHO [1] have indicated increasedmortalities of up 17.9 million deaths per year attributable to metabolic disease conditions such as obesity, diabetes mellitus and cardiovascular diseases, where, about 1.5 millionsof the deathswere caused by diabetes mellitus. Treatment of metabolic disorders often requires changes of lifestyle habits such as consumption of healthy diets and engaging in routine physical exercises[2][3]. However, it can be challenging for patients to adopt this lifestyle and faithfully adhere to it.

Despite the several industrial medications available to treat some metabolic disorders, there are still many challeges in treatment and management of these conditions[4][2]. For instance, finding the right medication and dosage for each patient can be a challenge. Additionally, some patients have adverse reactions to certain medicationswhile others have limited access to healthcare resources, [5][6]. These challenges necessitate for search of alternative therapeutic approaches including functional foods supplementationtowards preventing and controlling these conditions and other associated health risks. Several herbs, spices and natural remedies have been used as alternative therapeutics to these medications[7][8][9]. Chia seedsare among the commonly used natural remedies for controlling obesity, diabetes mellitus and cardiovascular diseases [10][11][12]. The seeds have been in used for instance for medicinal purposes by Mexican communities, among others since 3500 BC[13]. Nowadays, Chia seeds have extended use beyond medicine as used by ancient communities and have many industrial uses including;Oil industry to produce edible oil and mayonnaise, beverage industry where the seeds and products are used to enrich beverages, milk industry where it is use in butter, cheeses, and fresh dairy products such as yoghurt, packaging industry where the seeds are used to produce biodegradable films, meat, snacks and extrusion industries among others[14][15][16][17].

Chia is an annual flowering plant that belongs to a family Lamiaceae. The seeds are known to contain mainly; protein, fat, carbohydrate, dietary fibre, ash and dry matter contents[18][19]. Theyalso contain phenolic compounds, minerals and omega-3 fatty acids, and α-linolenic acid[20]. Omega-3 fatty acid are essential fatty acids known to have a large number of

physiological functions in human body[21][22],for instance, they function asantioxidants attributable to chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol [23]. These agents are believed to have renal, cardiac, hepatic protective effects, anti dyslipidemia, anti hypertension, anti-ageing and anti-carcinogenic characteristics [24][10].

Chia seed arealso known to improve insulin sensitivity, maintenance of bone mass, decrease inflammation of the tissue and decrease vascular reactivity[25][26][7]. They also contain high fiber content important in the control body weight gain and obesity[27][28]. Chia seeds however have a hardy seed coat which makes the hydrolysy of the coat lipids and proteins difficult hence difficult to release the bioactive components of the seed matrix. This makes an overall difficulty in bioavailaility of nutrients from the seeds [29].In addition, they are small in size and lowly cohesive, making them difficult to incoropearate into food and feed products [30].Processing of the seeds could simplify the release of the valuable chia seeds nutrients making them bioavailable. Grinding of the seeds is one of the simple ways to expose the seeds matrix from the hard seed coat to make the nutrients bioavailable [30] while preserving them. In this research we used chia seed powder after sun drying and mixing itwith commercial broiler chicken mash to compose experimental animalfeeds.

In this study, weevaluated the effect of ground dried chia seed onbody weight, selected hematological and biochemical parameters usingwistar albinorats as model animals.

Materials and methods

Study area

The study was conducted at College of Veterinary Medicine and Biomedical Science (CVMBS), Sokoine University of Agriculture (SUA), Morogoro.

Research design

An experimental study was done where, mature healthymale and female wistaralbino rats were fed on different percentages of chia seed meal and broiler chicken mash as basal diet to study the effects of the seeds on body weight and selected hematological parameters.

Chia seed meal preparation

Five chia seed feed formulations were prepared for the experiments at concentrations of 0, 5, 10, 15 and 20%in commercial broiler chicken feeds mash. The above formulations were prepared by addition of 0, 50, 100, 150, and 200g of chia seed powder to make 1,000g of the commercial broiler chicken mash for different feed formulations making up five formulations refered to as formulation 0 (F0) to formulation 5 (F4).The feeds were compounded three times (once a week) and stored in cool dry place without direct sun lightthrought the experiment period.

Preparation of experimental animals

A total of 100 mature male and female wistaralbino rats weighing between 180 to 200g were used for the experiments. The rats were divided into five subgroups each with 20 wistaralbino rats, designated as group 0 (G0) to group 4 (G4).G0 was used as the control group while G1-G4 were the treatment groups. All therats were housed in zoo-sanitary cages and had full access to potable tap drinking water and the formulated broiler chicken mash feeds with chia seed powder. Feeding the rats wasas follows:G0(Control group) was the control group and was fed on F0. The rest of the groups were fed on F1 for G1 to F4 for G4 respectively daily for 28 days. The room temperature and humidity conditions in the experimental rooms were adopted to the general environmental weather conditions at averages of 26°C temperature and 75% humidity of the theSokoine University of Agriculture environmental conditions. Dark and light conditions also adopted the environmental which approximated to 12 hours of light (day) and 12 hours of darkness (night).Windows to the experimental house were open and closed to provide the light and dark cycles.

Data and samples collection

Data was collected on feed intake andbody weights. These were recorded daily for all the rats in all cages during the entire period of the experiment.Whole blood samples were also collected. For the weights, the rats were weighed to the nearest grams using the Mettler PE 6000® Switzerland, weighing scale. For the blood samples, approximately 4 ml of intracardial blood samples were collected using procedures explained by Parasuraman*et al*.,[31] an approach to

collection of blood samples in small laboratory animals. The blood samples were collected on days0, 7, 14, 21 and 28 of the experiment. Briefly, the rats were anasthetised in chloroform where a piece of gauze was soaked in 1 ml of chloroform and introduced into a bell jar and closed with a lid. Each time of sampling, a rat was introduced into the jar for about sixty seconds to anasthesize the rats. Blood samples were collected through cardiac puncture of three rats from each group on every sampling day.About 4ml of blood was collected from each ratthrough cardiac puncture whereabout 1ml of the blood were transferred into vacutainer tubes containing ethyl diamine tetraacetate (EDTA) for haematological parameters analyses. 2ml of the blood samples from the respective rats and groups were immediately centrifuged at 3,000 revolution per minute (rpm) for 10 minutes to obtain fresh plasma. The plasma samples were used for analyses of hematological andbiochemical parameters using spectrophotometer (Cole Patner 1,100 rs). All sample collections were done in the mornings between 7 and 9 AMof the sampling day in order to reduce on diurnal variations that could affect the haematological parameters.Blood samples were collected in vacutainer tubes with anticoagulants and later analysedfor haematological parameters using theMS™4-s, (MS laboratories, France)automated hematological analyzer.For glucoseassays, the blood samples were collected in sodium florinated (NaF)vacutainer tubes to preserve glucose while for the other biochemical parameters, the blood samples were collected in heparinized vaccutainertubes.The blood samples were centrifuged to obtain plasma which were stored at 4°C prior to assays.

Determination of plasma glucose and lipid profiles

Blood glucoseand plasma total lipid profiles were done by assays of glucose and lipids using the Trinders methods [32] in the blood plasma. Briefly, for glucose assays, the Erba®Mannheim,(ErbaLachemas.r.o., Karásek 1d, 621 00 Brno, CZ) glucose kit which employs theglucose-oxidase principle were used. Plasma samples andTrinders reagents were added together and mixed thoroughly for about five seconds in a vortex mixer and immediately incubated for five minutes at in dark at 37°C. Together with glucose standard, the mixtures wereimmediatetly readfor absorbancesin the ColePalmer®1100RS Spectrophotometer (Product of the United Instrument Inc.).spectrophotometer following blanking of the spectrophotometer

with distild water.Glucose concentrations were determined using the formula, "Concentration of glucose = Absorbance of Sample/Absorbance of Standard * Concentration of Standard". For the lipid profiles, the Trinderscalorimetric enzymatic methods by (CELLBIOLABSINC., Creating Solutions to Lifescience Research)kits for HDL and LDL/VLDL cholesterol were used. Briefly, samples and cholesterol standards were incubated for 45 minutes and then readin the calorimeter. Concentration of the lipids in the samples were compared to the known cholesterol stardard curve. Values were expressed on mg/dl.

Data analysis

Data were statistically analyzed to check for differences of the means among the groups using one way analysis of variance (ANOVA). Differences were considered significant when *P values* were equal to or less than 0.05. Differences between the means of the groups were also analyzed for significancy using the student's *t*-test and a Tukey's Honesty Significance Difference (Tukey HSD) where differences were considered significant at P equal or less than 0.05.The means were expressed with there corresponding standard error of the means (mean ± SE). The data were analyzed in the statistical package for social sciences (SPSS) version 29, 2022 [33].

Ethical Clearance

The study and all procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Ethical Clearance Committee from the Directorate of the Post graduate, Research and Consultancy Committee of Sokoine University of Agriculture.

Results

Body weight

Feeddconsumption and body weight measurements were carefully monitored in all groups of rats throughout teh experimental period.Feeds consumption and body weight gain results for the control and experimental groups are indicated in Table 1. From the results,,mean body weights

of the rats in the control group wereslightly lower than, but not significantly different (p=1.069) from the treatment groups.

Table 1:Means ± SE of body weights (g) for control of the rats which were fed on basal diet and treatment groupswhich were fed on chia seed feed formulations for 28 days.

Groups	Treatment time in days				
	0	7	14	21	28
G0	155.9±16.5	193.8±14.5	177.9±12.3	179.7±12.6	185.3±12.3
G1	190.6± 15.2	182.5±17.7	180.5±12.1	173.1±10.5	175.1±14.2
G2	178.7± 1 4.3	183.4±12.8	170.3±17.5	175.0±14.4	167.5±21.7
G3	189.9± 12.9	183.7±17.8	184.0±12.3	178.6±21.2	165.9±13.4
G4	178.7±12.7	173.4± 22.0	165.3±13.5	177.0 ±14.4	177.9 ±11.7*

* = the mean of body weight differed significantly from the rest of the group means

Hematological results

Generally there were no signicant differences(P>0.05) in hematological parameters among the rats groups as inidcated in Table 2. the for red blood cells (RBC) count, blood hemoglobin concentration (Hb) and hematocrit (Hct). But there wassignificant dose dependent increase (p<0.05) in the white blood cell count (WBC), lymphocytes (LYM) and monocytes (MON) concentrations. The mean platelet volume (MPV) and platelets (PLT) were observed tobe significantly lower than (p = 0.008) the control group G0.

Table 2. Mean hematological parameters before and 28 days afteradministration chia seed in rats

Parameters	**G0**		**G1**		**G2**		**G3**		**G4**	
	Day 0	**Day 28**	**Day 0**	**Day 28**	**Day 0**	**Day 28**	**Day 0**	**Day 28**	**Day 0**	**Day 28**
WBC(M/mm 3)	5.9±0.3	5.8 ± 3.7	5.6 ± 1.0	6.2 ± 2.9	5.8 ± 0.5	8.5 ± 3.1	5.3 ± 3.4	10.1 ± 2.4	5.9 ± 0.3	7.7 ± 0.3
Lym(%)	60.8 ± 1.8	57.1 ± 10.5	66.0 ± 18.2	64.7 ± 12.0	59.9 ± 6.	68.8 ± 9.3	61.8 ± 3.1	68.6 ± 14.2	60.8 ± 1.8	68.9 ± 2.3
Mon(%)	12.5 ± 0.5	16.2 ± 4.5	14.4 ± 6.2	17.5 ± 6.1	18.1 ± 1.1	14.6 ± 4.2	18.4 ± 0.1	13.0 ± 3.9	12.5 ± 0.5	14.5 ± 1.2
Neu(%)	6.7 ± 1.3	8.7 ± 4.6	9.6 ± 12.90	7.6 ± 4.3	14.3 ± 2.4	6.5 ± 5.0	6.55 ± 0.2	13.6 ± 5.5	6.7 ± 1.3	10.5 ± 0.9
RBC(M/mm 3)	5.8 ± 0.1	6.3 ± 0.0	5.1 ± 0.2	6.1 ± 1.1*	6.5 ± 0.1	5.7 ± 0.6	4.2 ± 1.52	6.12 ± 0.2	5.8 ± 0.1	7.5 ± 0.4
MCV(ft)	54.9 ± 2.5	65.4 ± 1.5	55.7 ± 0.2	55.1 ± 1.2	61.1 ± 0.4	55.5 ± 1.3	45.5 ± 15.5	60.0 ± 2.7	54.9 ± 2.5	62.4 ± 0.8
Hct(%)	31.9 ± 2.0	41.6 ± 1.5	28.7 ± 1.2	33.5 ± 5.3*	39.6 ± 1.0	31.9 ± 4.2	25.1 ± 9.5	36.6 ± 0.4	31.9 ± 2.0	46.0 ± 2.2
MCH(pg)	24.2 ± 0.8	24.0 ± 0.1	25.7 ± 0.3	24.5 ± 1.7	24.2 ± 0.9	25.7 ± 1.5	20.2 ± 7.0	24.8 ± 1.8	24.2 ± 0.8	22.2 ± 0.5
MCHC(g/dL)	44.3 ± 0.5	36.7 ± 0.6	46.5 ± 0.3	34.5 ± 7.7	39.7 ± 1.8	46.5 ± 3.7	33.45 ± 11.3	41.3 ± 1.1	44.3 ± 0.5	35.7 ± 0.3
RDW	11.9 ± 1.3	14.3 ± 0.7	11.4 ± 0.3	11.2 ± 0.5	10.9 ± 0.7	12.1 ± 0.2	10.0 ± 0.6	10.4 ± 0.1	11.9 ± 1.3	10.8 ± 1.0
Hb(g/dL)	14.1 ± 0.7	15.2 ± 0.2	13.2 ± 0.4	14.9 ± 1.4	15.7 ± 0.3	14.7 ± 0.7	11.2 ± 4.2	15.1 ± 0.5	14.1 ± 0.7	16.5 ± 0.6
THR(M/mm 3)	1034.5 ± 51.5	2310.5 ± 46.0	1337.5 ± 16.5	1221.0 ± 61.0	1265.5 ± 36.4	1961.0 ± 87.0	1427.0 ± 36.0	997.7 ± 107.2	1034.5 ± 51.5	882.2 ± 12.2
MPV(fl)	9.0 ± 0	8.8 ± 0.0	8.6 ± 0.15	8.7 ± 0.1	8.9 ± 0.2	8.25 ± 0.1	7.6 ± 0.1	6.7 ± 0.2	9.0 ± 0	6.4 ± 0.0
PDW	7.6 ± 0.1	7.7 ± 0.0	8.4 ± 0.25	8.2 ± 0.5	6.85 ± 0.0	8.1 ± 0.0	6.4 ± 0.1	0.7 ± 0.1	7.6 ± 0.1	0.6 ± 0.0

Plasma glucose concentration

At baseline, the plasma glucose levels were not significantly different between the groups.The level of plasma glucose was observed to decrease significantly ($p<0.05$) in all treated groupsin a dose dependant manner. The decrease was more prominent on 7th day ofG3 and G4 (R^2=0.78 p = 0.032).

Table 3:The table indicates the mean plasma glucose levels (Mean ± S.E) for the control group of rats fed on basal diet treatment group of rats fed on different chia seed feed formulations()

Groups	**Treatment days**				
	0	7	14	21	28
G0	105.7±4.2	111.5±3.1	118.7±20.5	117.6±2.7	119.9±9.9
G1	113.5± 3.5	122.8±2.6	123.8±7.5	73.5±6.3	61.3±10.9
G2	116.5±2.0	118.7±4.7	122.1±14.9	102.9±5.6	80.3±12.9
G3	131.1±1.8	158.1±9.6	131.3±1.1	86.2±7.5	83.1±14.1
G4	138.5±0.8	110.7±4.7	107.1±4.9	89.9±5.9	80.3±12.9

Note: Normal value 76- 175 mg/dL

Plasma cholesterolconcentration

Results in figure 1 illustrates that there is significant in all lipid profile in a dose dependent manner (R^2=99.1; p = 0.000335) compared to the control group G0.The decrease was more in the low density lipoprotein (LDL) and very low density lipiprotein (VLDL).

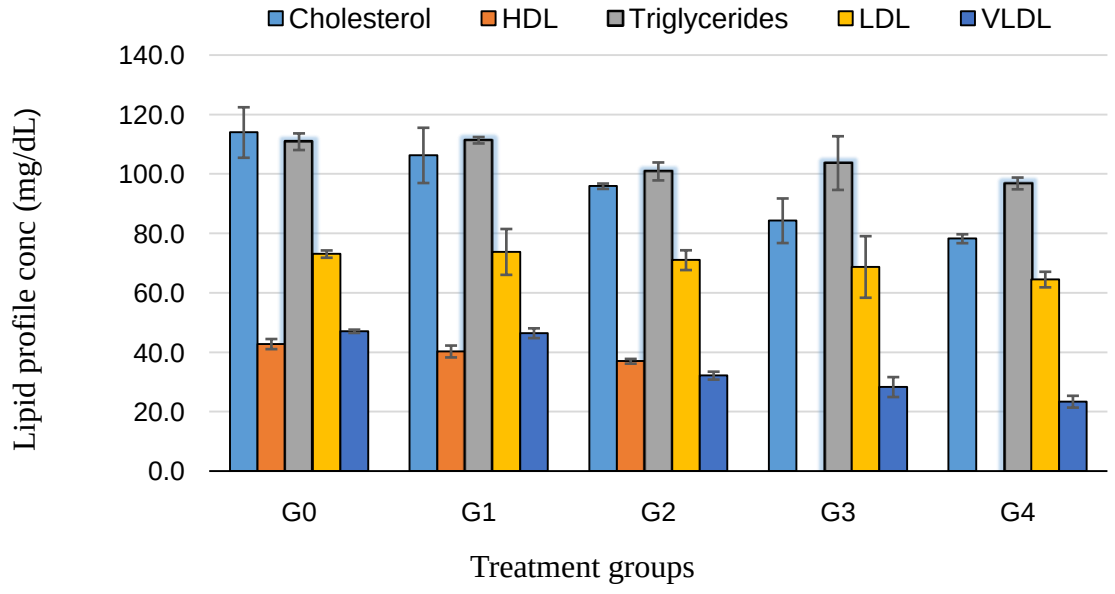

Fig 1: Plasma lipid profile of ratsfed with chia concentrate (Mean±S.E)

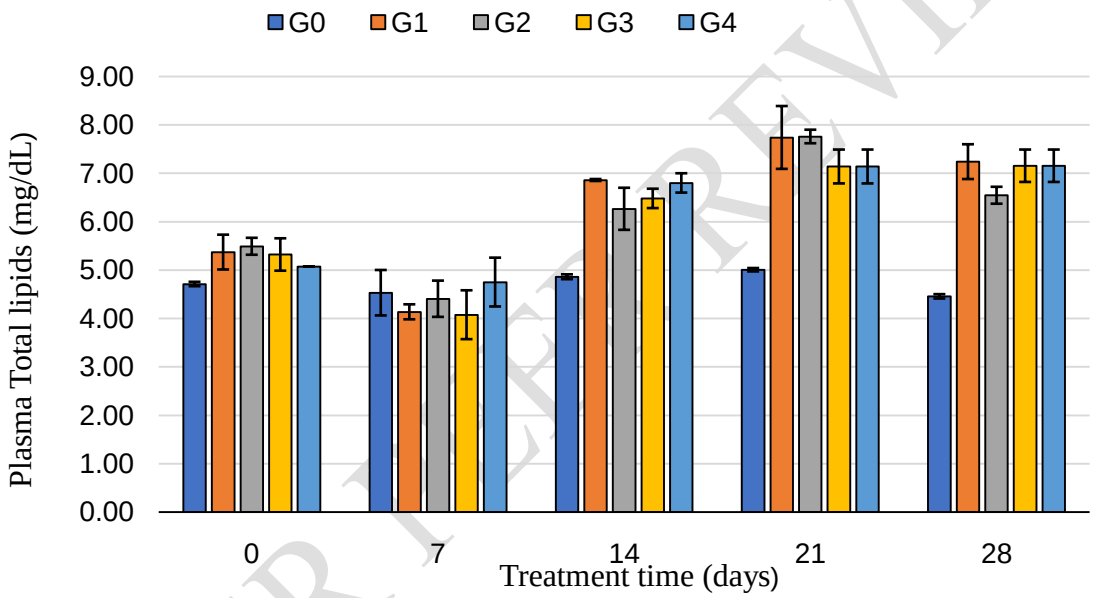

Fig 2: Plasma total lipids of rats fed with chia concentrate (Mean±S.E)

For plasma total lipids, results indicated there was significant increase (p=0.048) in the levelas compared to the control group G0.The level ranged from 4.71 to 7.16 g/dL for G0 to G4 that is, the rats fed with high concentration of chia seed concentrate. For the control groups, the value does not change insignificantly.

Discussion

The current study evaluated the effect of chia seed on body weight, some selected hematological and biochemical parameter using rats as model experimental animals. From thestudy results revealed that the chia seed concentrate had no significant change in body weight of rats fed with chia seed concentrate as compared to the control group. The results concurred with the findings of the study done by Marineli*et al.*,[34], Da Silva *et al.*,[35]and Alamri, [36]who reported insignificant variation of body weight following inclusion of chia seed in feeds.In the current study, the mean body weights for treated rats were lower than the control rats, however the differences were not significant, contrary to observations by other researchers who reported significant weight loss in rats fed on chia seeds. The weight loss in the other studies were linked to the presence of high fibre content in the seeds (more than 80%) [37]. This could also explain the observed weight loss in the rats in this current study. In the current study, dietary supplementation of chia seeds resulted into significant increasedin mean values of red blood cell indices such as RBC count, Hb, Hct and. RDW compared to the control group (G0). This could be linked to the high levels of micronutrients such as iron and calcium in the body [38] which are among factors promoting blood formation. The increased level of white blood cell indices observedsuch as WBC, lymphocytes and monocytes was the indication that chia seeds are capable of boosting the immune system [39][7][40].

All these results are in agreement with the study done by Alarcon *et al.*,[41]Alagawany*et al.*,[42]Mihafu*et al.*,[43]. who reported improvement of hematological markers following dietary supplementation with chia seed in different animals. The decreased levels of platelets in blood could be due to effect of omega 3 fatty acids which is the important in reducing the content of arachidonic acid in membrane phospholipids of platelets, endothelial cells, and inflammatory cells. Thisreduces the amount of pro-inflammatory factors that are derived from arachidonic acid. Also Alpha-linoleic acid is found to down-regulate the activity of a nuclear factor NF-ƘB which plays an important role in generating inflammatory responses which is known to cause cardiovascular diseases[10][44][43]. Alpha-linolenic acid decreases the risk of thrombosis by inhibiting platelet aggregation. They act upon the m RNA expression of the growth factors of

platelets, thus stop abnormal growth and proliferation of platelets and play a key role in preventing formation of clots and plaques in the arteries thus elp to prevent cardiovascular diseases[10][44][43].The plasma glucose and lipid lowering effect of chia seeds have been reported in different animal models as reported from astudy byGian *et al*.,[45]. Diyah, [46] and Radica [2016] indicates that thelowereing of blood sugar and lipids by chia seeds is attributable to their high fiber content, and antioxidants present in the chia seed flavonoids. They further indicate that the fibre contents of the seedscannot be broken down or absorbed. The fibresbinds glucose molecules thus preventing it from rapid absorbtion from the gut, a fact that prevents glucose spikes in the blood that can culminate to high blood sugar, diabebetes and elevated high plasma lipids levels.

Conclusion: Findings in this study illustrated thatchia seeds can be the good candidate for controlling body weight, blood glucose, lipid profile and improving hematological parameters in animals. This which are marked as goodindicators for controlling metabolic diseases such as as type 2 diabetes mellitus, obesity and cardiovascular diseases are increasing and causes death of both livestock and humans. Further researches are necessary for more confirmation of chia seed on their mode of action and toxicity in animal body.

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