International Research Journal of Biological Sciences E-ISSN 2278-3202
Vol. **5(6),** 43-51, June **(2016)** *Int. Res. J. Biological Sci.*

Metabolic Impact of Selected Minor Millets Fed Experimental Menopause Induced Rats

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Available online at: www.isca.in, www.isca.me
Received 28th April 2016, revised 31st May 2016, accepted 3rd June 2016

Abstract

Menopause is nothing but the cessation of menstrual activity which is accompanied by lower levels of estrogen and which gradually leads to weight gain. Postmenopausal weight gain is an alarm for women's health which leads to hyperlipidemia and glucose intolerance. These events further lead to lifestyle-related diseases such as diabetes mellitus, arteriosclerosis, hypertension and metabolic syndrome. The aim of the present research was to study the effect of four less explored minor millets (Paspalum scrobiculatumL., Panicum sumatrense Rothex Roem. and Schult, Eichinochola frumentacea Link and setaria italic(L.) P. Beauv.) which are less exploited for research on the metabolic profile of Ovariectomized rats. Twelve female wistar albino rats were grouped into four and only one group was fed with the feed prepared from the flour of the four minor millets mixture. The study was carried for 3 months and the body weight was measured weekly and blood sugar, HbA1c and lipid profile were measured monthly, whereas the other biochemical parameters were measured at the end of the experiment. The minor millets fed group showed significant (p‹ 0.05) improvement in the lipid profile and blood glucose whereas the body weight was under control. Thus the minor millets proved to be effective against the pathogenic condition of metabolic syndrome at the Menopausal stage.

Keywords: Minor millets, Menopause, Ovariectomy, Blood Glucose, Lipid profile.

Introduction

Menopause is the event in which the ovarian follicles get exhausted; as a result there is a decrease in the production of estradiol and other hormones[1]. Further, the much reduced estrogen during menopause increases the level of free fatty acid, which makes postmenopausal women easily prone to the metabolic syndrome and insulin resistance, which are the risk factors for cardiovascular disease[2]. Metabolic Syndrome and Cardio Vascular Disease which are more common in older women with notable increase in individual risk factors in the postmenopausal phase[3,4].

Women have a more tough phase of old age than men because of the dominant effects of hormonal changes caused by menopause. However, the public health care system does not concentrate the special health needs of older women. There has been enormous research on menopause in the West but in India only a few institutes have recognised the importance of research on the subject[5]. Nutrition is a basic need for human and an important factor to lead a healthy life. From the very early stages of life, a proper diet is essential for proper growth, development and to remain active[6]. Symptoms of menopause may be decreased by altering their nutritional status, which is one of the important environmental factors to lead a healthy life during menopause[7].

At different stages of life, the human body changes and it requires unique eating habits to sustain normal physiological functions. As indicated by these diverse stages, our ancestors had different foods that were healthy and nutritionally rich. Dating back to Indian civilizations and Indian old literature, every community that lived in India had a clear and separate food belief system[8]. The increasing urbanisation, westernization and mechanization in most countries has led to a sedentary lifestyle and a diet having high energy-dense and high fat foods[9,10].

Small millets, grown as a complement to existing crops, could offer an answer to the malnutrition and non-communicable diseases. They have good nutritional properties, including high micronutrients, low glycemic index, dietary fiber content[11], low digestibility, low carbohydrate content and water soluble gum content (b-glucan) which improves glucose metabolism. Thus they release sugar slowly in the blood and also slows the glucose absorption[12]. Thus they exhibit hypoglycemic and hypolipidemic effects[13].

The bilaterally ovariectomized rat model is the most popular choice of animal model for the study related to menopause, as it has been proven to represent some of the most important clinical features like postmenopausal bone loss[14], cardiovascular dysfunction[15], metabolic changes[16] and oxidative stress (OS)[17].

Though there are more number of pharmacological studies carried out worldwide on minor millets, no research work have been carried out in ovariectomized rats, an animal model of menopausal complications. This research work was done to study the potential effect of four minor millets when consumed as combined mixture on clinical and biochemical and clinical parameters of the metabolic syndrome in experimental menopause-induced rat model. Thus the aim of current study was to determine the metabolic impact of selected four minor millets when consumed as feed by ovarictomized rats.

Materials and Methods

Plant materials: Minor millets *(Paspalum scrobiculatumL., Panicum sumatrense Rothex Roem. & Schult, Eichinochola frumentacea Link and setaria italic (L.) P. Beauv.)* were purchased from a local shop in Erode, Tamilnadu, India and was identified and authenticated by Dr. K. Althaf Ahamed Kabeer, Scientist-D, Botanical survey of India, Southern regional centre, Coimbatore, Tamilnadu, India. All the minor millets were taken in equal proportion, subjected to cleaning with water for 10 minutes and then they are rinsed with distilled water and then air-dried overnight in an oven at 40°C. The Minor millets and the rat pellets are ground to coarse powder and the powder was mixed with water to form dough which was rolled and cut as the size of rat chow pellet .Then the pellets were subjected to baking overnight in an oven at 40°C to dry out most of the moisture content so that it can be stored until the end of the study. The pellet was packed in a tight plastic container to keep it fresh and free from humidity[18].

Phytochemical analysis of the aqueous extracts of the selected minor millets and its mixture revealed the presence of phenolic compounds, Phytosterols, Carotenoids, Flavanoids, Calcium, iron, steroids, alkaloids, carbohydrates and glycosides in extract. It was also found to have antioxidant activity.

Animals: Twelve Female wistar albino rats weighing about 170– 200 g in the age group of about 90 days were used and acclimatized to the experimental room at temperature 23±2 °C, controlled humidity conditions (50–55%) and 12-h-light/12-h-dark cycle. Animals were caged with a maximum of three animals each in a polypropylene cage and were fed with standard food pellets and water ad libitum. Ethical clearance was obtained from the Institutional Animal Ethics Committee (Proposal No. NCP/IAEC/No:07/2014-15)) for carrying out the animal study at Nandha Pharmacy College , Erode , Tamilnadu.

Experimental design: After seven days of acclimation, the rats were ovariectomized (OVX) or sham operated. The rat were anesthetized with ketamine hydrochloride (70mg/kg i.m), the ovaries were removed ventrally. Sham operation was performed in same manner but only exposing the ovaries. On sutures broad spectrum antibiotic were applied for ten days. Ten days after recovering from surgical damage, Experimental animals were divided randomly into four groups of three animals each. Group 1 was sham operated which served as basal control. All the other groups were ovariectomized and received treatment for 11 weeks starting from the fifteenth day of ovariectomy. Group 2 received the normal rat pellet diet and served as ovariectomized control. Group 3 was orally administered with Estradiol valerate (progynova tablets - contain the active component estradiol valerate which is a naturally occurring form of the main female sex hormone, oestrogen) (2mg/animal/day) orally for 11 weeks. Groups 4 were fed with the feed prepared from the four minor millets Body weights of all animals were measured weekly. At the end of 11 week treatment, all the rats were deprived of food. On next day, blood samples from all the groups were collected through sinus puncture. The blood samples were centrifuged at 2500 rpm for 15 minutes to separate serum and preserved (-200c) for analysis of blood glucose, HbA1c, Hemoglobin and lipid profile. Soon after collecting of blood, the animals were sacrificed by cervical dislocation and the heart was carefully removed, cleaned and weighed and preserved in 10% formalin solution for histopathological analysis[19,20] .

Figure-1
SHAM operated Wistar albino rat

Biochemical assays of serum: The level of blood glucose, HbA1c and the lipid profile in serum were determined by automatic analyser (Cobas C 111) using standard methods.

Analytical procedures: Estimation of blood glucose was done by UV Test, Enzymatic reference with hexokinase method[21,22]. HbA1c was estimated by Eross et al method[23]. Estimation of serum cholesterol was carried out by the method of Roeschlau and Allain[24,25]. Serum triglycerides were estimated by the method of Wahlefeld[26] and HDL cholesterol was estimated by the homogenous enzymatic colorimetric assay[27,28]. The VLDL cholesterol was calculated using the formula, TG/5mg/dl. The serum LDL cholesterol was estimated by the homogenous enzymatic colorimetric assay, an automated method[29]. Atherogenic index was calculated by using the formula, TC-HDL-C/HDL-C[30]. Estimation of lipid peroxidation was done by Thiobarbituric acid reaction[31].

Figure-2
Ovariectomised Wistar albino rat

Histological analysis: Heart specimen was fixed in 10% formalin for 24 hours at room temperature. Then the tissues were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Thin sections (4 µm) of the tissue were mounted on glass slides, dewaxed, rehydrated with distilled water, and stained with hematoxylin, and eosin. As a part of the histological evaluation, all slides were examined by a pathologist under a light microscope, without any knowledge of the treatment underwent.

Data analysis: Results were given as mean ± S.D. Data were analyzed using T test and one-way analysis of variance (ANOVA). A p-value of 0.05 or less was considered as indicator of a significant difference.

Results and Discussion

The effect of minor millets on body weight, blood glucose, HbA1c and Hb are summarized in Table-1. At the beginning of the study the body weight, blood glucose and HbA1c of the OVX and Sham operated rats were not significantly ($P < 0.05$) different but after the start of the study OVX control group showed significant ($P < 0.05$) increase in the body weight whereas the minor millets fed group were able to keep the weight under control within three weeks of the start of the study itself. The blood glucose and glycosylated hemoglobin level showed a dramatic reduced level in minor millets fed group compared to the ovariectomised control group. The Hemoglobin level was much reduced after ovariectomy but after the beginning of the study, there was a gradual improvement in the hemoglobin level in the minor millets fed group.

Total cholesterol, LDL, TG and VLDL level in the serum of the ovariectomized control rats was found to be markedly higher than in the sham-operated controls (Table-2 and 3). Minor millets fed group showed significant ($P < 0.05$) reduction in total cholesterol, LDL, TG, VLDL and atherogenic index compared to the OVX control.HDL level was significantly ($P < 0.05$) increased in the minor millets fed group.

Table-1
Effect of selected Minor millets on Body weight and serum biochemical markers in Ovariectomised rats

Group	Body weight(g)		Blood Glucose(mg/dl)		HbA1c (%)		Hb (mg/dl)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sham operated	199± 8.66	250.33± 7.57	82.73± 2.05	82.73± 2.05	4.23± 0.25	5.1± 0.44	15.77± 0.25	15.73± 0.21
Ovariectomised control	199± 14.73	286.67± 9.87	114.33± 1.53	116± 1.55**	7.63± 0.35	7.77± 0.81**	9.97± 1.0	10.37± 0.47**
Ovariectomised + standard drug	194.67± 5.03	213.67± 18.01	115.5± 0.5	91.2± 0.72	7.43± 0.93	5± 0.2	10.47± 0.6	14.5± 0.5
Ovariectomised + minor millet fed	200±2	203± 4.73*	117.9± 3.01	80.37± 0.55*	7.93± 0.23	4.5± 0.3*	10.37± 0.57	15.3± 0.3*

Mean ± S.D (n = 3). **statistically significant ($P < 0.05$) from the sham-operated control group, * statistically significant ($P < 0.05$) different from the OVX control group.

Table-2
Effect of selected Minor millets on serum Lipid Profile in Ovariectomised rats

Group	TotalCholesterol(mg/dl)		HDL(mg/dl)		LDL(mg/dl)		TG (mg/dl)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sham operated	185± 5	183.33± 2.08	70± 2	69.33± 3.79	86± 6.56	88.33± 2.08	158.33± 9.07	168.67± 1.15
Ovariectomised control	251± 9.87	260± 10**	37.67± 2.52	37.67± 2.51**	179± 11.53	183.67± 1.53**	262.67± 11.02	268.67± 3.2**
Ovariectomised + standard drug	266.67± 3.06	175.33± 5.03	33.33± 2.31	67.67± 2.5	177± 8.19	87.67± 1.53	258.67± 6.03	158±2
Ovariectomised + minor millet fed	279.33± 19.22	174.33± 8.14*	38±2	71.33± 1.5*	182.67± 8.33	85.67± 2.08*	266.33± 5.51	156.67± 9.87*

Mean ± S.D (n = 3). **statistically significant (P < 0.05) from the sham-operated control group, * statistically significant (P < 0.05) different from the OVX control group.

Table-3
Effect of selected Minor millets on serum lipid profile and Atherogenic Index in Ovariectomised rats

Group	VLDL (mg/dl)		CHO / HDL		Atherogenic Index	
	Initial	Final	Initial	Final	Initial	Final
Sham operated	26.53±0.42	26±0.2	2.64±0.08	2.71±0.17	1.64±0.08	1.71±0.17
Ovariectomised control	52.53±2.2	53.73±0.64**	6.68±0.21	6.93±0.61**	5.68±0.21	5.93±0.61**
Ovariectomised + standard drug	51.73±1.21	25.73±0.12	8.02±0.61	2.59±0.15	7.02±0.6	1.59±0.15
Ovariectomised + minor millet fed	51.67±0.58	25.67±0.5*	7.35±0.41	2.44±0.14*	5.78±0.6	1.44±0.14*

Mean ± S.D (n = 3). **statistically significant (P < 0.05) from the sham-operated control group, * statistically significant (P < 0.05) different from the OVX control group.

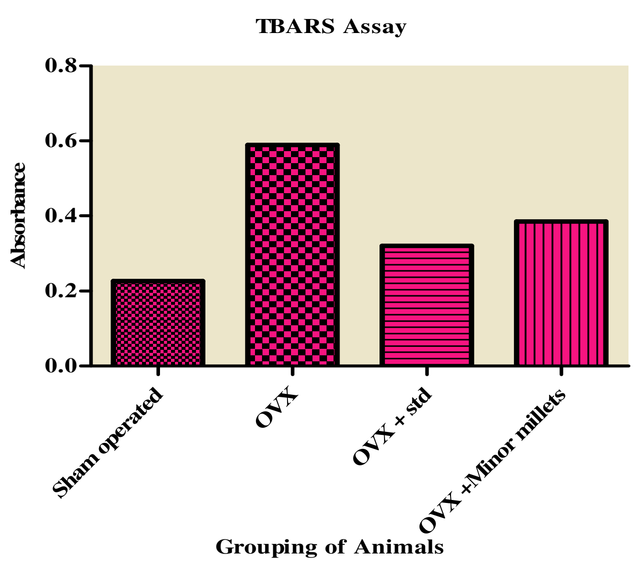

Figure-3
Effect of selected Minor millets on thiobarbituric acid reactive substances (TBARS) in Ovariectomised rats

Minor millets fed ovariectomised group showed decrease in the oxidative stress (TBARS). Present study result (Figure-3) showed significant increase in blood serum TBARS in Ovariectomised control rats compared to Sham control rats. The Standard drug (Estradiol Valerate) treated and Minor millets fed group prevented the formation of reactive oxygen species and induction of lipid Peroxidation by Ovariectomised rats.

Histopathology study: Histological examination of the hearts isolated from Group I, Group II, Group III rats revealed that cardiac muscle fibres were branched and regularly regularly arranged. They had pale acidophilic cytoplasm and apparent myofibrils. The myofibres showed a single vesicular nucleus in the centre (Figure-3). Histological examination of the hearts isolated from Group II rats revealed some areas with deeply acidophilic degenerated cardiac muscle fibres. The necrotic fibres revealed small pyknotic nuclei. Cardiac muscle fibres in some areas appeared in irregular arrangement with the destruction of their fibres. Mononuclear cellular infiltration was observed between the cardiac fibres.

Some blood vessels were ruptured and extravasated blood cells were observed in the interstitial tissue. Some damaged myofibres were seen as homogenous pale areas between the cardiac fibres (Figure-4). In contrast, the histological examination of the heart tissues isolated from Group III and Group IV rats revealed that most of the areas had regularly arranged branching and anastomosing fibres. Pale acidophilic cytoplasm was revealed. The nuclei were observed as single, central and vesicular; Destruction of few fibres was revealed with little connective tissue in between (Figure-5 and 6).

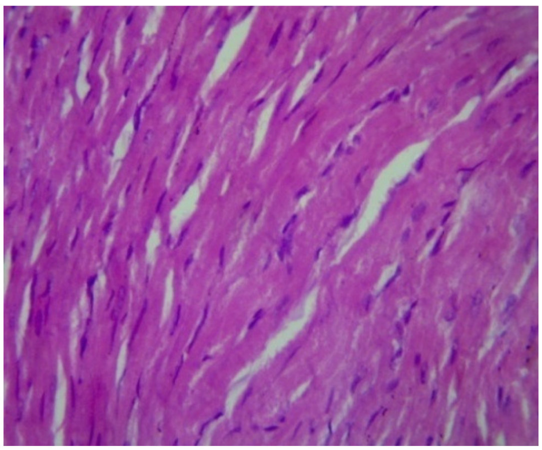

Figure-4
Photomicrograph (40X) of the heart isolated from Group I rats shows regularly arranged cardiac myocytes with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibres

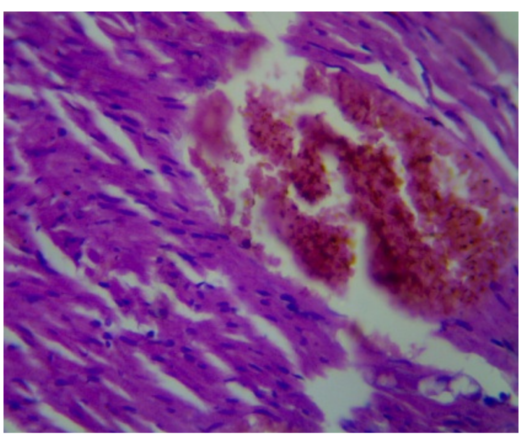

Figure-5
Photomicrograph of the heart isolated from Group II rats shows irregularly arranged cardiac myocytes with areas of degeneration and necrosis that are deeply stained, with blood extravasation and cellular infiltration

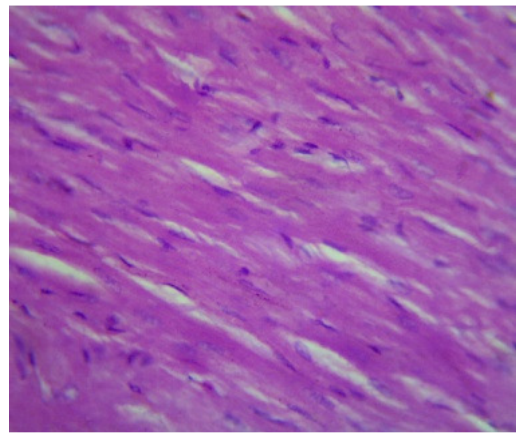

Figure-6
Photomicrograph(40X) of the heart isolated from Group III rats shows regularly arranged cardiac myocytes with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibres

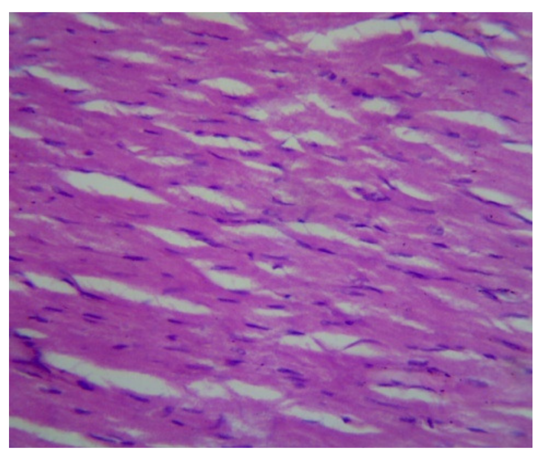

Figure-7
Photomicrograph (40X) of the heart isolated from Group IV rats shows regularly arranged cardiac myocytes with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibres

Discussion: Body weight, Blood Glucose, Hemoglobin and Lipid Profile: Postmenopausal status is associated with an increased risk of metabolic syndrome. Therefore, to prevent cardiovascular disease there is a need to evaluate metabolic syndrome and its components from the time of the menopause[32], in which Low HDL-cholesterol level, high abdominal obesity and low-grade chronic inflammation[33] are the most frequent characteristics in comparison to other metabolic components. The increased body weight in ovariectomized rats is well-documented in many studies[34,35]. Some previous studies in ovariectomised rats showed that estrogen deficiency significantly led to weight gain[36,37]. The results of the present work revealed the weight control ability of the minor millets in ovariectomised rats.

Estrogen also decreases blood glucose and glycosylated hemoglobin, a marker of long-term vascular damage in diabetes[38,39]. Hemoglobin level was found to be much reduced in ovariectomised rats which might be due to the increased formation of HbA1c. In poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin[40]. HbA1c was found to increase in patients with diabetes mellitus to approximately 16% and the amount of increase was directly proportional to the fasting blood glucose levels[41]. So, the measurement of HbA1c is a very sensitive index for glycemic control.

Total cholesterol, LDL-C and triglycerides were elevated and HDL cholesterol was decreased in post menopausal women[42,43]. Lipid profile and atherogenic index have been shown to be strong (significant) predictors for metabolic disturbances including Dyslipidemia, atherosclerosis, hypertension and cardiovascular diseases. Any changes in the levels of lipids make the individuals more likely to develop atherosclerotic cardiovascular diseases as well as endothelial dysfunction[44]. High density lipoprotein removes excess cholesterol from peripheral tissues and transports it to the liver, thus by cholesterol reverse transport, cholesterol homeostasis will be maintained[45,46].

Lowering of serum lipid levels by dietary or drug therapy seems to be associated with a reduced risk of vascular disease[47]. One of the major risk factors for developing cardiovascular disease is the elevated cholesterol. Ovariectomy lead to undesirable changes in the lipid levels thus leading to atherosclerosis and coronary heart disease[48,49]. The present work investigated whether the selected minor millets were effective in reducing the increased serum cholesterol level in ovariectomised rats.

Deficiency of estrogen have an indirect effect on lipid profile[50]. Coronary heart disease is directly linked with increased LDL cholesterol and inversely linked with increased HDL cholesterol[51]. Millets have been reported to be the rich sources of dietary fibre[52], resistant starch and low glycemic response[53,54] thus attributed to exhibit hypoglycemic and hypolipidemic effects[55,56]. Minor millets, with their low carbohydrate content, low digestibility and water soluble gum content (b-glucan) have been reported to improve glucose metabolism. These grains release sugar slowly in the blood and also diminish the glucose absorption[57,58]. These grains have also been demonstrated to exhibit beneficial effects on cholesterol levels, which is also due to their high dietary fibre and phytochemical content.

Elevated plasma glucose develops Reactive Oxygen Species (ROS) which leads to oxidative stress[59]. Oxidative stress leads to the pathogenesis of hypertension[60]. The beneficial effects of antioxidants in releaving oxidative stress and reducing elevated blood pressure is shown in various clinical studies[61]. And the antioxidants have also been proven to reduce the oxidative stress accompanied by decreased levels of plasma glucose and glycosylated hemoglobin[62], which is supported by the hypoglycemic effect of honey accompanied by reducing oxidative stress[63].

Similarly, the antioxidative properties of minor millets against hyperglycemia and oxidative stress are due to rich reserves of macro nutrient, micro nutrient and phytochemicals like phenolics, tannins, phytates, etc[64,65].

Histopathological examination of heart: In the present study, minor millets fed group appears to have preserved the myocardial structure and function. The histological examination of their hearts revealed less fibre destruction and a cardiac structure similar to the sham-operated rats due to less inflammatory changes and cellular infiltration.

Conclusion

Minor millets may be minor in their size but superior in its nutrients. They contain many health beneficial components which have been shown to reduce many degenerative diseases of the mankind like diabetes mellitus, cardiovascular diseases, cancer, etc.

This age of mankind can be better termed as “diseased period” because we are suffering from innumerable diseases of which source and cause are still under dark. We are not living but dying each and every second of our life by the fear of attack of the disease. Thus the only solution to be freed from such panic is to change the food habit and consume nutrient foods like minor millets.

Acknowledgement

I express my gratitude towards Dr C. Sengottuvel, Assistant professor, Nandha Pharmacy College, Erode for their personal interest, care, attention, continuous guidance and support in carrying out my research work. I like to thank my mother Institution Navarasam Arts & Science College for Women, Arachalur, Erode for being my backbone in all walks of my research work.

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