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Hypoglycemic and Hypolipidemic Effects of Watermelon (*Citrullus Vulgaris*) Seed Kernels on Male Albino Rats

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Abstract

The current investigation was planned to assess the hypoglycemic and hypolipidemic efficiency of watermelon seeds on male albino rats. Eight male albino rats weighing between 105-150g were randomly selected for the present experiment and were placed into two equal groups (n=4) designated as control and treated group. The control group received normal stock diet (20% protein, 5 % fat, 60 % carbohydrate). The treated group received a modified diet (90g Stock diet excluding groundnut oil + 9g watermelon seed kernel + 1g sugar). Percentages of protein, fat and carbohydrate in the modified diet were kept same as that of the stock diet. All animals were given water *ad libitum*. Rats were weighed at weekly intervals. After 28 days rats were anesthetized using chloroform anesthesia and blood samples were collected via cardiac puncture and serum was obtained for evaluation of some biochemical parameters. Result showed that serum glucose, triglyceride (TG) and very low density lipoprotein (VLDL) of the treated group were decreased significantly ($p < 0.01$, $p < 0.05$ and $p < 0.05$ respectively) compared to that of the control group. Liver glycogen, serum total cholesterol, low density lipoprotein (LDL) and AI (Atherogenic Index) were decreased (non-significant) whereas high density lipoprotein (HDL) increased (non-significant) in the treated group.

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Keywords

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Liver glycogen,
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Introduction

Dual burden of malnutrition exists in the Indian society. According to NFHS 3 (2005-2006) diabetes is highly prevalent in urban areas (1.374% women and 1.383% men) than in rural areas (0.641% women and 0.86% men). World Health Report 2002 speculated that cardiovascular diseases (CVDs) will be the largest cause of death and disability by

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2020 in India. On the other hand the magnitude of the problem found to be even more serious while giving a glimpse on national nutrition data. According to NFHS 3 (2005-2006) 40.4% children of below 3 years were underweight. Among the adults 36% of women and 34% of men are undernourished, with a BMI less than 18.5. This indicates an alarming situation of nutritional deficiency. In this present scenario recently more attention has been focused to recover valuable components from neglected food parts (food "losses", "wastes", "by-products") and reintroduce them inside the food chain. Watermelon *(Citrullus vulgaris*) seed, whose cultivar name is Sugar baby is one of such component.

Sample Description

Watermelon seeds are easily available during summer season. Though it is not an oilseed but researches have shown that these seeds contain about 52.6% oil[1], so these are good source of energy (628k.cal). C. Gopalan has listed watermelon seeds under nuts and oilseeds. *C. vulgaris* seed kernel contains highest amount of protein (34.1g/100g) compared to that of any other oilseeds. It also contains considerable amount of Arginine (900 mg/g of N) and branched chain amino acids (leucine, isoleucine and valine)[1].

Inspite of being highly nutritious and having some known medicinal values, these seeds have still not recognized and are less acceptable amongst the people as compared to other oilseeds. Previous work showed that watermelon seed kernel promotes growth due to the presence of good quality protein which was evidenced by significantly increased Protein Efficiency Ratio (PER) among the male albino rats, and therefore may be useful in prevention and treatment of Protein Energy Malnutrition (PEM)[2]. The nutritional importance of the seed kernels of *C. vulgaris* have been found in some discrete studies, but little work has been done on the effect of the seeds of *C. vulgaris* on different bodily systems of animals or humans. Hence the objectives of the present research are to:

- prepare a modified diet of male albino rats by adding watermelon seed kernel to the stock diet instead of groundnut oil so that its protein, fat, carbohydrate and calorie content would be same as that of the stock diet.
- study the effects of *C. vulgaris* seed kernels on serum glucose and liver glycogen of male albino rats.
- study the effects of *C. vulgaris* seed kernels on serum lipid profile of male albino rats.

Materials and Methods

Collection of Seeds and Preparation of Sample

Watermelons of specific species (*C. vulgaris*) were purchased during summer season (April to June) from local markets of Chetla and Rashbehari, Kolkata. The watermelons were cut with a clean, sharp knife, and the seeds were handpicked from them, washed thoroughly with tap water and then dried in shade at room temperature. Then the seeds were screened to remove the bad and immature ones. Finally the seeds were dehusked in a decorticator and the seed kernels were separated from the seed coat. The seed kernels were grounded in a mixer grinder and preserved in a dry, clean container for preparing the experimental diet.

Animal Experiment

Eight male albino rats weighing between 105-150g were used for the experiment with due approval of the Institutional Animal Ethical Committee (IAEC). They were assigned into 2 groups, (n = 4), labeled as control and treated group. The control group received normal stock diet (20% protein, 5 % fat, 60 % carbohydrate)[3]. The experimental group received a modified diet (90g Stock diet excluding groundnut oil + 9g watermelon seed kernel + 1g sugar) and the percentages of protein, fat and carbohydrate were kept almost same as that of the stock diet. All the animals were given water *ad libitum*. The detail composition of the stock and the modified diet were as follows-

The total experimental period was 28 days during which the rats were weighed at weekly intervals. After 28 days of feeding with the modified diet, the animals were anesthetized using chloroform anesthesia following ethical procedures. Blood samples were then collected via cardiac puncture and serum was separated for the analysis of glucose and lipid profile. Serum glucose, liver glycogen and lipid profile were analyzed by the following methods-

Table 1: Composition and Nutritive value of Stock diet[3]

INGREDIENTS	WEIGHT (g)	PROTEIN (g)	FAT (g)	CARBOHYDRATE (g)	CALORIE (K.cal)
Wheat flour (whole)	53	6.413	0.901	36.782	180.730
Skimmed milk powder	29	11.020	0.029	14.790	103.530
Sattu (Bengal gram)	10	2.250	0.520	5.810	36.900
Wheat Bran	3	0.405	0.487	1.455	12.577
Groundnut oil	5	-	5	-	45
TOTAL	100	20.088	6.937	58.837	378.737

Iodised salt- 0.5g/100g,
Vyceneral Vitamin syrup – 0.2ml/100g.

Table 2: Composition and Nutritive value of modified diet

INGREDIENTS	WEIGHT (g)	PROTEIN (g)	FAT (g)	CARBOHYDRATE (g)	CALORIE (K.cal)
Wheat flour (whole)	51	6.164	0.858	35.386	173.960
Skimmed milk powder	16	6.080	0.016	8.160	57.120
Sattu (Bengal gram)	20	4.500	1.040	11.620	73.800
Wheat Bran	3	0.405	0.487	1.455	12.577
Watermelon seed kernel	9	3.069	4.734	0.405	56.520
Sugar	1	-	-	1.000	4.000
TOTAL	100	20.218	7.135	58.026	377.977

Iodised salt- 0.5g/100g,
Vyceneral Vitamin syrup – 0.2ml/100g

Serum glucose by GOD – POD Method

Serum glucose level of all the rats were assessed by Glucose-Oxidase Peroxidase kit in protein free supernatant[4,5] provided by Sigma Aldrich Corporation, USA.

Liver glycogen was estimated by the standard method described in the book named "A Manual of Laboratory Techniques" of ICMR[3]

The liver was taken out rapidly from the animal & the excess blood was removed by blotting between folds of filter paper. Immediately 1g of liver was cut and weighed in digital balance and put into test tubes containing 2 ml of 30% KOH with a tissue concentration 2 ml/g of liver. After that the tissue of different rats were digested in a boiling water bath for 1hour and then cooled in ice cold water. 4 ml of 95% ethanol were then added & the mixture was heated just to boiling, taking care so that no spurting occurs during the process. It was then left to stand for overnight in the refrigerator. Next day the tubes were centrifuged and the supernatant was discarded. The precipitate was dissolved in 10 ml of warm water. 2 volumes of 95% ethanol were added to reprecipitate the glycogen. 60% ethanol was used for several washings of the centrifuged precipitate. 2 ml of 2N H_2SO_4 was added to it and was placed in boiling water bath for 3-4 hours for hydrolysis. Neutralization was done by adding NaOH. The solution was filtered and Glucose was estimated from the aliquot. The conversion factor 0.93 was used to obtain the value of glycogen from glucose.

Total cholesterol and HDL-C by CHOD-POD / Phosphotungstate method[6]

For Total Cholesterol:

Free Cholesterol and Fatty acids are obtained by hydrolysis of Cholesterol esters using Cholesterol Esterase (CE). In the next step, Cholest-4-en-3-one and Hydrogen Peroxide are liberated through the oxidation of the 3-OH group of free Cholesterol by Cholesterol Oxidase (CHOD). Red Quinoneimine dye is produced by the coupling of Hydrogen Peroxide with 4-Aminoantipyrine (4-AAP) and Phenol in presence of Peroxidase (POD). The absorbance of the produced coloured dye is measured at 505 nm in the spectrophotometer.

For HDL-Cholesterol

Polyethylene glycol 6000 (PEG) is added to precipitate the LDL, VLDL and chylomicron fractions. After centrifugation, the supernatant is used for HDL estimation by CHOD-PAP method.

Serum triglyceride (TG) by GPO-POD method with TBHA chromogen[7]

Glycerol and free fatty acids are obtained by the catabolism of serum triglycerides with the help of the enzyme Lipoprotein lipase. Liberated Glycerol is converted to Glycerol – 3 phosphate in presence of glycerol kinase and ATP. Hydrogen peroxide is formed by the action of Glycerol– 3 phosphate oxidase on Glycerol – 3 phosphate. This together with phenolic compound TBHA and 4 Aminoantipyrine in presence of peroxidase gives the red colour complex. The intensity of the colour is measured at 505 nm (500 – 546 nm) in the spectrophotometer.

VLDL = TG/5 mg/dl[8].

LDL = Total cholesterol – (HDL + VLDL)[8].

Atherogenic Index (AI) = LDL-C/HDL-C[8].

Statistical Analysis

Demographic characteristics of Control and Treated groups were similar. All statistical tests were two-tailed and were conducted at 5% significance level. For comparison of means Student's t-test was applied. Statistical analysis was done by using SPSS software version 16.

Results and Discussions

Serum glucose level decreased significantly ($p < 0.01$) among the treated group of animals than that of the control group but there was no significant difference in the liver glycogen content of the two groups of rats (Table-3). Type of fat intake, may be linked to insulin resistance, which can increase risk of developing type 2 diabetes and impaired postprandial lipid metabolism[9,10]. Previous studies showed that *C. vulgaris* seed kernel contained branched chain amino acids (BCAAs) i.e. leucine (400 mg/g of N), isoleucine (310mg/g of N) and valine

Table 3: Effect of the modified diet on the diabetes detecting parameters of male albino rats

PARAMETERS OBSERVED	CONTROL GROUP (n = 4)mean ± SEM	TREATED GROUP (n = 4) mean ± SEM	p- VALUE
Serum glucose (mg/dl)	123.016 ± 6.045	77.634 ± 8.367	0.005**
Liver glycogen (mg/100mg)	0.388 ± 0.024	0.360 ± 0.015	0.373NS

n = number of animals in each group. df = 6, NS = Not-significant, ** = $p < 0.01$.

Table 4: Effect of the modified diet on the lipid profile parameters of male albino rats

PARAMETERS OBSERVED	CONTROL GROUP (n = 4)mean ± SEM	TREATED GROUP (n = 4) mean ± SEM	p- VALUE
Total Cholesterol (mg/dl)	50.505 ± 2.353	48.532 ± 3.853	0.677 NS
HDL-C (mg/dl)	11.378 ± 1.213	18.484 ± 2.963	0.068 NS
TG (mg/dl)	115.540 ± 8.201	87.057 ± 5.626	0.029*
VLDL (mg/dl)	23.108 ± 1.640	17.411 ± 1.125	0.029*
LDL-C (mg/dl)	16.018 ± 4.601	12.636 ± 0.382	0.491 NS
AI	1.587 ± 0.574	0.764 ± 0.175	0.220 NS

n = number of animals in each group. df = 6, NS = Not-significant, * = $p < 0.05$.

(260 mg/g of N)[1] and 16.1 ± 0.88mg Vitamin E[11] per 100 g of seed kernels. There are reports that glucose metabolism can be modulated by BCAAs. In skeletal muscle of non-diabetic rats glucose is augmented by BCAAs through enhancement of translocation of glucose transporter 4 to the plasma membrane via phosphatidylinositol 3-kinase and protein kinase C pathways. The same study additionally reported that mammalian target rapamycin (mTOR) in the hepatocytes are upregulated by BCAAs. During long term insulin treatment mTOR in the skeletal muscles degrades insulin receptor substrates by insulin-mediated PI3K activity[12]. It has also been showed that the post-prandial blood sugar levels were decreased in the vitamin E supplemented patients of the type I DM patients over 24 months and also in the type II DM patients. This decline was statistically significant only at the end of 24 months[13]. Therefore, presence of substantial amounts of BCAAs and Vitamin E are probably the causative agents of hypoglycemic function of this seed.

The results obtained from this study reveal that after consuming the modified diet containing watermelon seed, serum triglyceride (TG) and VLDL-C of the treated group were significantly decreased ($p < 0.05$ and $p < 0.05$ respectively) in comparison to that of the control group. Serum total cholesterol, LDL and AI (Atherogenic Index) were decreased whereas HDL (good cholesterol) increased in the treated group compared to that of the control group (Table-4). Arginine (900 mg/ g of N) present in watermelon seeds reported to have positive effect on serum lipid profile parameters. Endothelial nitric oxide synthase (eNOS) helps in the production of Nitric Oxide (NO) from l-arginine. NO plays an important role in regulation of endothelium-dependent vasodilatation[14]. 59.64% of Linoleic acid is present in watermelon seed kernels[1] which can be metabolized into to gamma-linolenic acid (GLA) in the body. According to some preliminary evidence GLA, either alone or in combination with omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may help to reduce high blood pressure[15]. In mammalian metabolism Arachidonic acid (AA) is formed from Linoleic acid (LA) by subsequent Δ^6 desaturation, chain elongation and HelveticΔ^5 desaturation. In this metabolic pathway Δ^6 desaturation step is rate-limiting and the step can be by-passed by dietary γ -linolenic acid (GLA). Most of the GLA is elongated to dihomo- γ-linolenic acid (DGLA) by the action of elongase and a small amount of GLA is desaturated by Δ^5 desaturase into AA. The DGLA can enter into cycloxygenase pathway to form prostaglandins of the 1-series (PGE1) additionally or alternately it can enter into 15-lipoxygenase pathway to produce eicosatrienoic acid (15-HETrE). The PGE1 and 15HETrE have been proved to have suppressive effects on chronic inflammation, hypotensive effect through vasodilation and anti-atherosclerotic effect by retarding the vascular smooth muscle cell proliferation[16]. So, the presence of high amount of linoleic acid in watermelon seed kernels may be one of the possible reasons of improving lipid profile parameters.

Conclusion

Worldwide studies have been done to make use of herbal medicine. The proposed research work showed that consumption of watermelon seed kernel would improve lipid profile parameters, therefore may be useful in prevention and treatment of cardiovascular diseases. Presence of BCAAs and Vitamin-E in watermelon seed kernels are probably responsible for its hypoglycemic property. Thus, watermelon seed kernel may be used for the treatment of various ailments without any undesirable side effect, especially in those countries in which more classical therapeutic approach is not easily available. Still there is an ample scope of research to expose hitherto unknown bioactive ingredients and their mechanism of action responsible for the positive health benefits of these seeds.

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