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Biochemical Components of Makhana (*Euryale ferox* Salisb) and its Relevance to Anti-obesity Effect in Human

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ABSTRACT

Background: Makhana is generally used as a health promoting diet especially for weight loss and anti-aging effect. Identification and quantification of different biomolecules and their related acrivities in human cells are important.

Methods: In this study, two varieties of makhana *viz;* Superior Selection-1 (SS-1) and Swarna Vaidehi have been selected for proximate compositions and nutrient components especially for anti-obesity bio-molecules by using standard methods.

Result: The biochemical constituents of the SS-1 revealed moisture 12.38%, protein 11.02%, carbohydrates 73.50% and fat 0.45%. The minerals content of SS-1 were Ca^{2+} 149.18±1.68 mg/100 g, Mg^{2+} 20.0±0.81 mg/100g and S^{2+} 60.6±1.70 mg/100 g, which were higher than that of Swarna Vaidehi. The Swarna Vaidehi variety contained moisture 14.75%, protein 8.0%, carbohydrates 77.33% and fat 0.35%. The resistant starch, amylose of makhana ranged from 17.0±0.83 (SS-1) to 18.6±0.57% (Swarna Vaidehi). The free amino acids like glutamic acid, tyrosine, alanine, lysine, leucine were commonly found in both the varieties. In addition, the amino acids arginine and histidine content in SS-1 were 12.10±0.24 g/100 g p and 2.70±0.25 g/100 g p, respectively, whereas Swarna Vaidehi possessed 10.70±0.33 g/100 g p and 3.10±0.18 g/100 p, respectively. The amino acids tyrosine and phenylalanine of SS-1 were 2.70±0.41 g/100 g p and 5.88±0.49 g/100 g p as compared to Swarna Vaidehi (0.44±0.09 g/100 g p and 80±0.25 g/100 g p).

Key words: Amylose, Anti-obesity, Arginine, Lysine, Methionine, Magnesium, Makhana.

Abbreviations: ANOVA: Analysis of variance, ATP: Adenosine triphosphate, FFA: Free fatty acid, FOS: Fermentable fructo-oligosaccharide, ROS: Reactive oxygen species, TDP: Thiamine diphosphate, TRP-32: Thioredoxin-related Protein 32, Trx-1: Thioredoxin-1.

INTRODUCTION

Gorgon nut or makhana (*Euryale ferox* Salisb.) is a wonderful aquatic medicinal plant grown in the subtropical climate of India, China, South Korea, Japan, Russia and New Zealand. In China it was considered as an important medicinal plant about 3000 years ago. It has been used in Indian Ayurveda medicine from ancient times for having important ingredients to strengthen spleen and kidneys (Jha *et al.,* 1991). In addition, it also strengthens the heart and is useful against anemia and cardiovascular diseases (Das *et al.,* 2006). Cardio protective properties of makhana may be linked with the ability to induce TRP-32 and Trx-1 proteins and to scavenge reactive oxygen species [ROS] (Kumar *et al.,* 2016). It is very effective against diabetes and aging (Jana and Idris, 2018). Apart from these useful activities, the presence high Mg^{2+}, thiamine (B1) and amino acids makhana imparts anti obese effect. The amino acids lysine and methionine form carnitine in cells, which controls obesity in humans by burning fat. In many cases, the excess intake of energy rather than energy expenditure resulted in obesity (Verduin *et al.,* 2005 and Haslam and James, 2005) and according to WHO around 1.7 billion adults were overweight and 400 million obese. Obesity may be developed due to excess food intake and releases of free fatty acid FFA, thus, the adipose tissues get excess fat (Ghouse *et al.,* 2016). In adults, complicated obesity might be due to low serum and intracellular magnesium concentrations, which are associated with IR, impaired glucose tolerance and decreased insulin secretion (Ma *et al.,* 1985, Rosolova *et al.,* 2000 and Resnick *et al.,* 1990). According to WHO obesity is defined as a phenotypic manifestation of abnormal or it has been found high energy diet which have low Mg^{2+} content increased the incidence of obesity (Klop *et al.,* 2013 and Kopelman, 2000) Dietary taurine has a blood cholesterol-lowering effect in young obese and controls body weight by acting as anti-oxidant and helps in function of the central nervous system and (Huxtable, 1992 and Zhang *et al.,* 2015). Increasing dietary leucine reduces diet induced

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obesity and improves glucose and cholesterol metabolism in mice (Menge *et al.,* 2010 and Zhang *et al.,* 2004). Histidine enhances insulin sensitivity (DeNicolantonio *et al.,* 2017) whereas proline, lysine and isoleucine rectify stress related obesity through metabolism. People nowadays prefer the natural products for treating obesity in many Asian countries (Matson and Fallon, 2012). The scientists were trying for the safe and effective development of anti-obesity drugs or food from natural products for the treatment of obesity. In this situation, makhana could be an excellent alternative (Bhutani *et al.,* 2007) as it is enriched with a lot of bio-molecules. The aim of the study was to update data on the presence of potential anti-obesity agents or bio molecules in herbal medicinal plants of gorgon nut or makhana.

MATERIALS AND METHODS

Plant materials

The experiment was conducted in ICAR-RCER, Research Centre for Makhana, Darbhanga, during 2019. The genotypes under study were Superior Selection-1 (SS-1) and Swarna Vaidehi. Four samples of each genotype of makhana collected from a farm field and washed and fresh makhana kernel powders were analyzed in the following characters.

Proximate composition

Nutritive properties including moisture, ash, crude fat and crude protein contents were determined by AOAC methods (A.O.A.C., 2016). Crude protein was analyzed using Kelplus Elite Ex Micro Kjeldahl method using conversion factor 6.25. Carbohydrates (by difference) % weight was determined as followed by the method of Gopalan *et al.,* (1998). Total dietary fiber (TDF were determined by digesting the sample with α-amylase (AOAC method 991.43). All the biochemical analyses were carried out in four replications using fresh and dry makhana kernel powder.

Minerals estimation

Minerals content with regard to calcium, magnesium and sulfur were estimated. Dried samples (1.0 g) were digested with diacid mixture (HNO3: HClO4: 9:4). After digestion and extraction of samples, water-soluble Ca^{2+} and Mg^{2+} were determined by the Versenate method (Hesse, 1971). Sulphur was estimated by Turbidimetric method (Tabatabai and Bremer, 1970). All above cited minerals were estimated by (Ranganna, 1997) with an atomic absorption spectrophotometer (Analyst 100, Perkin Elmer and Norwalk, CT, USA) and expressed as mg mineral/100 g FW for individual elements.

Vitamins

In our experiment the vitamins *viz*; thiamine was estimated. Thiamine (B1) solution was extracted with the help of phosphate from bound form and passed through Base Exchange silicate column and finally oxidized by ferricyanide and measured by Fluorometrically (Rangnna, 1997).

Amino acids

Amino acid profile was obtained by the analysis of samples through adopting procedures of Bidlingmeyer *et al.* (1987) .

Amylose

The total amylose content of makhana raw seed was determined by adopting the method of Sowbhagya and Bhattacharye (1971).

Fat profile

Fat profiles including saturated fat, monounsaturated fat, trans fat and cholesterol were determined by A.O.A.C. methods.

Statistical analysis

The analysis of variance (ANOVA) was employed to compare means between genotypes/samples through the Fisher protected LSD with a significance level of 0.05%. Results were expressed as the mean of four (sample) measurements (replications) ± SD.

RESULTS AND DISCUSSION

Gorgon nuts (*Euryale ferox* Salisb.) had a number of anti-obesity bio-molecules including protein, vitamins and nutrition elements, especially, Mg^{2+}. Low magnesium level in blood serum resulted in obesity in humans in general. Magnesium absorption in human cells is enhanced with the help of vitamin B-I and fermentable fructo-oligosaccharide (FOS) present in food. High dietary Ca^{2+} intakes also control obesity.

Presence of biomolecules that control obesity in adult and children

Magnesium content of the seed of gorgon nut ranged from 11.2±1.10 in Swarna Vaidehi to 20.00±1.09 g/100g in SS-1 variety (Table 2). From the former analysis the value was a little bit low, it may be due to moist seed. The present study revealed that makhana possessed a moderate amount of Mg^{2+} which could mitigate magnesium deficiency and redress obesity. In the body, magnesium absorption depends on thiamine in most of the cases. To achieve TDP, thiamine needs ATP and magnesium to work with the enzyme thiamine pyro-phospho-kinase (Maguire *et al.,* 2018). Present study provides the information that the makhana seed flour contains very little Vitamin B1. Pharmacological doses of vitamin D, increase Mg absorption in both vitamin D-deficient and vitamin D-replete animals (Hardwick *et al.,* 1991). Deficiency in serum magnesium leads to general obesity in humans (Insel *et al.,* 2009) (Fig 1). In our present study, the amylose (resistant starch) content ranged from 17.0±0.83% in SS-1 to 18.6±0.57% in Swarna Vaidehi varieties, which act as FOS in the large intestine. In an experiment Syed and Singh, (2013), reported that the amylose content of lotus seed also ranged between 18.75 to 20.84%. Fermentable dietary fiber, such as fructo-oligosaccharides (FOS), have been demonstrated to increase Mg^{2+} absorption in humans by 10-25%, even though the underlying mechanisms remain

Table 1: Bio-chemical constituents of fresh makhana seeds (per 100 g kernel) of the variety SS-1 and Swarna Vaidehi.

Treatment	Moisture	Protein	Carbohydrates	Fat	Crude Fibre	Total Ash
	Range (%)	Range (%)	Range (%)	Range (%)	Range (%)	Range (%)
SS-1	12.38±0.43^{b}	9.70±0.62^{a}	73.50±0.82^{b}	0.45±0.05^{a}	0.35±0.43^{b}	0.51±0.08
Swarna Vaidehi	14.75±0.83^{a}	7.24±0.37^{b}	77.33±0.97^{a}	0.35±0.43^{b}	0.35±0.05	0.52±0.09
Fisher LSD (P>0.05)	0.53	0.71	1.33	0.08	NS	NS

*Results were expressed as mean of quadruplicate measurements±SD. Mean in the same column with common letters are not significantly different (P<0.05).

Table 2: Important vitamins and minerals of fresh makhana seeds (per 100 g kernel) of thevariety SS-1 and Swarna Vaidehi.

Treatment	Calcium	Magnesium	Sulphur	Thiamine	Amylose
	Range (mg/100 g)	Range (mg/100 g)	Range (mg/100 g)	Range (mg/g)	Range (%)
SS-1	50.4±1.66^{a}	20.0±0.81^{a}	60.6±1.70^{a}	BDL	17.0±0.83^{b}
Swarna Vaidehi	9.6±0.65^{b}	11.2±1.10^{b}	47.0±1.36^{b}	BDL	0.35±0.05
Fisher LSD (P>0.05)	1.88	2.10	4.11	-	18.6±0.57^{a}

*Results were expressed as mean of quadruplicate measurements±SD. Mean in the same column with common letters are not significantly different (P<0.05).

Table 3: Essential amino acids (per 100 g protein) in fresh makhana seeds of the variety SS-1 and Swarna Vaidehi.

Parameters	SS-1	Swarna Vaidehi	Fisher LSD (P>0.05)
	Range (g/100 g)	Range (g/100 g)	Value
Lysine	2.67±0.33^{b}	3.10±0.18^{a}	0.40
Threonine	3.75±0.29^{b}	4.50±0.41^{a}	0.53
Valine	5.87±0.32^{b}	9.30±0.40^{a}	0.56
Methionine	2.66±0.14^{b}	5.40±0.25^{a}	0.30
Isoleucine	4.80±0.24^{b}	5.30±0.32^{a}	0.42
Leucine	9.10±0.24^{b}	9.80±0.33^{a}	0.40
Phenyl Alanine	5.88±0.49^{a}	4.80±0.25^{b}	0.58
Histidine	2.70±0.25^{b}	3.10±0.18^{a}	0.33
Tryotophan	ND	ND	-

*Results were expressed as mean of quadruplicate measurements ±SD. Mean in the same column with common letters are not significantly different (P<0.05).

to be elucidated. on the other hand, some commonly consumed dietary compounds, such as phytate and oxalate, can inhibit Mg^{2+} absorption (Torsten, 2008). Synthetic activity and concentration of taurine in adipose tissues and plasma have been shown to decrease during development in obesity in humans and animals (Murakami, 2015). Taurine is a sulphur amino acid and synthesized from cysteine via cysteine sulfonic acid pathways. The cysteine content of makhana in the present study was less than 0.53±0.08 g / 100 g p in SS-1 and 1.80±0.50 g/100 p in Swarna Vaidehi. The similar results were also obtained by (Jha *et al.,* 1991) and (Kumar *et al.,* 2016). The Sulphur content of makhana seed was varied from 47.00±1.36 mg/100 g edible to 60.6±1.70 mg/100 g edible. The makhana seeds contain a very high amount of total divalent cation which promotes health.

Presence of biomolecules that burns fat

Fat burner carnitine could play an important role to reduce day to day intake of more energy food. Carnitine is generally synthesized from two simple amino acids lysine and methionine. From research station samples analysis, it was evident that makhana seed had lysine content ranging from 2.67±0.33 to 3.10±0.18 g/100 g p. Our results are similar to the results obtained by Kumar *et al.,* 2016 but less than the content published by Jha *et al.,* (1991). Another important amino acid methionine content was found to be the highest in Swarna Vaidehi 5.40±0.25 g/100 g p which was a little bit higher as obtained by Jha *et al.,* (1991). Therefore, the regular intake of makhana in the human body could lead to production of fat burner, a secondary amino acid, carnitine, in cells. It was found that the leucine content of makhana was very high, ranging from 9.10±0.24 to 9.80±0.33 g/100 g p, which could reduce diet induced obesity. According to Zhang *et al.,* (2004), the leucine played a great role in reducing diet intake obesity. Furthermore, makhana seed had the maximum protein content of 9.70±0.62% (Table 1) in which the sum of aspartic and glutamic acid was approximately 27.74 g/100 g p in SS-1, which are easily digestible and help in healthy body function (Table 5).

Presence of biomolecules that prevent diabetes related obesity

In our present study, the histidine content of makhana seeds was 2.70±0.25 to 3.10±0.18 g /100 g p (Table 3). The results were supported by the findings of Jha *et al*., (1991) and (Kumar *et al.,* 2016). Obesity due to diabetes can be alleviated by taurine and histidine supplemented foods through enhancement of insulin sensitivity (DiNicolantonio, 2017) and this may have been partially attributable to loss of body fat. Dietary taurine has a blood cholesterol-lowering effect in young overweight adults and children. Taurine is also essential for cardiovascular function and controls body

Table 4: Conditionally essential amino acids (per 100 g protein) in fresh makhana seeds of the variety SS-1 and Swarna Vaidehi.

Treatment	Proline	Glycine	Arginine	Cysteine	Tyrosine
	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)
SS-1	2.67±0.27^{b}	3.74±0.28^{a}	12.10±0.24^{a}	0.53±0.08^{b}	2.70±0.41^{a}
Swarna Vaidehi	3.3±0.49^{a}	3.6±0.48^{b}	10.70±0.33^{b}	1.80±0.50^{a}	0.44±0.09^{b}
Fisher LSD (P>0.05)	0.59	0.60	0.43	0.52	0.42

*Results were expressed as mean of quadruplicate measurements ± SD. Mean in the same column with common letters are not significantly different (P<0.05).

Table 5: Non-essential amino acids (per 100 g protein) in fresh makhana seeds ofthe variety SS-1 and Swarna Vaidehi.

Treatment	Alanine	Aspartic acid	Serine	Glutamic acid
	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)
SS-1	6.92±0.34^{a}	8.02±0.37^{a}	5.88±0.35^{a}	19.72±0.98^{a}
Swarna Vaidehi	5.60±0.33^{b}	7.60±0.41^{b}	5.30±0.25^{b}	17.30±0.82^{b}
Fisher LSD (P>0.05)	0.49	0.58	0.45	1.34

*Results were expressed as mean of quadruplicate measurements ± SD. Mean in the same column with common letters are not significantly different (P<0.05).

Table 6: Fat and dietary fibers in fresh seeds (per 100 g kernel) of the variety SS-1 and Swarna Vaidehi.

Treatment	Fat saturated	Fat mono unsat	Trans fat	Cholesterol	Dietary fibre
	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)	Range (g/100 g)
SS-1	0.77±0.46^{b}	1.67±0.37^{b}	0.00	<0.05	6.57±0.09^{a}
Swarna Vaidehi	0.83±0.05^{a}	1.74±0.15^{a}	0.00	<0.05	0.35±0.05^{b}
Fisher LSD (P>0.05)	1.88	NS	4.11	-	0.18

*Results were expressed as mean of quadruplicate measurements ± SD. Mean in the same column with common letters are not significantly different (P<0.05).

weight and helps in function of the central nervous system and diabetes (Huxtable,1992; Menge *et al.,* 2010 Zhang *et al.,* 2015;) Mg^{2+} deficiency in serum and intercellular level increases insulin resistance and leads to obesity. Total divalent cations in SS-1 was Mg^{2+} 20.00±0.81 g/100 g edible, Ca^{2+} 149.18 ±1.68 g/100 g and S^{2+} 47.00±1.34 g/100 g edible, which generally acted for preventing insulin resistant and played an important role in controlling diabetes and obesity. The makhana seeds are a store house of macro and micro nutrients and one of the most popularly consumed dry fruits owing to its low fat content and high fiber content (Bana and Gupta, 2015). The arginine content of makhana seed ranged from 12.10±0.24 g/100 g p in SS-1 to 10.70±0.33 g/100 g p in Swarna Vaidehi (in present study), which were little bit higher as obtained by (Jha *et al.,*1991) in local makhana and (Kumar *et al* 2016) in Swarna Vaidehi (previous). The amino acid arginine increases insulin sensitivity and controls diabetes by proper glucose metabolism (Liang et al, 2017) and decreases white adipose tissue to control obesity (Tan *et al.,* 2012) (Fig 2).

Presence of biomolecules reduce stress related obesity

Stress may play a major role in the development of obesity in individuals who have an increased glucocorticoid exposure or sensitivity (Eline *et al.,* 2018). Hypothyroidism is associated with decreased thermogenesis, metabolic rate and has also been shown to correlate with higher prevalence of obesity (Danforth *et al.,* 1979). For proper function thyroxine (T4) and triiodothyronine (T3) are necessary and the amino acid tyrosine plays a great role here. The appreciable amount of tyrosine was found in our present study 0.44±0.09 g/100 g p in Swarna Vaidehi to 2.70±0.41 g100 g p in SS-1 (Table 4). The requirement of tyrosine for hormonal tie-up is very moderate in quantity at cellular level. So the required amount of tyrosine is formed by the hydroxylation of phenylalanine in the liver. In our present study, the phenylalanine content of recent analysis was also very high (4.80±0.25 g/100 g p in Swarna Vaidehi to 5.88±0.49 g 100 g p in SS-1) as compared to the values obtained by Jha *et al.,* (1991) and Kumar *et al.,* (2016). Sometimes obesity due to oxidative stress can be rectified through amino acid proline, cysteine (N acetyl) and taurine where they act as antioxidants. Makhana had a unique amino acid combination including proline (2.67±0.27 to 3.3±0.49 g/100 g p). Lysine and leucine may be the good candidate to reduce the intracellular oxidative stress generation and glyco-oxidation (Anuradha, 2009) and their present was the maximum in Swarna Vaidehi makhana and these were about 3.10±0.18 g/100 g p and 9.80±0.33 g/100 g p, respectively. Apart from magnesium, calcium, useful amino acids, thiamine and amylase, makhana had considerable amount of crude fiber (0.40±0.06%) and mono-saturated fat

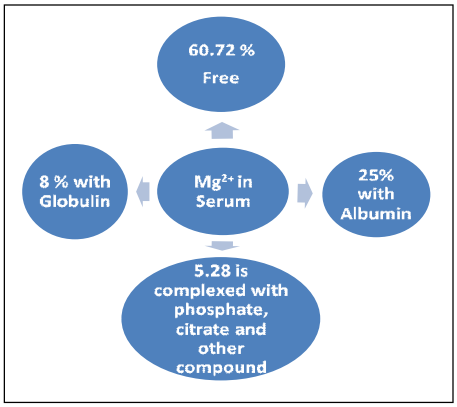

Fig 1: Free Mg $^{2+}$ in serum prevent obesity. About one-third of serum magnesium is bound to proteins, 25% with the albumin and 8% with the globulin. For the remaining two-third of serum magnesium, 92% is free and 8% is complexed with phosphate, citrate and other compound (Insel *et al,*.2009).

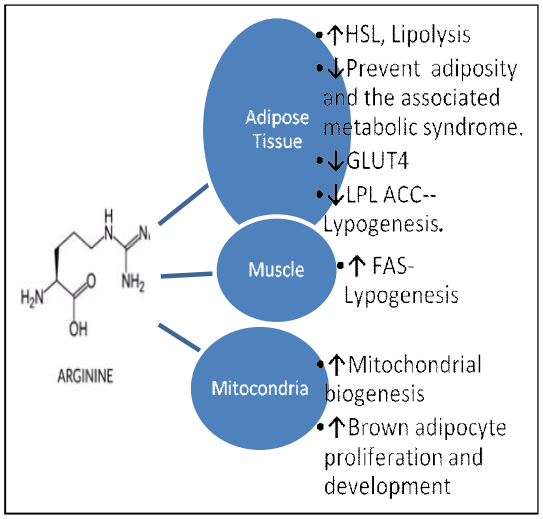

Fig 2: ACC, acetyl CoA caroxylase, FAS fatty acid synthase. GLUT4, glucose transporter4; HSL, Hormone sensitive lipage; LPL lipoprotein lipage. The symbol "↓" denotes an up regulation of Arginine on gene expression. The symbol "↑" denotes a down regulation of arginine on gene expression (Tan *et al.,* 2012; Liang *et al.,* 2017).

(0.45±0.05%) which are good indicators for normal body function and prevent obesity. Moreover, the makhana genotype SS-1 had less saturated fat (0.77±0.46 g/100 g) and high edible fiber content (6.57±0.09 g/100 g) as compared to Swarna Vaidehi variety. Apart from this, makhana contains a very low amount of saturated fat from 0.77±0.46 to 0.83±0.05% (Table 6), whereas Dietary fiber ranges from 6.24±0.12 to 6.57±0.09%. ***Weight gain due to corticosteroids, antidepressants (Serretti and Porcelli, 2018), seizure medicines, thyroid, hormone complications (PCOS) and use of estrogen /contraceptive (Norman *et al.,* 2004) are completely different and had no scope to discuss in this article.

Mechanism and Mode of Action (Mg and Arginine)

Function of Serum Magnesium

Function of L-Arginine in reducing white adipose cell

CONCLUSION

Makhana is a nutrient-dense functional food and fair source of fiber, Mg^{2+}, resistant starch, amino acids like arginine, histidine, lysine, methionine which control obesity due to diabetes , oxidative stress and hypothyroidism. The variety SS-1 was superior for having high arginine and phenylalanine and divalent cations over Swarna Vaidehi.

Conflict of interest: None.

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