Dietary Supplementation with Watermelon (Citrullus lanatus) Juice Enhances Arginine Availability and Modifies Hyperglycemia, Hyperlipidemia and Oxidative Stress in Diabetic Rats

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Abstract: Objective: Watermelon fruit is rich in L-citrulline an effective precursor of L-arginine. It has been shown to have a therapeutic properties. The present study was carried out to investigate the effect of watermelon juice on serum arginine availability, hyperglycemia, hyperlipidemia and oxidative stress in alloxan-induced diabetic rats. Material and Methods: Thirty female albino rats of Sprague Dawely Strain were used. The animals were divided into five groups: negative control group (N=6) and diabetic groups (N=24), which divided into four equal subgroups as the following: Untreated diabetic positive control group, diabetic control group treated with 0.2% L-arginine (as L-arginine-HCl) and treated diabetic groups with either 63% or 94.5% of watermelon juice for 30 days. Results: The beneficial role of watermelon juice was evaluated by orally administration to alloxan-induced diabetic rats. Diabetic untreated control group showed significant reduction in body weight gain as compared to normal and treated groups, enhancement in oxidative stress in rats as indicated by significant elevation p<0.05 in malondialdehyde (MDA) level, significant elevation of serum glucose, TAG, TC, LDL-C, VLDL-C and alkaline phosphatase (ALP) levels, whereas it was observed a significant reduction in liver glycogen content and serum HDL-C levels with respect to negative control and treated groups. Oral treatment of diabetic rats with L-arginine 0.2% or watermelon juice at 63% or 94.5%, induced significant decrease p<0.05 in serum MDA, ALP, remarkable glycemic control, increase liver glycogen and improvement in lipid profiles parameters as compared with diabetic control untreated group. Serum arginine was significantly increased in diabetic groups treated with 0.2% L-arginine or with either 63% or 94.5% of watermelon juice as compared to diabetic untreated control. Conclusion: The present study demonstrates that, watermelon juice enhances arginine availability, increase serum arginine concentration and has a significant hypoglycemic, hypolipidemic effects and significantly modify the oxidative stress with the best effect obtained with administration the high concentration of watermelon juice 94.5% in diabetic rats.

Key words: watermelon juice-arginine-hyperglycemia-hyperlipidemia-oxidative stress-diabetic rats.

INTRODUCTION

Chronic metabolic changes caused by diabetes establish an inflammatory state, accelerate atherogenesis, plus increase the risk of cardiovascular fatality (Creager et al., 2003). Watermelon is one of the few foods naturally rich source of citrulline with amounts varying from 0.7 to 3.6 mg/g of fresh weight, (Rimando and Perkins 2005). Collins, et al., (2007) demonstrated that plasma concentration of L-arginine improving cardiovascular, immunologic functions, and preventing the aging–associated increase in tissue oxidative stress. Wells et al., (2005), found that consumption of 1kg.watermelon flesh is equivalent to 40% of the mean daily arginine intake of 3.8g. for the American adult. Waugh et al., (2001) found that 1 to 3g. of oral citrulline increase plasma arginine concentrations as 50% in patients with sickle cell anemia. Arginine has a strong alkaline property in physiological solutions, its HCl salt or a mixture with acidic organic substances is generally used for animals to prevent an acid-base imbalance (Wu et al., 2007). However, high oral doses of arginine (>9g/d) is associated with nausea, gastrointestinal discomfort, and diarrhea in some subjects (Evans, et al., 2004). Oral administration of a high dose of citrulline could affect intestinal transport of some neutral amino acids 9 e.g., tryptophan) and thus potentially have a negative effect on protein nutrition in mammals (Wu et al., 2004).

Waugh et al., (2001) demonstrated that oral L-citrulline may portend very useful for palliative therapy
in sickle cell disease, and Side effects or toxicity from citrulline were not experienced.

Ma et al., (2010) demonstrated that, supplementing 0.5 or 1% arginine to the diet increased arginine concentration and increased cortisol level in serum, while enhancing antioxidative capacity and glutathione peroxidase activity in serum. Additionally, 1% arginine increased antioxidative capacity in skeletal muscle. Furthermore, 0.5 or 1% arginine decreased the cortisol receptor mRNA level in muscle.

Wu et al., (2007) studied the beneficial effects of watermelon pomace juice as a functional food for increasing arginine availability, reducing serum concentrations of cardiovascular risk factors, improving glycemic control, and ameliorating vascular dysfunction in obese animals with type 11 diabetes. Indeed, the vascular effects of dietary supplementation with 63% watermelon pomace juice were equivalent to those brought about by supplementation with 0.2% L-arginine in Zucker diabetic fatty rats (ZDF) rats. Thus, watermelon may be a functional food for ameliorating the metabolic syndrome of NIDDM. They also reported that oral administration of watermelon pomace juice has a beneficial effect on increasing the mass of brown adipose tissue, reducing excess white fat mass and serum concentrations of risk factors for cardiovascular disease, and enhancing nitric oxide (NO) dependent vessel reactivity in Zucker Diabetic Fatty Rats (ZDF).

L-arginine prevents metabolic effects of high glucose in diabetic mice, increasing nitric oxide (NO), bioavailability by L-arginine corrects the major biochemical abnormalities of diabetes (West et al., 2008).

Micol et al., (2007) suggested that watermelon extract stimulates antioxidant enzymes and improves glycemic and lipid metabolism. Watermelon (Citrus lanatus) is known during centuries for having a positive effect on health and ageing, and to improve the regulation of sugar/fat metabolic rate. Watermelon is indeed a source of known and characterized antioxidant molecules, such as carotenoids (lycopene and B-carotene), amino acids like citrulline (Rimando and Perkins 2005), minerals like potassium (Perkins and Collins., 2006), or superoxide dismutase (Bueno and Gimenez 1995). These compounds have been proposed to increase fat metabolic rate. Wu et al., (2007) reported that, concentrations of carbohydrates, protein, and fat in the watermelon pomace juice were 24, 0.87, and 0.45 g/L, respectively, as determined using the proximate analysis according to Wu et al.,(1999). Concentrations of free amino acids in the watermelon pomace juice (mg/L), analyzed using HPLC methods,(Fu et al., 2005) were as follows: citrulline, 2014; arginine, 1150; aspartate, 66; glutamate, 17; asparagine, 45; serine, 60; glutamine, 172; histidine, 88; glycine, 18; threonine, 34; alanine, 32; tyrosine, 37; tryptophan, 48; methionine, 39; valine, 78; phenylalanine, 89; isoleucine, 87; leucine, 79; ornithine, 18; lysine, 75; cysteine, 62; and proline, 74. Citrulline plus arginine accounted for 71% of total free amino acids (4.5 g/L) in the watermelon pomace juice.

MATERIALS AND METHODS

Materials:
Preparation of watermelon pomace juice concentrations: Watermelons were purchased from a local market in Cairo- Egypt. Pomace was prepared from the watermelon using a rack and cloth hydraulic press. Immediately before use, the pomace was squeezed using a juice maker. The resulting juice was filtered through a fine screen to obtain fluid, which was then added to drinking water for rats (63% and 94.5%).

Experimental Design and Animals: Diet:
Balance diets was prepared for all rats from fine ingredients per 100g. composition of a diet for growing rats,(14% casin, 10% sucrose, 5% corn oil, 5% fiber (cellulose), 3.5% salt mixture, 1% vitamin mixture, 0.3% DL-methionine, 0.3 % choline chloride and the remainder is corn starch according to Reveese et al., (1993).
Feed and drinking were provided ad-libitum through the experimental period 30 days.
Animals: Thirty adult female rats, Spargue Dawely strain were used in this study. Rats weighting 135-145g, and were obtained from the animal house of Research: Institute of Ophthalmology - Giza –Cairo. Rats were divided into five groups, six of each as the following:
Group 1: (Negative control), received drinking water
Group 2: Diabetic (untreated positive control), received drinking water
Group 3: Diabetic (treated control), received drinking water containing 0.2% L-arginine-HCl.
Group 4: (treated group) received drinking water containing 63%watermelon juice equivalent 0.2% L-arginine HCl.
Group 5: (treated group) received drinking water containing 94.5% watermelon juice equivalent 0.3% L-arginine HCl.
Groups, 2, 3, 4 and 5 were induced diabetic with single intraperitoneal (IP) dose of alloxan (140 mg/kg), which was freshly prepared immediately before injection. Rats were housed in a temperature and humidity
controlled facility on a 12-h-light: 12-h-dark cycle, then animals were allowed to drink 5% glucose solution overnight to overcome the death from hypoglycemia (Peschke et al., 2000). The diabetic state was confirmed by the measurement of fasting blood glucose concentration, 3 days after the alloxan treatment, using blood sample in eyes and glucose kits. Animals were considered diabetic at blood glucose levels of 226 mg./dl. At the end of experiments, rats were fasting 12-h- and blood sample was taken from eyes under ether anesthesia from each rat by heparinized capillary in a centrifuge tube and placed in room temperature at 37°C. Serum samples were separated by centrifugation at 3000 rpm for 20 min. and stored at -20 º C till analysis. Organs (liver, kidney, spleen and heart) were excised immediately, washed in cold saline solution (0.9% Na Cl), balotted between two layers of filter paper, weighed and stored at -20 º C till analysis.

Biochemical Analysis:

Serum glucose, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and Triacylglycerol (TAG), were determined by enzymatic colorimetric method according to Trinder, (1969), Fossati and Principe, (1982), Richmond, (1973), Lopes-Virella et al., (1977), and Friedewald et al., (1972), respectively. Serum very low density lipoprotein cholesterol (VLDL-C) concentration was calculated according to Friedewald et al., (1972) by the following equation:

\[
\text{Serum VLDL-C concentration (mg/dl) = Triacylglycerols/5}
\]

Serum alkaline phosphatase (ALP) was determined by kinetic method according to Wenger et al., (1984). The lipid peroxidation level was monitored by determined the end products of lipid peroxidation as malondialdehyde (MDA) by the (TBA) method according to Draper and Hadley, (1990). Liver glycogen was determined by method of Carrol, et al., (1956). Amino acids were determined by using an (Automatic AMINO ACID ANALYZER) according to the of Blook et al., (1958).

Statistical Analysis:

Statistical analysis of data was accomplished by means of the SPSS for Microsoft windows release 10 statistical software package. One way ANOVA (Analysis of variance and F-test were used for comparison of quantitative variables with each other p value (probability). All values are expressed as mean ± Standard division p < 0.05 significant p< 0.01 highly significant p< 0.001 very highly significant.

RESULTS AND DISCUSSION

Biological Evaluation:

Glycosuria, polyuria and diarrhea were observed from the first week in alloxan diabetic rats. Weight gain and relative weight of organs were illustrated in (Table 1): The results indicated that diabetic untreated group (G2) showed significant reduction (p < 0.05) in body weight as compared to negative and treated groups. Control group treated with L-arginine 0.2% (G3) and treated diabetic groups with 63% (G4) or 94.5% watermelon.(G5) showed highly significant increase in body weight (p < 0.01) as compared to untreated diabetic group (G2). There was a highly significant decrease in relative weight of liver in G5 compared with the normal control (G1) and untreated diabetic group (G2). No significant difference was found between all groups in relative weight of heart and spleen. Regarding to relative weight of kidney, there was slight significant differences between (G3) treated with L-arginine compared to normal group and highly significance increase (p < 0.05) in (G5) treated with watermelon juice 94.5% compared to normal control, diabetic treated control and treated diabetic groups.

Biochemical Assessment:

Treatment with citrullin rich in watermelon has been shown to regulate hyperglycemia. The levels of serum glucose, liver glycogen and ALP in negative, positive controls and treated groups were illustrated in table (2). The results show a significant elevation in serum glucose and ALP in addition. there was a significant reduction in liver glycogen in diabetic positive control compared to diabetic groups treated with 0.2% L-arginine HCl or watermelon juice (63 % and 94.5%). Oral administration of watermelon juice at different concentration (63% and 9 4.5%) and L-arginine 0.2% resulted in a significant reduction in serum glucose level (p < 0.05) and the more effect was found in diabetic groups treated with watermelon at level of 94.5 %.

The present study proved that the liver glycogen was significantly increased p < 0.05 ) in all treated groups compared to positive untreated diabetic control group (G2). No significant different in liver glycogen contents were found between groups treated with watermelon at 63% or 94.5% when compared to normal
control group whereas, the values statistically were similar to normal values. Regarding to ALP levels, it was found that a significant increase in serum ALP in untreated diabetic control group compared to normal group. Treatment with L-arginine 0.2%, 63% or 94.5% watermelon juice proved significant decrease in ALP level compared to untreated positive control, in contrast group treated with watermelon at 94.5% level recorded the lowest levels compared to all groups.

Table 1: Weight gain and relative weight of different organs in normal, treated and untreated diabetic rats (Mean ±SD g.%).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 normal control</th>
<th>Group 2 untreated Diabetic Positive control</th>
<th>Group 3 diabetic control treated with 0.2 g. L. Arg%</th>
<th>Group 4 treated with 63% Watermelon pomace juice</th>
<th>Group 5 treated with 94.5% Watermelon pomace juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain</td>
<td>30.75 ±3.86</td>
<td>17.25 ± 5.68abc</td>
<td>37.25± 5.68abc</td>
<td>75.0± 16.03abc</td>
<td>35.75 ± 5.32abc</td>
</tr>
<tr>
<td>Liver</td>
<td>3.18 ±0.32</td>
<td>2.66 ±0.32</td>
<td>2.69 ±0.25</td>
<td>2.58±0.23ab</td>
<td>2.58±0.23ab</td>
</tr>
<tr>
<td>Heart</td>
<td>0.28±0.03</td>
<td>0.34±0.11</td>
<td>0.37±0.11</td>
<td>0.20±0.007</td>
<td>0.20±0.007</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.47±0.004</td>
<td>0.52±0.003</td>
<td>0.56±0.001</td>
<td>0.63±0.10abc</td>
<td>0.63±0.10abc</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.27±0.003</td>
<td>0.23±0.007</td>
<td>0.23±0.004</td>
<td>0.26±0.005</td>
<td>0.21±0.007</td>
</tr>
</tbody>
</table>

All values are expressed as mean of 6 rats ± Standard division. p < 0.05 significant p< 0.01 highly significant.

The Effect of Watermelon on Lipids Profile and Lipid Peroxidation (MDA): Various parameters of lipid profiles were tested in alloxane induced diabetic rats in treated and untreated groups are shown in table (3). The levels of TC, LDL-C, VLDL-C, TAG and the serum lipid peroxidation as malondialdehyde (MDA) in diabetic untreated control rats were significantly elevated, whereas, HDL-C level was significantly reduced. In comparison administration of L-arginine (0.2 %) or groups treated with watermelon juice at 63% or 94.5% resulted in a significant reduction of (p <0.05) in TC, TG, LDL-C, VLDL-C and MDA and elevation in HDL-C.

Table 2: Effect of watermelon juice on serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triacylglycerol (TAG),VLDL-low density lipoprotein cholesterol (LDL-C) and malondialdehyde (MDA) (Mean ±SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 normal control</th>
<th>Group 2 untreated Diabetic control</th>
<th>Group 3 control treated with 0.2 g. L. Arg</th>
<th>Group 4 treated with 63% Watermelon pomace juice</th>
<th>Group 5 treated with 94.5% Watermelon pomace juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC mg/dl</td>
<td>144.49 ± 6.42</td>
<td>214.88 ±13.08abc</td>
<td>161.15 ± 4.63abc</td>
<td>164.97 ±5.98abc</td>
<td>152.79 ±4.07bd</td>
</tr>
<tr>
<td>HDL-Cmg/dl</td>
<td>72.47 ±0.45</td>
<td>57.37 ±8.7a</td>
<td>91.56 ±2.10abc</td>
<td>82.47 ±4.10bcd</td>
<td>95.10 ±4.92abc</td>
</tr>
<tr>
<td>TAG mg/dl</td>
<td>89.87 ± 1.38</td>
<td>124.97 ±3.06</td>
<td>100.35 ±2.62ab</td>
<td>101.83 ±4.57ab</td>
<td>94.48 ±1.78abc</td>
</tr>
<tr>
<td>VLDL-C mg/dl</td>
<td>17.97 ± 0.28</td>
<td>24.54 ± 0.78a</td>
<td>20.43 ±0.64ab</td>
<td>20.37 ±0.92ab</td>
<td>19.27 ±0.34abc</td>
</tr>
<tr>
<td>MDA nmol/l</td>
<td>2.42 ± 0.14</td>
<td>4.56 ±0.12abc</td>
<td>1.69 ±0.35abc</td>
<td>3.23 ±0.05abc</td>
<td>2.21 ±0.238abc</td>
</tr>
</tbody>
</table>

All values are expressed as mean of 6 rats ± Standard division. p < 0.05 significant p< 0.01 highly significant.

The Serum Amino Acids Contents: The results of serum amino acids profile obtained in this study were shown in table (4) as the following: Administration with 0.2% arginine or consumption of watermelon juice resulted in a significant increase in
serum arginine also a significant increase in serum proline, tyrosine as compared to untreated diabetic group. With no significant difference between all the experimental groups in the values of the remaining amino acids. Valine, glycine and glutamine were reduced in treated groups as compared to normal and diabetic control. Aspartic acid showed significant increase in G4 and G5 as compared to G3, while showed significant decrease as compared to G1 and G2.

Diabetes is one of the most challenging diseases facing health care professionals today. Its increasing prevalence puts a large burden on the public health sector, (Leroith and Smith, 2005). Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-immune destruction of pancreatic β-cell, and this disease is more likely to occur in relatives of an affected person (Bottini, et al, 2006).

Our results show that there was a significant decrease in body weight in diabetic control rats (G2). Whereas, there was an increase in body weight in groups supplemented with 0.2% arginine and 63% and 94.5% watermelon juice and the values were similar to the normal control group. Mendez and Hernandez (2005) who found that diabetic rats with alloxan (120 mg/kg, body weight) showed a decrease in body weight. In addition, the rats treated with either L-arginine or polyamine showed no significant difference from the normal control group. Theses finding was similar with the current study in groups treated with watermelon at different levels as compared with normal control.

Tan et al., (2008)., McKnight et al., (2010) showed that dietary l-arginine supplementation reduces adiposity in genetically obese rats, diet-induced obese rats, finishing pigs, and obese human subjects with Type-2 diabetes mellitus. The mechanisms responsible for the beneficial effects of l-arginine are likely complex, but ultimately involve altering the balance of energy intake and expenditure in favor of fat loss or reduced growth of white adipose tissue.

West, et al., (2008) showed that four weeks after STZ treatment, there was no change in heart weight/body weight ratio in mice treated with saline or L-arginine. (Jobgen et al., 2009) reported that arginine supplementation for 12 wk decreased the body weight gains of low fat (LF)- and high fat (HF)-fed rats by 60 and 40%, respectively, compared with control. The long-term arginine treatment did not result in any adverse effect on LF or HF rats.

**Effect of Watermelon on Blood Serum Glucose, Lipids Profile and ALP Activity:**

The study showed that group of rats received the high concentration of watermelon) 94.5% (was more effective for reducing serum blood glucose, lipids profile, MDA, LDL-C and elevated HDL-C than the other groups.

The present study is in accordance with many researchers. Chronic metabolic changes caused by diabetes establish on inflammatory stat. accelerate atherogenesis and increase the risk of cardiovascular fatality (Creager et al., 2003). Treatment with L-arginine has been shown before to regulate hyperglycemia and dyslipidemia (Mendez and Balderas 2001). L-arginine has been reported to protect rat βeTA cells against the diabetogenic effects of alloxan (Mendez and Hernandez 2005). Accumulation of sorbitol in diabetic animals was completely abolished by L-arginine, indicating that L-arginine inhibits the polyol pathway (West et al., 2008).

The most fundamental defect in diabetic patients is the resistance to cellular action of insulin. Insulin insensitivity enhances hepatic gluconeogenesis and glucose output, reducing suppression of lipolysis in adipose tissues, increasing hepatic very low density lipoprotein secretion and decreasing level of serum HDL-C (Avramoglu et al., 2006).

The present study proved that the liver glycogen was significantly (p > 0.05) reduced in diabetic positive control. Groups of rats received either oral administration with watermelon concentrations or received L-arginine (0. 2 %) resulted in a significant increase in liver glycogen contents as similar to normal group. In the diabetic positive group it was found a significant elevated blood serum glucose levels than that of normal control and treated groups. The elevation of glucose levels due to accumulation of acetylcholine in the adrenals following, inactivation of cholinesterase stimulate the release of adrenaline into the blood, adrenaline increases cell metabolism; it causes glycogenolysis in the liver and a consequent hyperglycemia Also, an accumulation of acetylcholine in some parts of brain, e.g., hypothalamus, humoral factord, are released systemically which cause mobilization of peripheral glycogen stores leading to hyperglycemia and decrease liver glycogen stores (Fox and Vigro 1986). (Jobgen et al., 2009) reported that arginine supplementation did not affect concentrations of lipids in adipose tissue or concentrations of glycogen in liver and gastrocnemius muscle .they also reported that dietary arginine supplementation reduced (P > 0.05) serum concentrations of glucose.

The hypoglycemic effect of watermelon juice is in accordance with that reported by Penelope et al., (2008) who showed that a diet enriched with citrulline/arginine or watermelon reduced glucose levels and improved
aortic flexibility in an animal model study. The high amounts of lycopene in red fleshed watermelon may be useful in blocking free radical damage, while the citrulline may improve vascular health. They reported that watermelon fruit contains the plant chemicals lycopene and citrulline, which may be helpful in preventing some chronic diseases. The amount of lycopene in watermelon is highly variable, but generally exceeds that of tomato. Citrulline is present in all parts of the fruit. Lycopene was found to be relatively stable in fresh cut watermelon, and could increase slightly in whole fruit held at room temperature. Seedless watermelon generally had more lycopene than seeded types, and lycopene was present in red fleshed fruit, with small amounts in orange fleshed watermelon, and none in yellow fleshed types. Subjects drinking six cups of watermelon juice per day had increased levels of arginine and lycopene content in their plasma. The hypoglycemic effect of watermelon can be attributed to the citrulline content of watermelon thus, citrulline can act indirectly by stimulating insulin secretion. It is noteworthy that insulin levels were significantly higher in citrulline-supplemented rats than in controls, even though insulinemia still remained lower than in the old healthy rats (Caldedef-Chezet et al., 2001). The increased levels of insulin could be a result of increased levels of arginine, which is well known to be a potent inducer of insulin secretion (Malaisse et al., 2004; Nakata and Yada 2003). showed that citrulline at a physiological concentration (0.1 mM) increased insulin release from rat isolated islets. Fu et al., (2005) found that dietary arginine supplementation with increased systemic NO synthesis and insulin sensitivity and decreased fat mass and serum concentrations of glucose.

Dietary arginine supplementation may increase insulin sensitivity and amplify its signaling mechanisms on net protein synthesis (Jobgen et al., 2006).

Miguez et al., (2004) showed that there was a significant increase in serum alkaline phosphatase activity in diabetic groups either treated with L-arginine or as compared to normal control. The present study agrees with these results whereas, it was found a highly significant decrease (p > 0.05) and differences between all diabetic groups and normal control. The lowest level in serum ALP was found in diabetic group treated with watermelon at level 94.5%. Unakami et al., (1990) they reported that if it is taken into account that the activity of this enzyme in the serum of diabetic animals is greater than normal, the decrease in the activity in the intestinal mucosa seems to be related to greater displacement of the enzyme to the serum.

Various parameters of lipid profiles were tested in alloxane – induced diabetic rats in treated and untreated groups are shown in table (3). The levels of TC, TAG, LDL-C, VLDL-C and the serum lipid peroxidation as malondialdehyde (MDA) in diabetic untreated control rats were significantly elevated, whereas. HDL-C level was significantly reduced in positive control. In comparison to control group administration of L arginine (0.2 %) or watermelon juice at different levels (63% or 94.5%) resulted in a significant reduction of TC, TG, LDL-C, VLDL-C, and MDA and elevated in HDL-C. The results indicated that the high concentration of watermelon juice (94.5%) was the best improvement and more effective compared to the other treatment groups.

Our results are in accordance with those of Rimando and Perkins (2005) who found that watermelon protected body from oxides formed by lipids oxidation, while in contrast to the results were found by Collins et al., (2004) who found that the supplemented diet with watermelon did not affect plasma lipid concentrations. However, Chareoonriri and Kongkachuichai (2009) assayed the total dietary fiber content of watermelon and it was 0.6 g/100 g edible portion which play role to reduce the lipid in body.

Hayashi et al., (2005) demonstrated that L-citrulline produced pharmacological effects that closely resembled those of L-arginine administration and NO action. L-citrulline caused a marked improvement in endothelium-dependent vasorelaxation in response to acetylcholine, and the combination of L-citrulline and L-arginine produced a synergistic response in elevating plasma NOX and cGMP, improving rabbit ear artery blood flow and slowing the progression of atherosclerosis. They also reported that, fatty diet-induced atherosclerosis and oxidative stress were reversed upon oral administration of L-arginine, L-citrulline, and antioxidants. These observations suggest that NO is the active species in reducing both the markers for oxidative stress and the progression of atherosclerosis. Cardiovascular disease is the leading cause of morbidity and untimely death both in men and women in the U.S. and may be largely avoidable and even reversible by adopting more sensible programs involving a healthy diet and moderate exercise. They demonstrated that, at least in rabbits, that chronic ingestion of L-arginine, L-citrulline, and antioxidants can reverse the progression of atherosclerosis.

(West et al., 2008) demonstrated that treatment with L-arginine decreased total and VLDL-associated triglyceride content, consistent with a direct effect of NO on VLDL rather than on chylomicron remnants. Decreases in HDL cholesterol content and size were also observed, which may be reflective of reversal of diabetes-induced changes. The decrease in VLDL triglycerides was; however, associated with a decrease in the concentration of large VLDL and an increase in LDL-C concentration. These changes most likely reflect a

1289

reduction in triglyceride concentration. Hence, the primary effect of L-arginine on lipoprotein subclasses reflects attenuation of hypertriglyceridemia. While the mechanisms by which L-arginine (or NO) affects triglyceride metabolism remain unclear, it is significant to point out that unlike L-arginine treatment, overexpression of GTP cyclohydrolase fails to normalize diabetes induced increases in triglycerides or HDL even though BH4 levels were restored. Thus, L-arginine supplementation may be an effective intervention for treating early dyslipidemia in diabetes.

Hayashi et al., (2005) reported that fatty diet-induced atherosclerosis and oxidative stress were reversed upon oral administration of L-arginine, L-citrulline, and antioxidants. These observations suggest that NO is the active species in reducing both the markers for oxidative stress and the progression of atherosclerosis. They also demonstrated that, at least in rabbits, that chronic ingestion of L-arginine, L-citrulline, and antioxidants can reverse the progression of atherosclerosis. Similar observations were made in humans with L-arginine and antioxidants (Cooke, 2003).

Míguez et al., (2004) investigated whether dietary supplementation with L-arginine, the endogenous precursor of nitric oxide, might affect serum lipid levels and activities of intestinal mucosa enzymes in animals, in which diabetes was induced by administration of streptozotocin. Control and diabetic rats were fed diets with or without 2% L-arginine supplementation for 4 weeks. Diabetic rats had significantly higher concentrations of serum triglycerides and LDL-cholesterol than control rats. These alterations were partially reduced by L-arginine supplementation. They also found that experimental diabetes did not influence the lactase and leucine aminopeptidase activity in the intestine, but the activity of alkaline phosphatase was increased. Furthermore, activities of maltase and sucrase in the intestinal mucosa were elevated in streptozotocin-induced diabetic rats and were restored to control levels after dietary L-arginine supplementation. On the basis of the present experimental evidence, dietary L-arginine supplementation appears to affect the metabolism of lipoproteins and might alleviate some gastrointestinal dysfunctions, commonly seen in diabetes mellitus.

physiological levels of arginine and nitric oxide have antioxidative function (Wu and Meininger 2008). For example, Wascher et al., (1997) reported that administration of L-arginine diminished superoxide release and copper induced lipid peroxidation in rats. Recently, Petrovic et al., (2008) demonstrated that L-arginine increased the antioxidative defense system in rats in response to cold acclimation. Importantly, dietary supplementation with arginine decreased the hydroxyl radical level in serum, while increasing the activity of GSH-Px Jan antioxidative enzyme (Wu et al. 2004a) in serum of pigs, indicating the enhancement of whole-body antioxidative function.

On contrast to our results Tan et al., (2008a) found that dietary supplementation with 1% arginine increased had no effect on serum concentrations of glucose, lipids or insulin.

Table 4: Serum amino acids profiles with normal and diabetic groups compared with treated groups with 63% or 94.5% of watermelon juice (Mean ± SD g.% n =3).

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Group 1 normal control</th>
<th>Group 2 untreated Diabetic</th>
<th>Group 3 treated with 0.2 g. L. Arg%</th>
<th>Group 4 treated with 63% Watermelon pomace juice</th>
<th>Group 5 treated with 94.5% Watermelon pomace juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>0.339 ±0.11</td>
<td>0.216 ±0.10</td>
<td>0.296±0.14 a</td>
<td>0.290±0.09 b</td>
<td>0.326±0.11 b</td>
</tr>
<tr>
<td>Lys</td>
<td>0.993 ±0.10</td>
<td>1.066 ±0.12</td>
<td>0.917±0.17 ab</td>
<td>0.687±0.20 ab</td>
<td>0.849±0.10</td>
</tr>
<tr>
<td>His</td>
<td>0.293 ± 0.07</td>
<td>0.324 ±0.09</td>
<td>0.281±0.10</td>
<td>0.271±0.08 ab</td>
<td>0.293±0.09</td>
</tr>
<tr>
<td>Phe</td>
<td>0.328±0.05</td>
<td>0.408±0.10</td>
<td>0.261±0.07</td>
<td>0.287±0.10</td>
<td>0.280±0.15</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.322 ± 0.09</td>
<td>0.169 ±0.10 acc</td>
<td>0.262±0.10</td>
<td>0.287±0.10</td>
<td>0.280±0.10</td>
</tr>
<tr>
<td>Leu</td>
<td>0.959 ± 0.10</td>
<td>1.049 ±0.10</td>
<td>0.686±0.09 ab</td>
<td>0.770±0.10 ab</td>
<td>0.803±0.10 b</td>
</tr>
<tr>
<td>Ile</td>
<td>0.247 ± 0.01</td>
<td>0.264 ±0.01</td>
<td>0.184±0.01 ab</td>
<td>0.189±0.0 ab</td>
<td>0.19± 0.01 ab</td>
</tr>
<tr>
<td>Val</td>
<td>0.604 ± 0.04</td>
<td>0.852 ±0.01</td>
<td>0.427±0.03 ab</td>
<td>0.458±0.02 ab</td>
<td>0.463±0.03 ab</td>
</tr>
<tr>
<td>Cys</td>
<td>0.052 ± 0.01</td>
<td>0.254 ±0.01</td>
<td>NA</td>
<td>0.123±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Ala</td>
<td>0.939±0.01</td>
<td>1.098±0.04</td>
<td>0.818±0.06</td>
<td>0.728±0.07</td>
<td>0.78±0.03</td>
</tr>
<tr>
<td>Gly</td>
<td>0.687±0.04</td>
<td>0.797±0.05</td>
<td>0.637±0.1 bc</td>
<td>0.554±0.1 bc</td>
<td>0.552±0.1 bc</td>
</tr>
<tr>
<td>Pro</td>
<td>0.027±0.01</td>
<td>0.012±0.01 acc</td>
<td>0.027±0.01</td>
<td>0.027±0.02</td>
<td>0.025±0.01</td>
</tr>
<tr>
<td>Glu</td>
<td>1.18±0.01</td>
<td>1.41±0.1 *</td>
<td>0.693±0.1 b</td>
<td>1.113±0.1 bc</td>
<td>1.058±0.01 bc</td>
</tr>
<tr>
<td>Ser</td>
<td>0.633±0.04</td>
<td>0.679±0.03</td>
<td>0.388±0.01 ab</td>
<td>0.456±0.06 b</td>
<td>0.507±0.03</td>
</tr>
<tr>
<td>Thr</td>
<td>0.578±0.01</td>
<td>0.569±0.1</td>
<td>0.415±0.05</td>
<td>0.422±0.08</td>
<td>0.485±0.04</td>
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<tr>
<td>Asp</td>
<td>1.14±0.01</td>
<td>1.254±0.10</td>
<td>0.774±0.01</td>
<td>0.866±0.01 ab</td>
<td>0.866±0.01 ab</td>
</tr>
<tr>
<td>Met</td>
<td>NA</td>
<td>NA</td>
<td>0.028±0.01</td>
<td>0.054±0.01</td>
<td></td>
</tr>
</tbody>
</table>

p < 0.05 significant a:Significant difference compared to group(1) b:Significant difference compared to group (2) NA: Not available data c: Significant difference compared to group (3) d:Significant difference compared to group (4) e: Significant difference compared to group (5)
The results of serum amino acids profile obtained in this study were shown in table (4) as the following: Administration with 0.2% arginine or consumption of watermelon juice resulted in a significant increase in serum arginine also a significant increase in serum proline, tyrosine as compared to untreated diabetic group. With no significant difference between all the experimental groups in the values of the remaining amino acids.

Collins et al., (2007) who reported that chronic consumption of watermelon is a safe and effective alternative to oral administration of L-arginine in raising its plasma levels in healthy subjects and may represent a novel and useful strategy for the management of obesity and diabetes. Provision of citrulline from watermelon also offers a unique advantage over the enteral supply of L-arginine for the following reasons. First, 40% of dietary L-arginine is catabolized by the intestinal tissues of adult humans and other mammals in the first pass (Wu, 1998).

In contrast, citrulline undergoes limited degradation in enterocytes of postweaning animals due to a low activity of argininosuccinate synthase in the cells (Wu, 1995). Thus, on the same equal molar basis, the entry of dietary citrulline into the portal circulation is much greater than that of dietary arginine in adults. Second, there is little uptake of circulating citrulline by liver and, therefore, nearly all the citrulline absorbed from the small intestine bypasses the liver and enters the systemic circulation (Morris, 2002). Third, in mammals, the synthesis of arginine from citrulline is the only pathway for its utilization by extrahepatic tissues, including predominantly kidneys as well as other tissue and cell types (e.g. heart, brain, macrophages, and endothelial cells (Wu and Morris, 1998). Indeed, the vascular effects of dietary supplementation with 63% watermelon. Watermelon pomace juice were equivalent to those brought about by supplementation with 0.2% L-arginine in ZDF rats. Thus, watermelon may be a functional food for ameliorating the metabolic syndrome of NIDDM.

In our study increasing dietary supplementation of watermelon pomace juice to 95% which were equivalent to those brought about by supplementation with 0.3% L-arginine lead to increased arginine concentration. These results are in accordance to those of (Collins et al.,2007) who showed that 3 wk of daily ingestion of 3 or 6 cups of watermelon juice, a natural source of citrulline, significantly increases plasma arginine concentrations in healthy subjects compared with controls. The authors conclude that watermelon juice may be an effective alternative to oral administration of arginine to humans, in situations where arginine supplementation may be beneficial. They refer as beneficial effects of arginine the improvement of cardiovascular and immunologic functions and a favorable regulation of whole-body metabolism of energy substrates; all these attributed to the role of arginine as a precursor of nitric oxide (Jobgen et al., 2006 and Lucotti et al., 2006).

(Hu et al., 2008; 2008a; Phang et al., 2008) reported that proline, glutamine, creatine and polyamines were important metabolites of arginine. Accordingly, dietary supplementation with 1% arginine increased serum concentrations of proline and glutamine, glutamate, as well as creatine (He et al. 2008). It is noteworthy that proline attenuates the stress response in the central nervous system of chicks (Hamasu et al. 2008), while reducing inflammatory responses and oxidative stress in mammals (Bassit et al. 2008; Gualano et al. 2008). Similarly, glutamine (Wang et al., 2008) and polyamines (Rider et al., 2007) enhance anti-oxidative function in cells.

Edmonds and Baker (1987) reported that arginine had an antagonistic effect on lysine absorption, but other investigators did not observe such a phenomenon (Tan et al., 2008a; Yao et al. 2008). This discrepancy may be explained by the differences in the contents of amino acids, including basic amino acids (Wu et al. 2008; 2008b). Studies with pigs have shown that dietary supplementation with 2% arginine (on dry matter basis) is generally safe and does not result in an antagonism among basic amino acids.(Wu et al., 2007a). In support of this view, found that supplementing 0.5 and 1% arginine to the basal diet increased the concentration of arginine in the serum of pigs but had no effect on that of lysine.

He et al., (2007) reported that arginine plays an important role regulating nutrient metabolism, but the underlying mechanisms are largely unknown. They found that dietary supplementation with 0 or 1.0% L-arginine to corn- and soybean meal-based diets. Serum decreased fat deposition and increased protein accretion in the body. They also showed that serum concentrations of low density lipoprotein, very low density lipoprotein, and urea were lower, but concentrations of creatinine, tricarboxylic acid cycle metabolites, ornithine, lysine and tyrosine were greater in arginine-supplemented than in control pigs. Additionally, the arginine treatment affected serum concentrations of nitrogenous and lipid signaling molecules (glycerophosphorylcholine and myo-inositol) and intestinal bacterial metabolites (formate, ethanol, methylamine, dimethylamine, acetate, and propionate). These novel findings suggest that dietary arginine supplementation alters the catabolism of fat and amino acids in the whole body, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial metabolism in growing pigs.
Wu et al., (2008) reported that, arginine is required for the detoxification of ammonia, which is an extremely toxic substance for the central nervous system. There is compelling evidence that arginine regulates interorgan metabolism of energy substrates and the function of multiple organs. Their results of both experimental and clinical studies indicate that arginine is a nutritionally essential amino acid (AA) for spermatogenesis, embryonic survival, fetal and neonatal growth, as well as maintenance of vascular tone and hemodynamics. Moreover, a growing body of evidence clearly indicates that dietary supplementation or intravenous administration of arginine is beneficial in improving reproductive, cardiovascular, pulmonary, renal, gastrointestinal, liver and immune functions, as well as facilitating wound healing, enhancing insulin sensitivity, and maintaining tissue integrity. Additionally, arginine or L-citrulline may provide novel and effective therapies for obesity, diabetes, and the metabolic syndrome. The effect of arginine in treating many developmental and health problems is unique among AAs, and offers great promise for improved health and wellbeing of humans and animals.

The amino acid profile of watermelon was reported by Tedesco et al., (1994) who quantify free amino acids extracted from 1 g wet weight of watermelon fruit yielded the following (in mmoles per gram wet weight): Phenylalanine, 1.25; histidine, 0.24; tryptophan, 0.35; lysine, 0.82; ornithine, 0.32; arginine, 11.36; aspartic acid, 0.97; threonine, 0.74; serine, 1.05; glutamate, 3.86; glutamic acid, 1.38; citrulline, 23.68; alanine, 1.15; valine, 0.17; isoleucine, 1.24; leucine, 0.24.

This is consistent with the knowledge that L-citrulline is converted to L-arginine by mammalian cells, including endothelial cells (Solomonson et al., 2003). This recycling pathway might be important in sustaining the production of NO in endothelial cells, especially when L-arginine becomes limiting, as is possible in atherosclerosis.

Osowska et al., (2006) found that among the 25 amino acids measured, concentrations of citrulline, ornithine, and arginine were the only ones that displayed significant variations according to the diet manipulations. They were higher in the plasma and the tibialis muscle in the Cit group after the renutrition period. They demonstrated that the citrulline-enriched diet exerted a remarkably stimulating effect on the protein synthesis in the muscle. citrulline supplementation in the old malnourished rats increases protein content of the muscle by stimulating protein synthesis. Citrulline can act directly (or via arginine production) on protein synthesis.

Marini et al., (2010) reported that Plasma arginine and ornithine are able to support citrulline synthesis during arginine-free feeding.

In accordance with our results Jobgen et al., (2009) reported that Serum concentrations of arginine, ornithine, and proline were higher, but those of glutamate, glutamine, and branched-chain AA (BCAA) were lower (P < 0.05) in arginine- than in alanine-supplemented rats. Dietary arginine supplementation did not affect serum concentrations of other measured AA including: Serum concentrations of alanine were 65% higher (P < 0.01) in alanine- than in arginine-supplemented rats.

Conclusion:
The present study demonstrates that watermelon juice enhances arginine availability, increase serum arginine concentration and has a significant hypoglycemic, hypolipidemic effects and significantly modify the oxidative stress, with the best effect obtained with high watermelon juice concentration 94.5% in diabetic rats.

REFERENCES


1294