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Effect of *Achyranthes Aspera (Linn)* Seeds on Membrane-Bound ATPases in Selected Tissues of Rats Fed with High Doses of Fructose

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Abstract

A high consumption of fructose diet alters the activities of membrane-bound ATPases in erythrocytes and tissues. In our earlier studies, we found that the *Achyranthes aspera* (*A. aspera)* extract decreased the hepatic lipid peroxidation without increasing the antioxidant enzymes and indicating the direct free radical scavenging action of the plant extract. In this study, we evaluated the influence of *A. aspera* seed extract on high fructose induced alterations in the activities of membrane bound ATPases in rats. For the experiments, adult male wistar rats were made insulin resistance (IR) by feeding them high fructose diet for 45 days and *A. aspera* was administered last 15 days to test its effects. In our study, the high fructose fed increased the levels of glucose, body weight, HbA1c and decreased level of plasma insulin and decreased the activities of total ATPases, $(Na^+ + K^+)$-ATPase, low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase in erythrocytes and tissues. Administration of *A. aspera* ameliorated the high fructose induced alterations of blood glucose level and changes in the activities of membrane-bound ATPases. Thus, our results show that the *A. aspera* normalises the activities of membrane-bound ATPases in various tissues, which could be mainly due to improved glycemic control by *A. aspera*.

Keywords: extracellular ATPase, hyperinsulinemia, hypertriglyceridaemia, insulin resistance, type-2 diabetes.

Abbreviation: *A. aspera - Achyranthes aspera*; ATPase - adenosine triphosphatase; Hb Heamoglobin; HFFD - High fructose fed diet; $(Na^+ + K^+)$-ATPase - total ATPases.

Introduction

A high intake of refined carbohydrates and sweeteners, including sucrose and high fructose corn syrup (HFCS), have been attributed to the growing epidemics of obesity and type-2 diabetes (T2D) in Western society [1]. Current evidence suggests that the consumption of added dietary sugars (including sucrose and HFCS) is currently stable or decreasing [2], however, it remains high. In India estimated that there are 22 million diabetics in 1990, and this number is likely to increase to 33 million in 2000 and 40.9 million in 2006 that may prediction of 69.5 million or more by the year 2025 [3]. This translates to between 26.8% and 39.3% of daily energy consumed from added sugar (based on 2000 Cal/day). Furthermore, sugar consumption is high in both children and adolescents [4], which have been identified as contributing to the alarming rates of T2D and obesity observed in this population. A high level of daily consumption of total fructose over the past two decades leads to a rise in obesity and metabolic disorders [5]. The utilization of high amounts of refined carbohydrates in food and beverage increases the risk of dyslipidaemia [6], obesity [7], insulin resistance (IR) [8] and heart disease [9]. The epidemiological examinations in human found an association between diabetes prevalence and sugar availability [10]. In experimental models such as rats, fructose-rich diet mimics the pathological conditions in human by causing IR, hyperinsulinaemia, glucose intolerance, hypertriglyceridaemia and hypertension [11].

The ubiquitous membrane bound enzyme as like us $(Na^+ + K^+)$-adenosine triphosphatase (ATPase) is responsible for the maintenance of both intracellular sodium and potassium concentrations [12]. The function of this enzyme is to transport three ions of sodium from the intracellular space to the extracellular environment and vice versa, at the same time allow two ions of potassium to enter the cell. The high- affinity Ca^{2+}-ATPase is the one of the major active calcium transport protein responsible for the regulator of normal intracellular calcium levels in various cell types. The maintenance of the cation gradient by high affinity Ca^{2+}-ATPase is important to the control of hydration, volume, nutrient uptake and fluidity of cells, and is also essential for the contractility and excitability of muscles [13]. Low-affinity Ca^{2+}-ATPase is having major responsible for the shape and deformability of the erythrocyte membranes [14]. The Insulin response is standout amongst the hormones that control those amalgamations [15]. The amount and the activity of $(Na^+ + K^+)$-ATPase in the plasma membrane are reduced in diabetic animals and insulin administration restores near normal conditions in experimental group compared to control rats [16].

A persistent hyperglycemia activates the polyol pathway and increased accumulation of sorbitol leads to reduction of $(Na^+ + K^+)$-ATPase activity. Several alterations in structural and dynamic properties of

erythrocyte membrane are reported in both type 1 and type 2 diabetes, respectively [17]. Of particular interest, the alterations in the activity of erythrocyte membrane (Na^++K^+)-ATPase in type 1 and type 2 diabetes [18]. The membrane bound (Na^++K^+)-ATPase or those sodium pump also contributes to manage the activity of molecules involved in cell division functions that depends upon the intra and extracellular Na^+ and K^+ concentrations. Those particle gradient ion produced by (Na^++K^+)-ATPase impetus for cell volume and osmotic pressure, which acts as a driving force for inward co-transport of amino acids and monosaccharides, and also induction of the active co-transport of ions such as H^+, Ca^{2+} and K^+. Impaired (Na^++K^+)-ATPase is a feature of diabetes mellitus in many cell types and is believed to be a pivotal regulator of various cell functions. It is widely believed that an impairment in (Na^++K^+)-ATPase activity may play a major role at the cellular level in the pathophysiology of many late complications of diabetes mellitus: neuropathy, nephropathy and retinopathy [19] and in the development of diabetic vascular complications [20]. *A. aspera* Linn. Belonging to family Amarathaceae, is commonly found as a weed on way side throughout India. It is known as Apamarg in Sanskrit, Chirchitta in Hindi and Prickly chaff flower in English, Naayuruvi in Tamil. *A. aspera* seeds are used to treat snakebites, hydrophobia and itching. Seeds are emetic and used as a brain tonic painful delivery. The juice of the plant is used to stop bleeding of wounds [21]. *A. aspera* is having multiple capacities as like as anti-inflammatory, antimicrobial, antinociceptive, antifungal, immunomodulatory antifertility, antiurolithiatic and anxiolytic activities [22]. *A. aspera* is having phytoactive constituents and reduction of lipid peroxidation and enhancement in free radical scavenging activity of the herbal seed crude powder [23]. In our previous work have been *A. aspera* exhibit potent hypolipidimic, active against HFFD rats [24]. Dependent upon these perceptions, we analyzed the impacts of *A. aspera* in body weight, glucose, insulin, Hb, HbA1c, Total-ATPase, Na^++K^+--ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase.

Materials and methods

***Animals*:** Healthy male adult albino rats (Wistar strain) 6-7 weeks old, weighing 160-180g was procured from "Sri Venkateswara Enterprises", Bangalore, India. They were housed in a clean sterile polypropylene cages with proper aeration and lighting (12 ± 1 hr day / night rhythm) throughout the experimental period. During the course of the experiments, the temperature was maintained between 27ºC ± 2ºC. The animals were fed with commercially available pelleted rat feed (Gold-Mohur, M/S Hindustan Lever Ltd, Mumbai, India) during the acclimatization period and water *ad libitum*. The usage and handling of experimental rats was done according to the rules and regulations given by the Institutional Ethics Committee.

After one week of acclimatization the animals were divided into two batches. One batch was provided with a control diet containing starch as the source of carbohydrate and the other was fed a fructose-enriched diet for 45 days. They were fed either a control diet, containing 60% corn starch, 20% casein, 0.7% methionine, 5% groundnut oil, 10.6% wheat bran, 3.5% salt mixture and 0.2% vitamin mixture, or a high-fructose diet, which had the same composition as the control diet, except that corn starch was replaced with an equal amount of fructose. The total experimental duration was 45 days. Supplementation of *A. aspera* (100mg kg^{-1} body weight) was given orally for the last 15 days of the experimental period. This dose selected based on our previous study [25]. The rats were divided into four groups and consisting of six rats each.

Experimental Design

Group I : Normal control rats.

Group II : Control rats treated with the crude powder of *A.aspera* seeds (100mg kg^{-1} body weight) twice daily for a period of last 15 days of the experimental period.

Group III : High Fructose fed rats (>60% fructose for 45 days).

Group IV : High Fructose fed rats treated with the crude powder of *A.aspera* seeds (100mg kg^{-1} body weight) twice daily for last 15 days of the experimental period.

Chemicals : Fructose, bovine serum albumin, glucose-6-phosphate, g-glutamyl paranitroaniline, nicotinamide adenine dinucleotide (NAD^+, NADH), nicotinamide adenine dinucleotide phosphate ($NADP^+$, NADPH), reduced glutathione, oxidized glutathione, adenosine triphosphate (ATP), adenosine monophosphate (AMP) and 1,2,4-aminonapthol sulphonic acid were obtained from Sigma Chemical Company, ST. Louis, MO, USA.

All other chemicals and reagents used were of analytical grade with highest purity. They were obtained from Glaxo Laboratories, Mumbai, SD Fine Chemicals, Mumbai and Sisco Research Laboratories, Pvt. Ltd., India.

***Collection of Samples*:** At the end of experimental period, the rats were fasted overnight and killed by cervical decapitation under mild ether anesthesia. Blood was collected in heparinised tubes to separate the plasma. Liver and kidney was perfused *in situ* with cold 0.15M NaCl at 37ºC before collection.

To collect the erythrocytes, the overnight fasted animals were anesthetized by giving an intramuscular injection

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of ketamine (24 mg/kg) and sacrificed by decapitation between 8 a.m and 9 a.m. Blood was collected in the tubes with EDTA and erythrocytes were separated by washing with 0.15 M sodium chloride solution. Erythrocytes and tissues (liver, kidney and heart) were collected for the measurement of membrane bound ATPases such as total ATPases, $(Na^+ + K^+)$ - ATPase, low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase. The tissues were homogenized in Tris buffer prepared in deionised water. The deionised water was used throughout the experiment to avoid interference to phosphorous estimation in the assay of ATPases.

Analytical method: Plasma insulin was assayed using ELISA kit by the method of Burgi *et al*. [26]. Blood glucose, Hb and HbA1c were estimated by the methods of Ramesh *et al*. [27] respectively. The activity of total ATPases, phosphate $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase was measured by the method of Ramesh and Pugalendi [28].

Statistical analysis: Values or mean ± SD for six rats in the each group and statistical differences between mean values were determined by one way analysis of variance (ANOVA) followed by DMRT test for multiple comparison. The values of $P < 0.05$ were considered to be significant. Statistical Package for Social Studies (SPSS Inc., Chicago, IL) 19.0 versions were used for this analysis.

Results

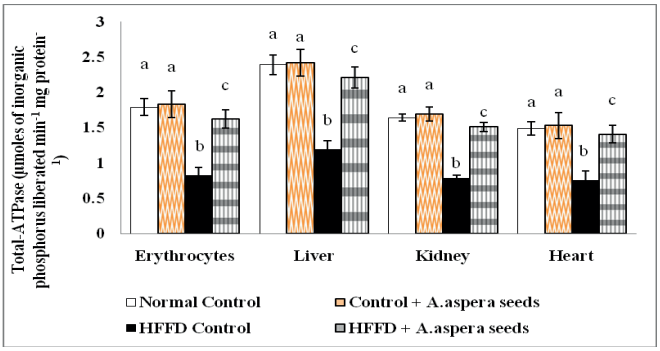
Table 1 illustrates the effect of *A. aspera* seeds on body weight, glucose, and insulin in normal and fructose fed rats. The insulin level decreased significantly while the level of glucose and body weight significantly increased in fructose fed rats when compared with normal rats. A significant improvement in insulin level with a marked reduction in fructose-induced elevation in the levels of glucose and body weight was observed in Table - 2 Effect of A.aspera seeds in diet on Hb and HbA1c in normal and fructose fed animals.

Group	Hb (g/dL)	HbA1c (mg/g of Hb)
I (Normal Control)	11.09 ± 0.97 a	0.36 ± 0.02 a
II (Control + A.aspera seeds)	11.18 ± 0.91 a	0.34 ± 0.03 a
III (HFFD Control)	5.92 ± 0.36 b	1.38 ± 0.11 b
IV (HFFD+ A.aspera seeds)	10.61 ± 0.82 c	0.41 ± 0.03 c

rates administered with *A. aspera* seeds.

Table 2 illustrates the effect of *A. aspera* seeds on Hb and HbA1c levels in normal and fructose fed rats. The level of Hb significantly decreased along with a significant

Fig. 1. Effect of A.aspera seeds in Total-ATPase in Erythrocytes. Liver, Kidney and Heart of Control and Experimental Rats



Columns are means + SD for six rats in each group. Columns not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT).

Table - 1 Effect of A.aspera seeds in diet on body weight, blood glucose and insulin in normal and fructose fed animals.

Group	Body weight (g)		Blood glucose (mg/dL)		Insulin (µU/ml)
	Before treatment	After treatment	Before treatment	After treatment	
I (Normal Control)	188.25 ± 16.1 a	218.59 ± 18.13 a	81.54 ± 7.82 a	84.91 ± 7.92 a	18.45 ± 1.67 a
II (Control + A.aspera seeds)	188.68 ± 15.3 a	189.47 ± 13.57 a	78.47 ± 5.3 a	79.42 ± 6.18 a	18.93 ± 1.44 a
III (HFFD Control)	261.41 ± 19.5 b	311.25 ± 19.12 b	248.62 ± 21.32 b	314.05 ± 20.16 b	5.18 ± 0.4 b
IV (HFFD + A.aspera seeds)	263.59 ± 21.1 b	190.07±18.87 c	251.12 ± 15.73 b	94.41 ± 6.38 c	17.42 ± 1.47 a

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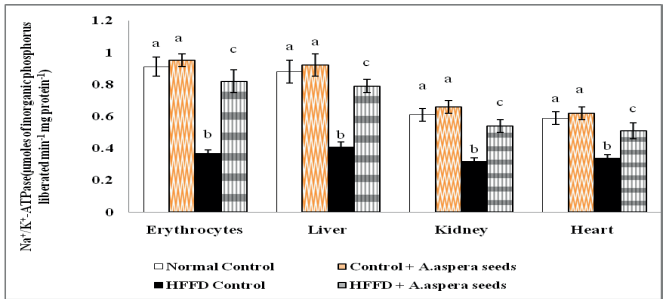
increase in HbA1c was observed in fructose fed rats. The administration of *A. aspera* seeds significantly improved the Hb level and decreased the level of HbA1c

Fig. 2. Effect of A.aspera seeds in Na+/K+-ATPase in Erythrocytes. Liver, Kidney and Heart of Control and Experimental Rats



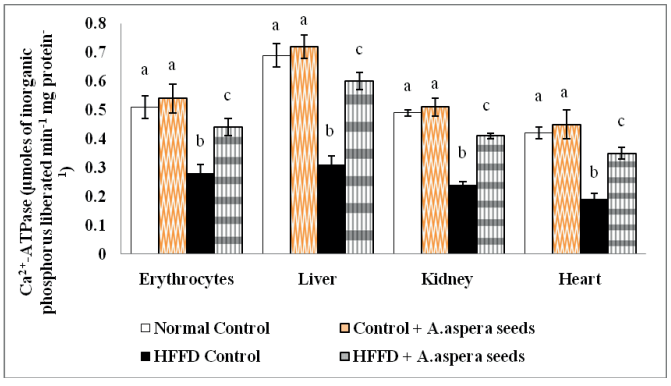
Columns are means + SD for six rats in each group. Columns not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT).

in fructose fed rats.

The activities of total ATPase and (Na^++K^+)-ATPase in the erythrocytes and tissues (liver, kidney and heart) of control and experimental rats are given in Fig. 1 and 2, respectively. In rats fed with high fructose diet, the activities of total ATPase and (Na^++K^+)-ATPase in the erythrocytes and tissues were significantly decreased and treatment with *A. aspera seeds* restored the activities of total ATPase and (Na^++K^+)-ATPase to near normalcy.

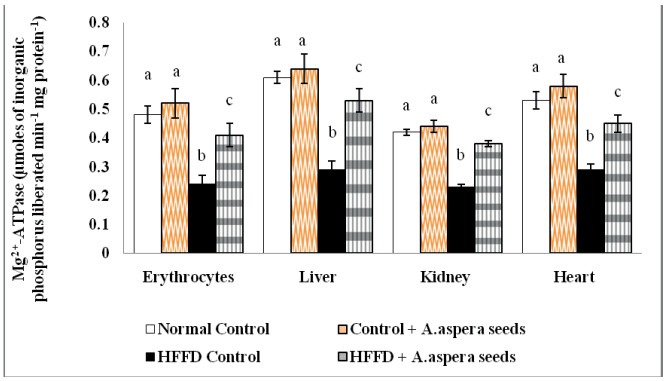
Fig. 3 and 4 represent the activities of low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase in the erythrocytes and tissues (liver, kidney and heart) of control and experimental rats. The activities of Mg^{2+}-ATPase and low affinity Ca^{2+}-ATPase were decreased in erythrocytes and tissues (liver, kidney and heart) of high fructose fed rats. While treatment with *A. aspera* improved their activities to near normalcy levels. *A.aspera* seed extract (100 mg/ kg of B.W) is effective dose for all parameters significant

Fig. 3. Effect of A.aspera seeds in Ca2+-ATPase in Erythrocytes. Liver, Kidney and Heart of Control and Experimental Rats



Columns are means + SD for six rats in each group. Columns not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT).

Fig. 4. Effect of A.aspera seeds in Mg2+-ATPase in Erythrocytes. Liver, Kidney and Heart of Control and Experimental Rats



Columns are means + SD for six rats in each group. Columns not sharing a common superscripts (a, b and c) differ significantly at P < 0.05 (DMRT).

effect in HFFD rats as compared to control rats. *A.aspera* in normal control rats didn't show any significant.

Discussion

A very high level of plasma glucose connected with hyperinsulinaemia impedes insulin action in fructose-fed rats. Insulin response largely needed for anabolism and it fortifies protein amalgamation and retards protein degradation [29]. Previous reports have shown that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin [30], which may be partly responsible for the decreased level of Hb in diabetic rats. HbA1c comprises 3.4 percent to 5.8 percent of total Hb in normal human red cells [31], but it increases in diabetic patients up to 16 percent [32]. In fact, monitoring the level of HbA1c is a reliable index of glycemic control in diabetes [33]. A raise in the level of HbA1c and reduced level of Hb is observed in our study revealed that diabetic animals had prior high blood glucose level. The administration of *A.aspera* to fructose fed rats increased Hb level and decreased the level of HbA1c, which may be due to the reduction of the blood glucose level. This may be due to presence of saponin and flavnoids *in A. aspera*.

Among different types of ATPases, (Na^++K^+)-ATPase, low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase play important role in the membrane stability, cell homeostasis and functions. Insulin and catecholamines are vital mediators of acute hormonal control of Na'/K^+-ATPase [34]. In fructose fed rats, we observed a reduction in the level of (Na^++K^+)-ATPase in the tissues, which correlates with previous report from other research group [35]. This reduction could be connected with lack of insulin, as insulin administration partially restored (Na^++K^+)-ATPase [16]. The oxidative damage of proteins causes their inactivation and degradation. As the (Na^++K^+)-ATPase is rich in thiol groups, the oxidation of thiol groups can inhibit its enzymatic activity[36]. It is well known

that hyperglycemia leads to glycosylation of proteins and increases cellular lipid peroxidation processes, which, in turn, can cause inhibition/reduction in the activities of $(Na^+ + K^+)$- and Ca^{2+}-ATPases. This, in turn, influences the intracellular concentrations of Na^+, K^+, and Ca^{2+} and alters the signal transduction pathways, which consequently leads to cellular dysfunctions[19]. In fructose fed rats, a decrease in the activity of low affinity Ca^{2+}-ATPase is a consequence of interaction of glucose with this enzyme [37]. The low-affinity Ca^{2+}-ATPase is responsible for the shape and deformability of the erythrocyte membranes[14]. Treatment with *A. aspera* restored the activity of low affinity Ca^{2+}-ATPase, which might be associated with improved insulin effects and reduced blood glucose. Erythrocyte $(Na^+ + K^+)$-ATPase plays a central role in the regulation of intra- and extracellular cation homeostasis. $(Na^+ + K^+)$-ATPase is an integral protein in the red cell membrane; its catalytic activity is dictated by the vicinal activation of phospholipids. The alteration in this transport system is linked to several complications of diabetes [38]. Concerning illustration $(Na^+ + K^+)$-ATPase is an integral protein in the red cell membrane; its catalytic activity can be dictated by the vicinal activation of phospholipids. As there is a direct interaction between enzyme and phospholipids [39], the changes in lipids in the erythrocyte of diabetics could lead to decreased $(Na^+ + K^+)$-ATPase activity [40]. Interestingly, *A. aspera* administration restored $(Na^+ + K^+)$-ATPase, low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase along with a marked reduction in lipidperoxidative damage to membrane phospholipids. .

In this context, the normalization of membrane bound ATPases by *A. aspera*-treatment may protect erythrocytes from the risk of deformability, preserve specific properties of tissues and prevent diabetic complications. Many of the well-characterized membrane ATPases (P type ATPases - Na^+, K^+- and Ca^{2+}-ATPases; V type ATPases- lysosomes;) are transport proteins and they utilise intracellular ATP to drive active ion transport. However, F-type ATPases are involved in the formation of ATP in mitochondria. In contrast, the ubiquitously expressed cell surface ATPases, hydrolyze extracellular ATP (and UTP, ADP) [41]. Similarly, Ecto ATPases (extracellular ATPases) are cell surface ATPases that hydrolyze extracellular ATPs. E-type ATPase activity is another class of ATPase that was first designated as Mg^{2+}-ATPase [42]. In our study, the high fructose diet induced decrease in the activities of total ATPases was significantly improved by *A. aspera*, which might be mainly due to improved blood glucose level and its homeostatic processes.

Conclusion

In conclusion, our results show that *A. aspera* in the diet reduces blood glucose and HbA1c level by increasing insulin secretion and/or activity, and thereby it improves the activities of $Na^+ + K^+$-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase in erythrocytes and tissues of rats fed with high fructose diet.

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Conflict of interest

None declared

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