EFFECT OF APPLE (MALUS DOMESTICA) ON Na⁺/K⁺-ATPase ACTIVITY IN LIVER, KIDNEY AND HEART OF ADULT WISTAR RATS

ABSTRACT

Aim: The effect of crude extract of apple on the activity of Na⁺/K⁺-ATPase in the liver, kidney and heart tissues of rats was investigated.

Methodology: Two separate test groups of animals were fed a standard laboratory diet, water and given 1 ml and 1.5 ml/ kg body weight of crude extract of apple respectively. The control animals were fed the diet and water. The animals were treated for 6 weeks and then sacrificed. The liver, kidney and heart were excised from the rats and subsequently used for analysis of Na⁺/K⁺-ATPase.

Results: The results obtained indicate that there was no significant (p>0.05) change in body weights of rats administered 1 ml and 1.5 ml/ kg body weight of apple extract. Similarly no significant (P>0.05) change was observed in the weight of liver, kidney and heart of test rats relative to control. Examination of the data reveal that the activity of the enzyme was significantly (P<0.05) decreased in the kidney but not in the liver and heart of the rats treated with the extract as compared to control. The renal Na⁺/K⁺-ATPase activity was lowest in rats treated with an extract dose of 1.5 ml / kg body weight of extract.

Conclusion: The present study has shown that the Na⁺/K⁺-ATPase activity in the kidney was inhibited by apple extract. The inhibition of the enzyme is suspected to be due to some phytochemicals present in apple. However, this suggestion remains to be scientifically confirmed.

Keywords: Whole apple extract, Flavonoids, Na⁺/K⁺-ATPase activity, Wistar rats.

1. INTRODUCTION

Apples (Malus domestica) are consumed more in the developed countries than in developing countries. Apple constitutes one of the five fruits recommended for daily consumption [1]. Apples are often eaten raw, and except for the seeds, which are slightly poisonous, the whole fruit including the skin is suitable for human consumption [1]. Apples bred for the purpose of eating are termed dessert or table apples. Apples can be canned or juiced, and are milled to produce apple cider (non-alcoholic, sweet cider) and filtered for apple juice. The juice can further be
fermented to make cider (alcoholic, hard cider) and vinegar [2]. Through distillation, various alcoholic beverages have also been produced from Apple, which include drinks such as Applejack, Calvados, and Apple wine [3]. Pectin and Apple seed oils may also be produced from Apple.

Apart from its nutritional value, apples are a good source of antioxidants [3]. Report has shown that apples have the next highest concentration of antioxidant activity amongst fruits eaten in the United States [3]. The Medicinal value of apple is attributed to the presence of different phytochemicals which are known to protect against many of the disease known to man [4, 5]. The phytochemicals are the non-nutritive component of the fruit and this include flavonoids, isoflavonoids and phenolic acids [3]. Data has also shown that catechin and epicatechin are constituents of apple [6]. In terms of phenolic content, it has been shown that apple has the next highest of concentration of phenols compared to other fruits, and also has the highest concentration of free phenol [7]. Apple also remains a very important source of dietary flavonoids. A Finnish study involving approximately 10,000 people, associated flavonoid intake to low total mortality [8]. In that study, apple turned out to be the highest source of dietary flavonoids.

The ‘Zutphen Elderly Study’ [9], showed that the intake of flavonoids was sturdily connected with a reduced mortality from heart diseases in elderly men, and also was negatively linked to coronary heart disease. In this study, tea was however the main source of the flavonoids, even though flavonoids from apple contributed 10% of total ingested flavonoids. Flavonoids from apple were equally connected to a decreased risk of death from coronary heart disease [9]. This previous finding by Hertog et al. [9] was corroborated in another study by Sesso et al. [10] which reported a 35% decrease in the risk of cardiovascular diseases in subjects that consumed the highest quantity of dietary flavonoids.

In addition to the above effects of flavonoids on cardiovascular disease, some studies also demonstrated that flavonoids can influence some disease states linked to the activity of the ion transporting ATPases like the Ca-ATPase in sarcoplasmic reticulum and Na⁺/K⁺-ATPase [11]. The later plays a crucial role in maintaining the homeostasis of Na⁺ in the organism. Since ion transport mediated by Na⁺/K⁺-ATPase is the major consumer of metabolic energy in the kidney, the enzyme is considered as critically important to renal function. Changes in the activity of this enzyme may have major impact on basic cellular functions leading to either functional benefits or pathological consequences. The function and regulation of Na⁺/K⁺-ATPase in the kidney is altered in various forms of spontaneous hypertension [11]. The majority of cardiovascular disease (CVD) is caused by risk factors such as hypertension [12].
The literature contains numerous studies on the effect of individual phytochemical constituents of apple on Na⁺/K⁺-ATPase activity in tissues of rats [9]. However there is a paucity of research information on the effect of the crude extracts of apple on activity of Na⁺/K⁺-ATPase in the tissues of rats.

In the light of the above, the present study examined the effect of crude extract of whole apple on the activity of Na⁺/K⁺-ATPase in the liver, kidney and heart of male Wistar rats.

2. MATERIAL AND METHODS

2.1 Collection and preparation of aqueous extract of apple (Malus domestica)

Fresh whole apple (Malus domestica) fruits weighing about 180-200 g were bought from the market in Abraka, Nigeria. Each apple fruit was washed and chopped into small sizes before it was blended with the aid of electric blender. The aqueous extract or juice (golden-colouration) was extracted by gravity-aided filtration using a clean white cotton cloth. This process of extraction was done on a daily bases because the juice often oxidizes quickly, with notable change in colour. This occurred even when stored in the refrigerator. Each of the whole apples produced 15 - 20 mls of the aqueous extract giving a total aqueous extract of 200 mls.

2.2 Animals

A total of eighteen (18) male Wistar rats weighing between 100-160 g were procured from the breeding colony of the animal facility of the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were housed in well ventilated cages, at room temperature (24 ± 2°C) with a 12:12 hour light/dark cycle, and were allowed free access to clean drinking water and growers mash (Guinea Food Nigeria Ltd). The animals were acclimatized for two weeks and received humane care in compliance with the ethical guide for the care and use of Laboratory Animals approved by the College of Health Sciences, Delta State University, Abraka, Nigeria (DELSU/CHS/EC/14/108).

2.3 Experimental design

The animals were randomly allocated to three experimental groups of rats each, and were administered their feeds and extract (test groups) on a daily basis for 6 weeks.

**Group 1**- (Control Group) Fed on growers mash and water only.

**Group 2**- (Test Group) Fed on 1.0 ml/kg body weight of aqueous extract of Apple plus growers mash and water.

**Group 3**- (Test Group) Fed on 1.5 ml/kg body weight of aqueous extract of Apple plus growers mash and water.
2.4 Sample preparation
At the end of 6 weeks of treatment, all rats were weighed and were sacrificed under chloroform anaesthesia. The abdomen and the thorax were opened and the heart, liver and kidney of each rat were collected. Each of the collected organs was washed thoroughly in ice cold physiological saline (0.9% (w/w) NaCl, blotted on filter paper, weighed and frozen. A ten percent homogenate was prepared from each frozen organ, using ice cold 1.15 % (w/w) KCl. The homogenates were centrifuged at 4°C, and aliquots of the supernatants obtained were used for biochemical assays.

2.5 Na⁺,K⁺-ATPase activity assay
The reaction mixture for the Na⁺/K⁺-ATPase assay contained 5.0 mM MgCl₂, 80.0 mM NaCl, 20.0 mM KCl, and 40.0 mM Tris–HCl buffer, pH 7.4, in a final volume of 200 microliter. The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0 mM. Control was assayed under the same conditions with the addition of 1.0 mMouabain. Na⁺/K⁺-ATPase activity was calculated by the divergence between the two assays [13]. Released inorganic phosphate (Pi) was measured by the method of Chan et al. [14]. Enzyme specific activity was expressed as nmol Pi released per min per mg of protein. All assays were performed in duplicate and the mean was used for statistical analysis.

2.6 Statistical Analysis
Data are expressed as Mean ± SEM from 6 animals in each group (n=6). Differences between the experimental groups were evaluated using the non-parametric Man-Whitney U-

3. RESULTS AND DISCUSSION
Table 1. Effects of aqueous extract of apple (Malus domestica) on the body weight of adult Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Change in weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>113±4.63</td>
<td>121±3.67</td>
<td>8.00±0.52</td>
</tr>
</tbody>
</table>
Group 2 (1 ml/kg Body weight) 111±6.40 123±8.30 12.00±0.42*
Group 3 (1.5 ml/kg Body weight) 121±5.56 119±9.27 -2.00±0.31*†

Data values for % change were expressed as Mean ± SEM. P <0.05 (Mann-Whitney U-test) compared to control (*), compared to rats treated with 1ml / Kg body weight (†).

Table 2. Effects of aqueous extract of apple (Malus domestica) on organ weight of adult Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney weight (g)</th>
<th>Liver weight (g)</th>
<th>Heart weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>1.18±0.37</td>
<td>7.87±0.69</td>
<td>2.46±0.18</td>
</tr>
<tr>
<td>Group 2 (1 ml/kg Body weight)</td>
<td>1.02±0.08</td>
<td>6.04±0.42</td>
<td>2.24±0.13</td>
</tr>
<tr>
<td>Group 3 (1.5 ml/kg Body weight)</td>
<td>1.12±0.66</td>
<td>7.06±0.38</td>
<td>2.32±0.29</td>
</tr>
</tbody>
</table>

Data values for organ weight were expressed as Mean ± SEM. P <0.05 (Mann-Whitney U-test) compared to control (*), compared to rats treated with 1ml / Kg body weight (†).

Table 3. Effect of apple (Malus domestica) on Na+/K+-ATPase activity in liver, kidney and heart of adult Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na+/K+ATPase Kidney (µmol/hr/mg/protein)</th>
<th>Na+/K+ATPase Liver (µmol/hr/mg/protein)</th>
<th>Na+/K+ATPase Heart (µmol/hr/mg/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>73.52±16.95</td>
<td>98.40±4.21</td>
<td>90.20±4.46</td>
</tr>
<tr>
<td>Group 2 (1 ml/kg Body weight)</td>
<td>52.40±20.53*</td>
<td>74.80±8.54</td>
<td>75.40±3.65</td>
</tr>
<tr>
<td>Group 3 (1.5 ml/kg Body weight)</td>
<td>28.80±3.59*†</td>
<td>85.60±1.63</td>
<td>77.4±3.72</td>
</tr>
</tbody>
</table>

Data values for Na+/K+ ATPase were expressed as Mean ± SEM. P <0.05 (Mann-Whitney U-test) compared to control (*), compared to rats treated with 1ml / Kg body weight (†).

The effect of crude extract of apple on body and organ weights of rats is shown in Tables 1 and 2 respectively. The results obtained indicate that there was a significant (p>0.05) change in body weight gain of rats administered 1 and 1.5 ml/kg body weight of apple extract relative to control. Rats administered 1.5 ml/kg body weight actually had a negative weight gain. Conversely, no significant (P>0.05) change was observed in the weight of liver, kidney and heart of test rats relative to control. The finding on body weight loss observed in rats treated with a higher dose of...
apple extract (1.5 ml/kg body weight) in this study is in agreement with available reports by Omole and Ighodaro [15] which indicate that apple reduced the body weight of experimental rats. The authors attributed this effect to the ability of fruits to help in body weight reduction through ease of excretion. It is also noteworthy that consumption of apple by high fat diet-induced obese rats improved body weight and body fat loss and blood lipid profiles [16]. Changes in organ weights have often been used as indices of toxicity of xenobiotics [17]. Thus the lack of significant change in the weight of organs of apple treated rats is a likely indication of the safety of apple which is in consonance with previous reports [1].

The effect of aqueous extract of apple on the activity of Na\(^+\)/K\(^-\)-ATPase in the liver, kidney and heart is presented in Table 3. Examination of the data reveal that the activity of the enzyme was significantly (P<0.05) decreased in the kidney but not in the liver and heart of the rats treated with the extract as compared to control. The renal Na\(^+\)/K\(^-\)-ATPase activity was lowest in rats treated with a dose of 1.5 ml / kg body weight of extract. The decreased kidney Na\(^+\)/K\(^-\)-ATPase activity is a possible indication of an abnormality as it could impact on the electrophysiological and biochemical functioning of this organ [18] with severe consequences. For instance, activation of renal Na\(^+\)/K\(^-\)-ATPase activity has been implicated as a possible event in the processes that lead to spontaneous hypertension [11]. In this work, though, the blood pressure of the rats was not measured but available reports in literature indicate that apples contain components that can lower high blood pressure [19].

The phytochemical constituents of apples have been studied [7] just as their specific effect on various disease state has also been elucidated [4,5]. The influence of some of the phytochemicals of apple on some of this disease state has been linked to their antioxidant activity as well as the modulation of Na\(^+\)/K\(^-\)-ATPase activity [11,20]. As well as being the highest source of dietary flavonoids [8], apple is also known to have the highest concentration of phenols compared to other fruits with the concentration of free phenols in apple, the highest ever [7]. There is no doubt that flavonoids affect cell membrane transport system such Na\(^+\)/K\(^-\)-ATPase [21]. Na\(^+\)/K\(^-\)-ATPase is considered a specific receptor for cardiac glycosides [22]. However the mechanisms by which flavonoids inhibit the activity of Na\(^+\)/K\(^-\)-ATPase is yet to be elucidated [23]. The inhibition of the enzyme by flavonoids has been postulated to be connected with the non-cardiac glycoside specific binding sites of Na\(^+\)/K\(^-\)-ATPase [23]. It is probably due to a precise hydroxylation design of the ‘B’ ring of the flavonoles. It is believed that the inhibitory activity of flavonoles is increased in relation to Na\(^+\)/K\(^-\)-ATPase, just as was demonstrated in mast cell secretion [18]. It is also conceivable that the mechanism by which flavonoids inhibit Na\(^+\)/K\(^-\)-ATPase activity is by changes in membrane composition and fluidity in the immediate Na\(^+\)/K\(^-\)-ATPase domain as was suggested in a previous study on Na\(^+\)/K\(^-\)-ATPase activity with coconut oil and palm kernel oil supplemented diets [24].
In conclusion, it has been shown in the present study that the Na⁺/K⁺-ATPase activity in the kidney was inhibited by the action of apple extract. The inhibition of the enzyme is suspected to be due to some of the phytochemicals present in apple. However, this suggestion remains to be scientifically confirmed.

4. CONCLUSION

In conclusion, it has been shown in the present study that the Na⁺/K⁺-ATPase activity in the kidney was inhibited by the action of apple extract. The inhibition of the enzyme is suspected to be due to some of the phytochemicals present in apple. However, this suggestion remains to be scientifically confirmed.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

I hereby declare that "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the Delta State University's ethical committee for the use of laboratory animals.

I hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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