INTRODUCTION

Intestinal enzymes promote cell differentiation, decrease inflammation and uptake of nutrients through the gut mucosa. Alkaline phosphatase interacts with bacterial flora in the gut, detoxifying lipopolysaccharides, cytosine-guanosine dinucleotides and flagellin, subsequently maintaining intestinal homeostasis. Intestinal enzymes interact with dietary nutrients, which influence their expression and ultimately have an effect on normal gut flora. An experiment carried out on rat intestines showed that the secretion of intestinal alkaline phosphatase is influenced by the levels of fats, carbohydrates and proteins in the diet. Adenosine triphosphatase, as part of the Na+/K+ ATP-ase complex, aids in the absorption of intestinal sodium and water.

Spices and herbs are a common ingredient in South Asian cuisine, used for their flavour enhancing properties, color and also for medicinal purposes. Some common spices that are a dominant ingredient in everyday cooking are onions, garlic, ginger,
pepper, turmeric and chillies. These spices have been found to have several different beneficial effects on the body. Some of these include anti-oxidant, anti-tumorigenic, antimicrobial, and anti-inflammatory properties. There is a paucity of studies on the effects of spices used in South Asian cuisine on the levels of intestinal enzymes. While the effects of certain spices on tumor growth, inflammation during autoimmune disease processes such as rheumatoid arthritis and H. pylori growth has been researched, this aspect of their activity in the body has not been fully determined. The objective of this study was to find out the effect of fourteen seasonal spices on the three intestinal phosphatases: alkaline phosphatase, adenosine triphosphatase (ATPase), and acid phosphatase.

Fourteen commonly used spices were selected for these investigations. These were identified according to systematic (botanical) names as shown in Table 1. These spices were cleaned, powdered and stored in wide mouthed bottles.

### METHODS

#### Experimental Animals:
Animals selected for these investigations were male rabbits. Although rabbits are herbivorous, they were preferred due to the following reasons:
1. Rabbits are abundant in local areas and therefore easily available
2. Rabbits have not been previously exposed to spices and therefore the exact effect of spices on their intestinal phosphatases can be easily detected. Rabbits were obtained from the local market regardless of their size and age. Prior to experimental assays the animals were sacrificed and homogenate of the upper part of the intestine was prepared.

#### Lipid extraction
20 grams of each powdered spice was weighed and poured in a thimble to extract 40ml lipid in a soxhlet apparatus so that each ml of extract would contain 0.5 grams of spices. Aqueous extracts were prepared from the defatted spces. A 10% aqueous extract was prepared and centrifuged at 15000rpm. Supernatant was taken as a 10% extract of the spices.

#### Enzyme assays
General diagnostic kits were used for a calorimetric estimation of alkaline phosphatase, ATPase and acid phosphatase, according to manufacturer’s instructions (for alkaline phosphatase Assay Kit (Colorimetric) (ab83369) by Abcam, for acid phosphatase Assay Kit by Merck, and for ATPase, Activity Kit (Colorimetric) by Novus biological can be used). Assays were performed separately for lipid extract and aqueous extracts of the spices. Controls were run with each assay without homogenate.

### TABLE 1: COMMON NAMES, BOTANICAL NAMES AND PARTS OF THE PLANTS USED AS SPICES

<table>
<thead>
<tr>
<th>COMMON NAME OF SPICE</th>
<th>BOTANICAL NAME OF SPICE</th>
<th>PART OF THE PLANT USED AS SPICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANISE</td>
<td>PIMPENELLAANISUM LINN</td>
<td>FRUIT</td>
</tr>
<tr>
<td>BLACK PEPPER</td>
<td>PEPPERANIGRUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CARAWAY</td>
<td>CARUMCARVI</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CARDAMOM (LARGE)</td>
<td>ELETTARICARDAMOMIUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CARDAMOM (SMALL)</td>
<td>ELETTARICARDAMOMIUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CHILLIES</td>
<td>CAPSICUM ANNUN</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CINNAMON</td>
<td>CINNAMOMUMZELANICUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CLOVE</td>
<td>EUGENIA CARYOPHYLLATA</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CORIANDER</td>
<td>CORIANDRUMSATIVUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>CUMIN</td>
<td>CUMINUMCYMIMUM</td>
<td>FRUIT</td>
</tr>
<tr>
<td>GARLIC</td>
<td>ALLIUM SATIVUM</td>
<td>BUD</td>
</tr>
<tr>
<td>GINGER</td>
<td>ZINGEBEROFFICINALE</td>
<td>RHIZOME</td>
</tr>
<tr>
<td>ONION</td>
<td>ALLIUM CEPA</td>
<td>LEAVES</td>
</tr>
<tr>
<td>TURMERIC</td>
<td>CURCUM LONGA</td>
<td>RHIZOME</td>
</tr>
</tbody>
</table>
RESULTS
The results for this study were analysed using SPSS version 20. Since the data was non-parametric, the median and interquartile range were calculated. A p-value of <0.05 was considered statistically significant.

Biochemical analysis of Spices
The highest lipid content was found in cinnamon (0.44 gms/gm), and similar high values were also observed in anise, black pepper, coriander, cloves and chillies. Lower values were observed in ginger, garlic, cardamom (large) and onion i.e. from 0.009 gms/gm in ginger and 0.005 gms/gm of cardamom (large).

The highest water soluble content was present in cardamom (large) with a value of 0.639 gms/gm of cardamom (large). Cinnamon contained the lowest value of 0.005 gms/gm.

Chillies carry the highest moisture content with a value of 0.54 gms/gm, and the highest caloric content was calculated as 5.77 kcal/gm, found in cumin.

Activity of acid phosphatase, alkaline phosphatase and adenosine triphosphatase in the intestine of rabbit
The enzymatic assay at baseline, i.e. without intake of spices, served as a control for the study and aided in determining the effect of spices on the activity of these enzymes. The levels of intestinal acid phosphatase, alkaline phosphatase and adenosine triphosphatase showed significant differences. Acid phosphatase was present in the rabbit’s intestines between a range of 41.07-50 IU. Alkaline phosphatase levels ranged from 96.7-125 IU, while the amount of adenosine triphosphatase was calculated to be 3.1-4 IU (62-82 gms per 100 mg of tissue).

Acid phosphatase
The results of effects of lipid and aqueous extracts are summarised in Tables 2-5. In general, it appears that the effect of the lipid extract of spices on this enzyme is inhibitory. Although chillies, garlic and cardamom (small) are activating the enzyme, the effect of only chillies and garlic are significant.

Black pepper, cinnamon, cumin and turmeric moderately decrease the activity from 12 to 16%.

As seen with lipid extracts, the aqueous extracts of spices demonstrated an inactivating effect on the activity of acid phosphatase.

Lipid extracts of black pepper, turmeric, cardamom (small), garlic, ginger, cardamom (large) and onions exhibited a fall in enzyme levels from 31.40 to 39.23 IU, which is a 4-23% decrease in activity, however these results were not statistically significant due to a high standard error. Inactivating effects produced by cumin, chillies, cinnamon and coriander range from 27.5 to 45.5% decrease. A very significant decrease in activity was observed in the presence of Anise. At an enzymatic level of 14.5 IU, there was a 64.7% decrease in activity. However, this difference was not statistically significant.

Alkaline phosphatase
The results show that half of the spices investigated had an activating activity, while the other had inhibitory effects on the enzyme. The results of chillies and coriander are very significant, their levels, ranging from 119 to 125 IU demonstrated a 23-29% increase in enzymatic levels.

In general, the effect of aqueous extract on the enzyme is inhibitory. Cardamom influenced no change in the activity of enzyme. Cloves, garlic and cinnamon inhibited the enzymes activity appreciably demonstrating a 13.6 to 16% decrease in activity.

ATP-ase
The results of lipid and aqueous extracts are shown in Tables 2-5. The lipid extracts of garlic and onion are the only spices that increased the activity ranging between 27.4% to 38.7%. Chillies, cumin and cinnamon activate the enzyme from 1.69 to 2.15, demonstrating a 30-45% decrease. Although not statistically significant, these changes are noticeable.

In general, the effect of aqueous extracts is also inhibitory. Black pepper and cardamom have no effect on the activity. The most significant decrease in activity is demonstrated by caraway, cumin and clove with levels ranging between 1.2 – 2.1 gms, which is a 51 to 72% decrease in activity.
<table>
<thead>
<tr>
<th>Spice</th>
<th>ACID</th>
<th>ALK</th>
<th>ATP</th>
<th>MA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Pepper</td>
<td>0.28</td>
<td>0.29</td>
<td>0.04</td>
<td>0.008</td>
<td>0.060</td>
</tr>
<tr>
<td>Caraway</td>
<td>0.26</td>
<td>0.26</td>
<td>0.06</td>
<td>0.020</td>
<td>0.070</td>
</tr>
<tr>
<td>Cardamom (large)</td>
<td>0.25</td>
<td>0.26</td>
<td>0.06</td>
<td>0.020</td>
<td>0.070</td>
</tr>
<tr>
<td>Cardamom (small)</td>
<td>0.25</td>
<td>0.26</td>
<td>0.06</td>
<td>0.020</td>
<td>0.070</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.26</td>
<td>0.26</td>
<td>0.06</td>
<td>0.020</td>
<td>0.070</td>
</tr>
<tr>
<td>Clove</td>
<td>0.26</td>
<td>0.26</td>
<td>0.06</td>
<td>0.020</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Table 2: Levels of enzymes with anise seed, black pepper, caraway and cardamom (large, small).

Table 3: Levels of enzymes with cardamom (small), chilies, cinnamon, clove.
### Table 4: Levels of enzymes with coriander, cumin, garlic, ginger

<table>
<thead>
<tr>
<th></th>
<th>CORIANDER</th>
<th>CUMIN</th>
<th>GARLIC</th>
<th>GINGER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACID</td>
<td>23.50, 10.12</td>
<td>0.109</td>
<td>38.80, 26.90</td>
<td>0.465</td>
</tr>
<tr>
<td>ALK</td>
<td>115.50, 52.75</td>
<td>0.068</td>
<td>0.068</td>
<td>27.75, 52.88</td>
</tr>
<tr>
<td>ATP</td>
<td>1.65, 0.51</td>
<td>0.068</td>
<td>2.20, 2.45</td>
<td>0.068</td>
</tr>
</tbody>
</table>

### Table 5: Levels of enzymes with onion and turmeric

<table>
<thead>
<tr>
<th></th>
<th>ONION</th>
<th>TURMERIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACID</td>
<td>51.50, 13.75</td>
<td>0.593</td>
</tr>
<tr>
<td>ALK</td>
<td>91.00, 49.63</td>
<td>0.141</td>
</tr>
<tr>
<td>ATP</td>
<td>4.80, 1.68</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Table 4: Levels of enzymes with coriander, cumin, garlic, ginger

Table 5: Levels of enzymes with onion and turmeric
DISCUSSION

Intestinal phosphatases play a vital role in the digestion and absorption of nutrients through the gut wall. While the biological functions of intestinal phosphatases in humans are still largely unknown and under study, the role of alkaline phosphatase has been identified in some experimental studies. Alkaline phosphatase is essential for the maintenance of gut microbiota and ultimately regulates the equilibrium between diet, intestinal flora and mucosal lining. A study on the intestinal mucosa of mice demonstrated that mice with low levels of alkaline phosphatase in their gut absorbed fat at a much faster rate than mice with adequate levels of enzyme. This indicates that intestinal alkaline phosphatase is involved in an essential rate limiting step during mucosal fat absorption. An increase in the levels of intestinal alkaline phosphatase in response to a high fat diet has been observed. This increase in levels, however, occurs in a negative feedback manner as an increased fat consumption resulted in an inhibition of alkaline phosphatase expression and an upregulation of inflammatory markers.

In our study, the levels of intestinal alkaline phosphatase increased by 20-23% with the ingestion of chillies and coriander. This increase in alkaline phosphatase levels could be attributed to the high lipid content in chillies, however studies using other spices with low to moderate lipid content have also resulted in the same findings. An increase in fat and protein digestion was observed in rats ingesting high quantities of spices. Not only was bile acid secretion increased, the mobilization of lipid from adipose tissue was also augmented due to an increase in the activity of hormone sensitive lipase. Additionally, enhanced secretion of bile and pancreatic lipase has been demonstrated in rats with high dietary ginger intake. These findings are indicative of the presence of a trophic effect of dietary spices on gut mucosal enzymes. The influence of spices on dietary nutrient absorption and metabolic activity has been demonstrated in several other studies. While onions and fenugreek, in combination, have a hypoglycemic effect in patients with type 2 diabetes, the flavonoids and sulfur compounds present in onions have hypolipidemic and antioxidant effects. A high dietary intake of onions results in significant reductions in body weight, visceral fat and hepatic steatosis (animal study). These effects of onions on lipogenesis may be attributed to the identification of a bioactive component in onions that influences a decrease in the collection of lipids in the liver. Garlic, like onions, also exhibits hepatoprotective properties.

Capsaicin, an essential compound found in red chillies and chilli peppers, catabolises lipids. A study conducted on Asian and Caucasian subjects revealed that a diet high in red chilli peppers resulted in appetite suppression therefore alleviating the risk of weight gain and subsequent obesity. Additionally, chilli peppers are rich in vitamins and nutrients such as vitamins A, C, K, B6 and potassium. They also induce an anti-inflammatory effect on adipose tissue therefore reducing the synthesis of cytokines such as IL-6 and TNF-a.

Turmeric also has profound beneficial effects in the body. Curcumin, the primary compound in turmeric, has anti-inflammatory, anti-tumorigenic, anti-oxidant, anti-bacterial as well as anti-diabetic effects. Dietary curcumin suppresses the growth of adipose cells while simultaneously modulating the metabolism of energy in these cells, resulting in significant reductions in weight gain. This effect may be attributed to the suppression of the differentiation of pre-adipocytes into mature adipocytes, however this hypothesis has not been fully confirmed and warrants further study.

Dietary spices have been found to have several immunomodulatory functions. Black pepper, although identified as a luminal irritant resulting in epigastric pain upon ingestion, has immunomodulatory effects on the body. Peppers are seen to reduce the synthesis and expression of interleukins 1b, 6 and 12, as well as TNF-a and GM-CSF. Ginger oil has also been utilized for its therapeutic effects in reducing inflammation and pain in patients with rheumatism. Additionally, turmeric helps protect the mucosal barrier of the gut wall and results in a reduction in systemic inflammation. Due to these properties, the use of turmeric is encouraged in patients with chronic kidney disease and associated disorders.

The influence of intestinal phosphatases on gut microbiota and the anti-microbial effects of certain spices raised the question of a possible correlation between the two, in this study. Although no significant association was found, and the antimicrobial effects of spices may be exerted independently of intestinal enzymes, their benefits are noteworthy. The use of spices in Egyptian folk medicine is common, and a study conducted to demonstrate their benefits showed that the compounds eugenol and cinnamaldehyde found in cinnamon have an antibacterial effect on the growth of Bacillus anthracis. Additionally, curcumin in turmeric, as well as ginger, caraway and cumin as well as a few other spices, have also displayed an ability to reduce the activity of H. pylori in the gut. The protective effects of dietary spices are further evidenced by a study conducted in Tokyo in which mycelial growth was significantly inhibited in the presence of black cumin, cloves, cardamom and caraway. Star anise, also a component of South Asian cuisine, is commonly used in East Asia as a traditional herbal medicine. Its beneficial effects are attributed to its antibacterial, anti-inflammatory, anti-bacterial and anti-tumorigenic properties.
Commonly used as an antiseptic in the treatment of otalgia, star anise is also known to alleviate the inflammatory effects of rheumatism.20

In the South Asian population, it is commonly believed that dietary spices exert a simulant effect on the digestive system and enhance the absorption of nutrients.21-23 This effect has been demonstrated in an experimental study conducted on rats in India. Although an increase in the activity of pancreatic amylase, lipase and chymotrypsin was seen in the experimental subjects with a high dietary intake of spices, the effect on phosphatases was not studied.23 Our study findings did not indicate that the digestive stimulation properties of dietary spices can be explained by fluctuations in intestinal phosphatase levels, however further studies need to be conducted to study the effects of phosphatases in the gut to elucidate this finding.

There was no statistically significant change in the levels of enzymes in our study subjects, however this finding can be attributed to certain limitations of our study. While a significant percentage decline in activity of enzymes was observed, the results were not statistically significant. This could be attributed to a high standard error.

CONCLUSION

The selected spices, though an essential and regular component of cooking in the Pakistani population, were found in general suppressing the activity of intestinal enzymes. More studies are required with focused analysis of each spice, its effect on the body and the mechanisms through which they exert these effects. This can allow us to encourage the consumption of certain spices for therapeutic purposes in the treatment chronic inflammatory gut disorders such as Crohns disease and ulcerative colitis, as well as metabolic disorders like obesity, diabetes mellitus and hyperlipidemic syndromes.

REFERENCES

12. McCart M, DiNicolantonio J, O’Keefe J. Capsaicin may have important potential for promoting vascular and metabolic health: Table 1. Open Heart 2015;2(1):000262.