Role of Fenugreek Leaves and It's Extract as Anti-Acidity in Experimental Rats

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Abstract:

The present study aimed to investigate the potential impacts of fenugreek leaves and its aqueous extract as a natural anti-acidity agent on the experimental rats. Twenty five male rats weighing between (160±10 g) were randomly distributed into five groups as follows: 1st: fed on basal diet (negative control), 2nd: fed on basal diet mixed with white bread powder (15%) of diet (positive control), 3rd: fed on basal diet mixed with white bread powder (15%) of diet + 5% of fenugreek leaves powder, 4th: fed on basal diet mixed with white bread powder (15% of diet) + 10% of fenugreek leaves powder, 5th: fed on basal diet mixed with white bread (15% of diet) +1 ml aqueous extract non concentrated of fenugreek leaves (FLE).

Results showed that the application of fenugreek leaves (FL) in rat diets a significant increase (P<0.05) in final weight and body weight gain comparing with the other groups. The positive control recorded the lowest level of bicarbonate (23.56 ±0.13), when the addition of FL to the rat diets, and FLE observed increased of bicarbonate level compared to negative control. It was noted that the acidity of the stomach and blood were affected by FL and FLE through the current results, thus there was a significant difference between the positive control group and the other experimental groups. Group 4 which fed on diet supplemented with 10% of FL recorded the highest mean value of calcium. The application of FL and its extract reduced MDA and increased TAC. It could be concluded that the use of FL as a dietary supplement it’s useful in maintaining stomach and blood pH and preserving a good oxidative status which positively reflects on general health.

Keywords: Fenugreek leaves, stomach, blood, acidity, antioxidative indices

Introduction:

Fenugreek (Trigonella foenum) has a long history as both a culinary and medicinal herb since long time ago. Utilization of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies (Semalty et al., 2015). The Greeks and Romans used it for cattle fodder (Trigonella foenum graecum meaning). In traditional chinese medicine, fenugreek seeds are used as atonic, as well as a treatment for weakness and medicinal herb (Semalty et al., 2015 and Bazzano et al., 2016). In India, fenugreek is commonly consumed as a condiment and used as a lactation stimulant (Kahar, 2004). Fenugreek seed contains 20-30% proteins high in lysine and tryptophan, 45-60% carbohydrates, mainly...
mucilaginous fiber (galactomannans), 5 - 10% fixed oils (lipids), pyridine alkaloids, mainly trigonelline (0.2 - 0.38%), choline (0.5%), gentianine and carpaine, the flavonoids, luteolin, apigenin, vitexin, orientin, quercetin, and isovitexin, free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 - 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin), vitamins A, B1, C and nicotinic acid and 0.015% volatile oils (n-alkanes and sesquiterpenes), cholesterol and sitosterol (Mehrafarin et al., 2010). Which are thought to account for many of its presumed therapeutic effects (effect of fenugreek is thought to be largely due to its high content). The hypoglycemic of soluble fiber which acts to decrease the rate of gastric emptying, thereby delaying absorption of glucose from the small intestine (Yin et al., 2003, and Zargar, 2014).

One of the historical uses of fenugreek leaves is the treatment of gastric acidity and ulcer (Pandian et al., 2002). The aqueous extract and a gel fraction derived from fenugreek showed significant protective effects compared to those on omeprazole. The researchers found that the cytoprotective effect of fenugreek may be due to the anti-secretory action and to the effects on mucosal glycoproteins (Choi et al., 2007).

Previous reports confirmed the use of vegetables, fruits and some plants to maintain gastric acidity. Thus, the present study aimed to investigate the potential impacts of fenugreek leaves and its aqueous extract as a natural anti-acidity agent on the experimental rats.

Materials and Methods:

Materials:
The leaves of fenugreek (Trigonella foenum – graecum L.) were obtained from local markets in Zagazig government, Sharkia, Egypt and grinding to obtain the fine powder. The plant material were authenticated in department of Botany, Faculty of science, Zagazig university, Egypt. A voucher of leaves (voucher number: PRC\2017|2022).

Fenugreek leaves powder was analyzed to its proximate components according the method of AOAC (1990). The white bread was obtained from a local market and roasted at 100°C and grinded to powder.

Animals:
Twenty five male rats weighing between (160±10 g) were randomly distributed into five groups. All the experimental rats were not suffering from any pain or disease of any kind during the experiment. Rats were
obtained from the Agricultural Reached Center, Giza, Egypt. Rats were housed in wire cages under the normal condition. Rats were fed on basal diet and used distilled water as a drink.

**Methods:**

**Experimental design:**

Rats were divided into five groups each group has five rats as follow.

1- Rats were fed on basal diet as a negative control.

2- Rats were fed on basal diet mixed with white bread powder (15% of diet) to induced acidity according to (Robertson *et al.*, 2007). As positive control.

3- Group 3 fed on basal diet mixed with white bread (15% of diet) and 5% fenugreek leaves powder.

4- Group 4 fed on basal diet mixed with white bread (15% of diet) and 10% fenugreek leaves powder.

5- Group 5 fed on basal diet mixed with white bread (15% of diet) and take 1ml oral aqueous extract non concentrated leaves.

**Experimental diet:**

The composition of basal diet was casein 14%, corn oil 10%, and salt mixture 4%, vitamin mixture 1%, cellulose 5%, and starch 66%. The composition of salt and vitamin mixtures were applied according to Campbell, (1961).

**Biological Evaluation:**

The experiment lasted for 8 weeks, at the end of experiment; the rats were slaughtered and drawing samples from hepatic partial vein in clean centrifuge tubes. Serum was separated by centrifugation at 3000r.p.m. for 15 minutes and kept at -18C until analysis.

**Chemicals analysis:**

**Calcium content:** Absorption method (pye Unicom mod AW serum were measured at. Calcium determination using the atomic 422 nm was according to Pearson (1970).

**Phosphorus content:** Phosphorus was determined in serum according to the colorimetric methods as described by Page (1982).

**pH value:** pH value of the blood and stomach were determined by direct immersion of pH electrode in blood and gastric fluid at the room
temperature (25°C) using the digital pH meter model 3020 Dunmou (Jenway, Essex, UK)

**Bicarbonate** ($HCO_3^-$): Bicarbonate was determined in serum according to Tietz (1999).

**MDA -TAC**: Total antioxidant capacity (TAC) and malondialdehyde (MDA) were determined in serum according to Koracevvic et al. (2001) and Sotah (1979).

**Statistical analysis:**

The data obtained were subjected to Analysis of Variance (ANOVA) appropriate for a completely randomized design, using the statistical program SPSS.20® (IBM Cooperation, USA) to assess the significant differences with Duncan’s multiple range test.

**Histopathological examination:**

Specimen from stomach, kidney, liver and bone of rats from all groups were fixed in 10% (v\(\text{v}\)) neutral buffered formalin, dehydrate in ethyl alcohol and cleared in xylo. Tissue section 4-6 u thick was stained with hematoxylin and erosin stain (H and Ex200) according to carlton et al., (1967).

**Results and Discussion:**

**Chemical composition of fenugreek leaves:**

The chemical composition of fenugreek leaves is illustrated in Table (1). Fenugreek leaves (FL) content recorded the highest value for protein (26.13 % ±0.990). The content of fiber was 17.36 % ±1.29. For fat content, it was 1.55 % ±0.07. The FL content of ash and moisture were 9.80 % ±1.29, 12.36% ±0.29, respectively. The results agree with Rekha Sinha, (2018), who found that moisture, protein, fat, ash, fiber and carbohydrate contents of fenugreek seeds and leaves were 86.36, 28.76, 5.60, 9.10, 7.30 and 49.30 %, respectively.

**Table (1): Chemical composition of fenugreek leaves**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fenugreek leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>26.13 ±0.99</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>17.36 ±1.29</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.55 ±0.07</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>9.80 ±1.29</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>12.36 ±0.29</td>
</tr>
</tbody>
</table>
Final weight and body weight gain:

Data presented in Table (2) showed the impacts of treatments on final weight (FW) and body weight gain (BWG). It is noticeable that FW and BWG were significantly (P<0.05) affected by the treatments. The use of 5 and 10 % FL gave the best values of FW (309 and 303.4 g) and BWG (145 and 138.60 g), respectively compared to the other treatment groups and the control group. The improvement of the FW and BWG may be related to the constituents of the fenugreek leaves that are a rich source of vitamins, minerals and antioxidants, which may be protect the body’s cells from damage by free radicals. Also, the high-fiber content of fenugreek leaves promote digestion, improve appetite and to support respiratory health, supports for healthy bowel function and its lecithin content promotes fat metabolism (Roberts, 2011).

In line with our results, Abd El Rahman (2014), who found that the use of dietary fenugreek leaves in rat diets increased (P<0.01) weight gain and showed enhancement in nutritional status as compared to the negative control group.

Table (2): Mean values ± SE of initial weight, final weight and body weight gain of the experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Body weight gain %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (NC)</td>
<td>165.00 ± 2.02</td>
<td>299.00 ± 5.74b</td>
<td>134.00 ± 7.16ab</td>
</tr>
<tr>
<td>Group 2 (PC)</td>
<td>167.00 ± 0.89</td>
<td>291.20 ± 9.37b</td>
<td>124.20 ± 9.79b</td>
</tr>
<tr>
<td>Group 3 (5 % FL)</td>
<td>164.00 ± 1.58</td>
<td>309.00 ± 3.97a</td>
<td>145.00 ± 3.82a</td>
</tr>
<tr>
<td>Group 4 (10 % FL)</td>
<td>164.80 ± 1.50</td>
<td>303.40 ± 3.89a</td>
<td>138.60 ± 2.99a</td>
</tr>
<tr>
<td>Group 5 (1 % FLE)</td>
<td>166.40 ± 1.63</td>
<td>296.80 ± 10.16b</td>
<td>130.40 ± 9.99ab</td>
</tr>
</tbody>
</table>

Means in the same column within each classification bearing different letters are significantly different (P<0.05 or 0.01). NS= not significant, *= significant (P<0.05), **= significant (P<0.01).
Bicarbonate, stomach pH and blood pH:

The mean values of bicarbonate, stomach pH and blood pH of the experimental groups tabulated in Table (3). As for bicarbonates values, the positive control group affected by acidosis, and recorded the lowest mean value of bicarbonate (23.56 ±0.13), when the addition of (FL) to the rat diets, and aqueous extract by oral, observed significantly increase of bicarbonate level like negative control which recorded 26.20±0.3. Metabolic acidosis induced by the reduction in the serum level of bicarbonate (HCO₃) then reduction of blood pH (Eustace et al., 2014).

The bulk of the HOCO₃ is generates in the proximal convoluted tubule (PCT) as a result of NH₃ production and its excretion in urine as NH₄ (Born, 2006). With respect to stomach pH, it is noted that the acidity of the stomach affected by FL and its aqueous extract through the current results, thus there was a significant difference between the positive control group (the lowest value of stomach pH) and the other experimental groups.

On the other side, the negative control group recorded the highest mean value (4.10 ±0.34) of stomach pH. These results clearly indicated that FL able to equate the stomach acidity. This is consistent with the study prepared by Helmy et al., (2011) who explained that gastro-protective effect of fenugreek enriched fraction against indomethacin induced gastric ulcer was by increasing mucine secretion.

From the same table, it can noticed that blood pH of negative control group was 7.11±0.14, significantly higher than the positive control group 6.61±0.11, then with the addition of FL and aqueous extract blood pH value increased morally for the other groups compared to positive control. There is a scientific study by Toffaletti and Rackley (2016), who confirmed that the blood pH and bicarbonate are clearly associated with the acid–base status. Human life requires a tightly controlled blood pH level about 7.4 (a slightly alkaline range of 7.35 to 7.45) to survive (Waugh and Grant, 2007).

The improvement in acidity may be due to the high content of the fenugreek (leaves, seeds and it's aqueous) on flavonoids, and the most important of cumarins, which has alkaline effect. Our results are agree with Selim et al., (2012); Ouf et al., (2014) they found that treatment of acute acidosis by alkali therapy is usually indicated to raise and maintain the plasma PH to greater than 7.20 using methoxy cumarins nucleus which change plasma pH from acid to basic as from obtained results anti-inflammatory activity by hind paw edema methods which exhibited a 87% of edema inhibition.
Table (3): Mean values ± SE of bicarbonate, stomach pH and blood pH for the experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variable</th>
<th>Bicarbonate</th>
<th>Stomach pH</th>
<th>Blood pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (NC)</td>
<td></td>
<td>26.20 ± 0.37a</td>
<td>4.10 ± 0.34a</td>
<td>7.10 ± 0.14a</td>
</tr>
<tr>
<td>Group 2 (PC)</td>
<td></td>
<td>23.56 ± 0.31b</td>
<td>3.50 ± 0.41c</td>
<td>6.61 ± 0.11b</td>
</tr>
<tr>
<td>Group 3 (5 % FL)</td>
<td></td>
<td>26.40 ± 0.51a</td>
<td>3.77 ± 0.31a</td>
<td>7.03 ± 0.07a</td>
</tr>
<tr>
<td>Group 4 (10 % FL)</td>
<td></td>
<td>27.40 ± 0.40a</td>
<td>3.84 ± 0.21a</td>
<td>7.13 ± 0.15a</td>
</tr>
<tr>
<td>Group 5 (1 % FLE)</td>
<td></td>
<td>26.80 ± 0.58a</td>
<td>3.55 ± 0.26c</td>
<td>7.18 ± 0.06a</td>
</tr>
</tbody>
</table>

Significance

- NC: Negative control; PC: Positive control; FL: Fenugreek leaves; FLE: Fenugreek leaves extract.
- Means in the same column within each classification bearing different letters are significantly different (P<0.05 or 0.01). NS= not significant, *= significant (P<0.05), **= significant (P<0.01).

Calcium (Ca) and phosphorous (P):

Table 4 show the values of calcium and phosphorous. The negative control (10.33±0.26) significantly higher than positive control (8.83±0.19). All the experimental groups were insignificantly increased compared to positive control. Group 4 which fed on diet supplemented with 10% of FL recorded the highest mean value of calcium in serum.

On the other hand when adding FL to the rat diets did not affected on the level of phosphorus and therefore no significant differences between all studied groups. Our results are consistent with Joanna et al. (2014), who found that calcium and phosphorus content in the bone mineral were significantly increased in rats which fed on trigonelline (Foenum greacum L.). Another study indicated that diosgenine, a steroidal sapogenine in fenugreek leaves may have a positive effect on bone minerals, especially calcium and phosphorus (Alcontara et al., 2011 and Folwar et al., 2012).
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Table (4): Mean values ± SE of the Ca and P for the experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium (g/dl)</th>
<th>Phosphorous (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (NC)</td>
<td>10.33 ± 0.26b</td>
<td>5.70 ± 0.37</td>
</tr>
<tr>
<td>Group 2 (PC)</td>
<td>8.83 ± 0.19d</td>
<td>6.82 ± 0.23</td>
</tr>
<tr>
<td>Group 3 (5 % FL)</td>
<td>9.58 ± 0.40bcd</td>
<td>6.76 ± 0.21</td>
</tr>
<tr>
<td>Group 4 (10 % FL)</td>
<td>11.22 ± 0.35a</td>
<td>6.35 ± 0.15</td>
</tr>
<tr>
<td>Group 5 (1 % FLE)</td>
<td>9.64 ± 0.13bcd</td>
<td>6.38 ± 0.25</td>
</tr>
</tbody>
</table>

Significance  **NS**

- Means in the same column within each classification bearing different letters are significantly different (P<0.05 or 0.01). NS= not significant, *= significant (P<0.05), **= significant (P<0.01).

Total antioxidant capacity (TAC) and malondialdehyde (MDA):

Results of antioxidative indices (TAC and MDA) are summarized in Table (5). As for total antioxidant capacity (TAC) and malondialdehyde (MDA), there were significant differences among the groups. Mean value of TAC was 1.14±0.05 for negative control, then decreased significantly compared to positive control (0.99±0.01) by enhancing food in the fenugreek leaves and aqueous orally. There is a significant increase in groups 3 (5% leaves) and 4(10%leaves).

Concerning MDA, it can noticed that positive control was 4.36±0.18 which considered the highest mean value of MDA compared to negative control which recorded the lowest value (3.78±0.23). While, there was no significant differences between positive control and the experimental groups except group 5 which takes oral aqueous extract showed a significant decrease compared to positive control.

Malondialdehyde has a very devastating process altering the structure and function of cell membranes Nair and Nair, (2015), Koc (2003), demonstrated that the formation and increase of MDA level can lead to oxidative mechanisms, high cytotoxicity and inhibitory actions. MDA acts as a tumor promoter and co-carcinogenic agent. On the other side, the increase of total antioxidant capacity (TAC) is evidence of improved antioxidative status according to Chaturvedi et al. (2013) and Joshi et al. (2015). Authors also found that fenugreek can reduce MDA levels and increase antioxidant enzymes.
Table (5): Mean values ± SE of the total antioxidant capacity (TAC) and malondialdehyde (MDA) for the experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Antioxidative indices</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAC (nmol/g)</td>
<td>MDA (nmol/g)</td>
</tr>
<tr>
<td>Group 1 (NC)</td>
<td>1.14 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.78 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 2 (PC)</td>
<td>0.97 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.36 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 3 (5 % FL)</td>
<td>1.24 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.32 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 4 (10 % FL)</td>
<td>1.05 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.54 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 5 (1 % FLE)</td>
<td>0.96 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.88 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Means in the same column within each classification bearing different letters are significantly different (P<0.05 or 0.01). NS= not significant, *= significant (P<0.05), **= significant (P<0.01).

**Histological examination of the stomach:**

Microscopically, stomach of rats from group 1 (negative control) revealed the normal histological structure of gastric layers (mucosa, submucosa and musculosa) Photos 1 & 2. While, stomach of rats from group 2 (positive control) revealed focal necrosis of gastric mucosa and submucosal oedema with inflammatory cells infiltration Photos 3 & 4.

However, when adding FL and aqueous to groups 3 & 4 revealed no histopathological changes Photo 5, whereas, other sections from these groups showed sub mucosal inflammatory cells infiltration. Stomach of rats from group 5 revealed no histopathological changes. The obtained results showed that the positive control group recorded mucosal necrosis; however, all fenugreek leaves treated groups did not record any mucosal necrosis. It is speculated that the polysaccharide composition of the gel and/or the flavonoids are responsible for the gastro-protective and anti-secretary activities of fenugreek (Madar and Shomer, 1990).
Photo (1): Stomach of rat from group 1 showing the normal histological structure of gastric layers (mucosa, submucosa and muscolosa) (H & E X 100).

Photo (2): Stomach of rat from group 2 showing focal necrosis of gastric mucosa and submucosal edema associated with inflammatory cells infiltration (H & E X 100).

Photo (3): Stomach of rat from group 3 showing no histopathological changes (H & E X 100).
Photo (4): Stomach of rat from group 4 showing no histopathological changes (H & E X 100).

Photo (5): Stomach of rat from group 5 showing no histopathological changes (H & E X 100).
References


European calcified tissues society, 39 Annual congress, Stockholm, 5158 -5159.


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