Anti-inflammatory activity in vitro and in vivo of ethanolic extracts of Stevia rebaudiana Bertoni and Trigonella foenum-graecum

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ABSTRACT

This study was based on evaluating anti-inflammatory activity of ethanolic extracts from Stevia leaves (EESL) and Fenugreek seeds (EEFS) (in vitro and in vivo). In the in vitro experiment, the results exhibited potent anti-inflammatory activity. The combination of both extracts (1:1, 100 μg) showed to be the most potent inhibitor of COX-2 with inhibition % (83.64%). This result was comparable with the standard Celecoxib showed higher level of inhibition, (86.63%, 100 μg). While, EESL showed the highest activity as COX-1 inhibitory activity (74.35%, 100 μg), which it is higher than standard Celecoxib (67.64%, 100 μg). In vivo anti-inflammatory activity was performed on Wistar rats at the doses 200 and 400 mg/kg body weight using the carrageenan-induced rat paw edema modal. Combination of (SLEE + FSEE, each 100 μg) displayed the highest significant anti-inflammatory activity (0.46± 0.025, p<0.05) after 4 h, compared with indomethacin (0.44± 0.025, p<0.05). The results indicated that these plants may have a role for discovering new anti-inflammatory natural drugs. Furthermore, Stevia leaves and Fenugreek seeds can be subjected to further investigations as antioxidants.

KEYWORDS: Anti-inflammatory, COX-1, COX-2 isozyme, fenugreek- Stevia.

1. INTRODUCTION

Inflammation is known, as the Localized response of living mammalian tissue to injury due to a factor and exhibit in the shape of painful tumefy associated with some skin changes (Arya et al., 2012). On the other side, the available anti-inflammatory medications present a loose range of aspect effects for which the primary reason is nonselective repression of COX-1 and (COX-II) (Vane and Botting, 1995). The inflammation response happens in two stages meaning, the emission or liberation of histamine, serotonin, and bradykinin in the first stage, and followed by prostaglandin liberation in the second stage (Deraedt et al., 1976). The carrageenan-induced paw edema shape is widely used for determining the critical stage of the inflammatory. Histamine, 5-hydroxytryptamine, and bradykinin are the first detectable mediators in the Initial stage of carrageenan-induced inflammation, whilst prostaglandins are tangible in the final stage inflammation (Jain et al., 2010).

The medical herbs are in persistent need in the developed world for initial health care for their efficacy, safety, and few side effects. These plants are rich as secondary metabolites and involved triterpenes, sterols, and alkaloids others. Many of these phyto-components showed different biological activities viz., antidiabetic, wound healing activities (Assaf et al., 2019), antimicrobial (Mahmoud et al., 2019), anti-inflammatory and anti-hyperglycemia (Mohammed et al., 2016), hepatoprotective (Wahid et al., 2016), etc.

Stevia rebaudiana Bertoni, (Family: Asteraceae), Known for its abundance as the sweet leaves, is an herbaceous perennial shrub. source is in South America, it is widely cultivated and used mostly as a sweetener in many parts of the world, including Central America, Paraguay, Thailand, China, and Bangladesh (Gupta et al., 2013). Besides its sweetening property, Stevia rebaudiana is also known for its medicinal properties (Debnath, 2008).

Fenugreek (Trigonella foenum-graecum L.), are family Fabaceae has a long and esteemed history history of medicinal uses in Middle East and Persian medicine. The hypocholesterolaemic and hypoglycaemic effect of fenugreek were attributed to its major steroidal sapogenin, diosgenin and its major alkaloid (trigonelline). (Mehrafari et al., 2010), anti-inflammatory activities of methanolic extract of callus and intact plant part of stevia rebaudiana Bertoni were studied for the first time (Arya et al., 2012).

Stevia leaves and fenugreek seeds contains fiber, alkaloids, flavonoids, phenolic compounds, saponins and steroids (Mahmood and Yahya, 2017; Howladar et al., 2016).

The current work Seeks to study the anti-inflammatory activities of ethanolic extracts of both
Stevia leaves (SLEE) and Fenugreek seeds (FSEE) in vitro and in vivo.

2. MATERIAL AND METHODS

2.1. Plant material:

2.1.1. Stevia plant:

The leaves of stevia plants (Stevia rebaudiana Bertoni), were obtained from Sugar Crops Research Institute, Agricultural Research Centre, Giza, Egypt. The leaves were removed from the plants, washed with water, and dried at room temperature 25-30 °C.

2.1.2. Fenugreek plant:

Seeds of Trigonella foenum graecum were provided kindly rom Prof. Dr. Gamal Abdel Aziz professor in Research Institute, Agricultural Research Centre, Giza, Egypt.

2.2. Preparation of ethanolic extracts:

Finely ground the Stevia leaves and Fenugreek seeds. Each sample (100 g) was soaked separately in 1 liter of ethyl alcohol at a ratio of (1:10) in a closed flask for 24 h. Then, they were quickly filtered with precautions against the loss of solvent. The ethanolic extracts were concentrated for drying in a rotary evaporator under low pressure and controlled temperature (40-50 °C) to yield Stevia leaves (39.92 g, SLEE) and Fenugreek (16.53 g, FSEE). The ethanolic extracts were stored in the refrigerator at 4 °C until further use.

2.3. Anti-Inflammatory activity:

2.3.1. In vitro cyclooxygenase inhibition action:

The colorimetric COX (ovine) method by Ghorab et al., 2017.

2.3.2. In vivo carrageenan induced paw edema

Fifty-four Male adult albino rats (150-200 g) were obtained from the animal house of Faculty of Medicine, Assiut University, Egypt and used for anti-inflammatory studies. They were housed at the temperature 24±2°C in groups of six animals each group. The fasted of animals were over night before the experiment (Kulkarni, 2007). The anti-inflammatory activity method by (Perez et al. 1990).

Experimental design:

Rats were divided into 9 groups (6 rats each) as following:

**Group 1**: Normal (distilled water).

**Group 2**: Carrageenan (injection of Carrageenan).

**Group 3**: Carrageenan + (Standard drug Indomethacin 7 mg /kg b.wt) Yazdinezhad and Andalib, (2017) revealed that the indomethacin decrease inflammation. The rats were treated with indomethacin at a dose of 10 mg/kg (Mondal et al., 2019).

**Group 4**: Carrageenan + (SLEE 200 mg/kg b.wt).

**Group 5**: Carrageenan + (SLEE 400 mg/kg b.wt).

**Group 6**: Carrageenan + (FSEE 200 mg/kg b.wt).

**Group 7**: Carrageenan + (FSEE 400 mg/kg b.wt).

**Group 8**: Carrageenan + (Combination of (SLEE + FSEE, each 100 mg/ kg b.wt).

**Group 9**: Carrageenan + (Combination (SLEE + FSEE, each 200 mg/ kg b.wt).

Indomethacin and ethanolic extracts were injected with a single dose at 0, 1, 2, 3 and 4 h after the carrageenan injection.

The percentages of edema inhibition were calculated according to the following equation (Arya et al., 2012).

**Percentage of Inhibition**

\[
\frac{\left(V_T - V_O\right)\text{Control} - \left(V_T - V_O\right)\text{Treated group}}{\left(V_T - V_O\right)\text{Control}} \times 100
\]

\(V_O = \) paw volume of the rat before administration of Carrageenan.

\(V_T = \) paw volume of the rat after the administration of Carrageenan at different time intervals. Percentage inhibition of paw edema was found to be proportional to anti-inflammatory activity.

2.4. Statistical analysis

The outcomes were considered in mean ± SD. One-way variance analysis (ANOVA) followed by Dunnett’s test, using SPSS 13.0. The findings were considered as statistically significant at the level of p<0.05.

3. RESULTS AND DISCUSSION

3.1. In vitro anti-inflammatory activity:

Among all sample as cyclooxygenase-II (COX-2) inhibitory percentage activity at 100g, the combination of (SLEE 50% + ESEE 50%) and SLEE exhibited the highest activities with (83.64 and 82.15%, respectively). These results are comparable with the standard (celecoxib) (86.63%). Also, they displayed higher activities as COX-1 inhibitory percentage activity at 100 µg as 72.92% and 74.35%, respectively, while celecoxib) (67.64%). These results are demonstrated in Table 1. Prostaglandins are basic middleman of the body’s response to soreness, and inflammatory Consists from essential fatty acids found in cell membranes. This reaction is Stimulate by cyclooxygenase, a membrane-associated enzyme occurring in two isofoms, COX-1 and COX-2. Non-steroidal of anti-inflammatory Medicines (NSAIDs) Operate by inhibiting the activity of COX (Taylor et al., 2002).
Table 1. *in vitro* COX-1 and COX-2 inhibitory activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract of <em>Stevia rebaudiana</em> (SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>74.35</td>
<td>82.15</td>
</tr>
<tr>
<td>10</td>
<td>59.05</td>
<td>59.92</td>
</tr>
<tr>
<td>1</td>
<td>41.80</td>
<td>43.44</td>
</tr>
<tr>
<td>0.1</td>
<td>32.36</td>
<td>38.54</td>
</tr>
<tr>
<td>100</td>
<td>66.99</td>
<td>78.74</td>
</tr>
<tr>
<td>0.1</td>
<td>54.17</td>
<td>56.37</td>
</tr>
<tr>
<td>Ethanolic extract of fenugreek (FE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>42.51</td>
<td>46.71</td>
</tr>
<tr>
<td>0.1</td>
<td>35.16</td>
<td>35.91</td>
</tr>
<tr>
<td>Combination composed from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE 50% + FE 50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>72.92</td>
<td>83.64</td>
</tr>
<tr>
<td>1</td>
<td>58.72</td>
<td>61.88</td>
</tr>
<tr>
<td>0.1</td>
<td>41.08</td>
<td>46.81</td>
</tr>
<tr>
<td>Celecoxib (standard)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>49.80</td>
<td>58.71</td>
</tr>
<tr>
<td>0.1</td>
<td>42.51</td>
<td>49.69</td>
</tr>
</tbody>
</table>

Safayhi and Sailer, (1997) reported that diterpenes, triterpenes and pentacyclic triterpenes in inflammatory plants have been act as anti-inflammatory factors. *S. rebaudiana* as food can treat as diseases of the immune system, such as rheumatoid arthritis (Jeong *et al.*, 2010). The anti-inflammatory properties of fenugreek may be due to existence of flavonoids and saponins. Flavonoids used as antioxidant and inhibitors of cyclooxygenase (COX), and lipoxygenase (Handa *et al.*, 2005; Sharififar *et al.*, 2009).

Cyclooxygenase (COX) is the locate enzyme in prostaglandin synthesis, there are two types, COX-1 and COX-2 enzyme, compared to standard (Celecoxib). Data in Table (2) revealed that the most effective treatment is a combination (50% SLEE + 50% FSEE, 100 μg), which cause 50% inhibition of the COX-1 and COX-2 enzyme, compared to standard (Celecoxib).

### in vitro cyclooxygenase-II selectivity index (COX-1/COX-2):

Table 2. IC_{50} and S.I of plant extracts against Cox-1 / Cox-2

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC_{50} μg/ml</th>
<th>S.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract of fenugreek (FE)</td>
<td>33.68</td>
<td>2.24</td>
</tr>
<tr>
<td>Ethanolic extract of Stevia rebaudia(SE)</td>
<td>23.34</td>
<td>1.89</td>
</tr>
<tr>
<td>Combination 50%FE+50%SE</td>
<td>16.81</td>
<td>2.20</td>
</tr>
<tr>
<td>Celecoxib (Stander)</td>
<td>11.77</td>
<td>8.93</td>
</tr>
</tbody>
</table>

3.2. activity of plant extract as Anti-inflammatory *in vivo*:

Carrageenan is widely act as an actual example for inflammation-causing paw edema in rats when investigating a new medicine efficacy as an anti-inflammatory. The anti-inflammatory activity of SLEE and FSEE opposite carrageenan-induced hind paw edema has been shown in tables 3 and 4. In the groups studied, edema volume (cm) was evaluated at different intervals (0, 1, 2, 3 and 4 hours). The results in table 3 showed that the injection of carrageenan in rats caused a significant increase (p<0.05) in edema weight compared to the control group and this was in agreement with the findings of the previous reports (Haddadi and Rashtiani, 2020).

The injection of SLEE and FSEE decreased significantly paw volume (p<0.05), reaching a maximum reduction at the end of the experiment after 4 h, when compared to the carrageenan group (Table 3). On the other hand, the edema volume in the combination of (SLEE + FSEE) group (200
Table 3. *In vivo* - Anti-inflammatory activities of plant extracts and reference drug indomethacin:

<table>
<thead>
<tr>
<th>Anti inflammatory</th>
<th>Mean value of paw edema thickness (cm)</th>
<th>Combination</th>
<th>ST 50% + FE 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>control</td>
<td>Indo (7mg)</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.30b ± 0.0</td>
<td>0.72a ± 0.029</td>
<td>0.71a ± 0.025</td>
</tr>
<tr>
<td>1 hr</td>
<td>0.30d ± 0.0</td>
<td>0.77a ± 0.029</td>
<td>0.59c ± 0.047</td>
</tr>
<tr>
<td>2 hr</td>
<td>0.30f ± 0.0</td>
<td>0.80a ± 0.050</td>
<td>0.53c ± 0.029</td>
</tr>
<tr>
<td>3 hr</td>
<td>0.30c ± 0.0</td>
<td>0.80a ± 0.050</td>
<td>0.49d ± 0.047</td>
</tr>
<tr>
<td>4 hr</td>
<td>0.30e ± 0.0</td>
<td>0.83a ± 0.029</td>
<td>0.44f ± 0.025</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.D. Means of various litters within the same row are considerably different at (P<0.05).
mg/kg b.wt) was significantly lower than the control and indomethacin groups.

Data in Table 4 indicated that (SLEE + FSEE) at a dose (200 mg/kg b.wt) showed 56.60 and 64.14%, respectively inhibition carrageenan-induced rat paw edema. The inhibition at a dose (200 mg/kg b.wt) showed anti-inflammatory activity compared to a dose (400 mg/kg of b.wt) that appeared 50.94 and 54.72%, respectively inhibition of carrageenan-induced rat paw edema.

The results also showed that the combination of (SLEE + FSEE) with a concentration (200 mg/kg b.wt) showed 69.81% inhibition of carrageenan-induced rat paw edema. This result indicated that the mixture SLEE and FSEE at this concentration (200 mg/kg b.wt) showed a maximum anti-inflammatory activity compared to the mixture of (SLEE and FSEE) with a concentration (400 mg/kg b.wt), which showed only 60.38% inhibition of carrageenan-induced rat paw edema.

Inflammatory is the response of living tissue to deteriorate, which involves activating various enzymes, moderators freeing, cell emigration, tissue separated, and repair (Katzung, 2004). The first stage of edema is attributed to the emancipation of histamine and 5-hydroxytryptamine. (Balasubramanian et al., 2005).

These results were in agreement with Arya et al., (2012) that SLEE at the test doses 100, 200 and 400 mg/kg body weight reduced the edema induced by carrageenan by 28, 37 and 42%, respectively at 4 h. The Fenugreek seeds' excerpt could also inhibit paw-edema, indicating their prominent mechanism of inhibitory effects on prostaglandins and bradykinin, which are responsible for the second stage of edema (Yoshimoto et al., 1983). Rehman and Ghauri, (2018) reported that the results of the study fenugreek (Trigonella foenum-graecum L.) are decrease (85%) inflammatory of the paw in formaldehyde and carrageenan-induced paw edema. This inhibitory activity may be due to the natural phenolic and flavonoid compounds, which play antioxidants activities by different mechanisms. The high contents of these phytochemicals in both extracts can exhibit anti-inflammatory activity (Bairagi et al., 2012). Flavonoids frustrate Cox-I enzymes contributory in the initiation stage of inflammation reactions (Dames et al., 1985).

4. CONCLUSION

Recent studies have reported that Trigonella foenum-graecum L. (Fenugreek) seed extracts had potential anticancer properties (Nagulapalli et al., 2017). Our findings indicated that the SLEE and FSEE can be used to as anti-inflammatory agent to reduce inflammation alleviate pain. The results demonstrated that these plants may play an important role for discovering new anti-inflammatory natural drugs. Also, these medicinal plants can be subjected to further investigations as anticancer natural products.

5. REFERENCES


Wahid A, Hamed AN, Eltahir HM, Abouzied MM (2016). Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl4-induced chronic
hepatotoxicity in rats. BMC Complementary and Alternative Medicine, 16: 263.


الملخص العربي

قياس النشاط المضاد للالتهابات معملياً و داخل الأنسجة الحية للمستخلصات الإيثانولي لـ استفيا و الحلبة

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استندت هذه الدراسة إلى تقييم الأنشطة المضادة للالتهابات للمستخلصات الإيثانولية من أوراق ستيفيا (EESL) وبذور الحلبة (EEFS) تحت ظروف المعمل وفي أنسجة فئران التجارب الحية. في التجربة المخبرية أظهرت النتائج أن جميع المعاملات كانت انقائيا لإنتيزم COX-2 وأظهرت نشاطًا قويًا مضادًا للأنتييمات. كانت التوليفة المكونة من كلا المستخلصين (1:1، 100 ميكروجرام) هي الأكثر فعالية EESL (83.64% COX-2)، بينما أظهر ttتلتثيث 2 (64% COX-2) أقل نشاطاً. كان النتائج مشابهة لعدة معايير مع كال عاملين (القياسي) ، وأعلى من كل من CELECOXIB (6.74%)، 100 ميكروجرام. تم إجراء الدراسات في فئران بجرعات 200 و 400 مجم / كجم من وزن الجسم باستخدام الورمية التي تسببها الكاراجينان.

أظهرت النتائج أن هذه النباتات قد يكون لها دور في اكتشاف عقاقير طبيعية جديدة مضادة للالتهابات. علاوة على ذلك، يمكن أن تخفض أوراق الاستفيا وبذور الحلبة لمزيد من الدراسات كعوامل مضادة للسرطان.