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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 mg) displayed the highest significant anti-inflammatory activity ($0.46^{ef}\pm$ 0.025, $p<0.05$) after 4 h, compared with indomethacin (0.44^{f} ± 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 anticancer agents.

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- I 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). (Mehrafarin *et al.*, 2010). anti-inflammatory activiteis 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); Howlader *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℃ 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

$$= \frac{(VT-VO)Control - (VT-VO)\text{Treated group}}{(VT-VO)Control} \times 100$$

$\mathbf{V_O}$= paw volume of the rat before administration of Carrageenan.
$\mathbf{V_T}$= 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 cyclooxgenase-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 isoforms, 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

Sample	Concentration (µg)	Inhibition (%)	
		COX-1	COX-2
Ethanolic extract of *Stevia rebaudiana* (SE)	100	74.35	82.15
	10	59.05	59.92
	1.0	41.80	43.44
	0.10	32.36	38.54
Ethanolic extract of fenugreek (FE)	100	66.99	78.74
	10	54.17	56.37
	1	42.51	46.71
	0.1	35.16	35.91
Combination composed from SE 50% + FE 50%	100	72.92	83.64
	10	58.72	61.88
	1	41.08	48.41
	0.1	40.04	40.96
Celecoxib (standard)	100	67.64	86.63
	10	53.65	75.26
	1	49.80	58.71
	0.1	42.51	49.69

Safayhi and Sailer, (1997) reported that diterpenes, triterpenes and pentacyclic triterpenes in inflammatory plants have been act as anti -atory factors. *S. rebaudiana* as food can treat as diseases immunet system, such as rheumatoid arthritis (Jeong *et al.*, 2010). The anti-inflammatory propertes 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).

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

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).

Cyclooxygenase (COX) is the locate enzyme in prostaglandin synthesis, there are two types, COX-1 is an enzyme found in widely tissues, while COX-2 whose synthesis can be up-regulated by cytokines, and growth agents. (Fosslien, 2000).

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

Sample	Cox-1	Cox-2	S.I.
	Ic_{50} g/ml	Ic_{50} g/ml	
Ethanolic extract of fenugreek (FE)	33.68	15.05	2.24
Ethanolic extract of Stevia rebaudia(SE)	23.34	12.35	1.89
Combination 50%FE+50%SE	16.81	7.66	2.20
Celecoxib (Stander)	11.77	1.32	8.93

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:

Anti inflammatory	Mean value of paw edema thickness (cm)								
	Normal	**control**	**Indo (7mg)**	**ST**		**FE**		**Combination ST 50% + FE 50%**	
				200 mg	**400 mg**	**200 mg**	**400 mg**	**(200 mg) 100mg+100 mg**	**(400 mg) 200mg+200 mg**
Zero time	0.30^{b} ±0.0	0.72^{a} ± 0.029	0.71^{a} ±0.025	0.71^{a} ±0.025	0.73^{a} ±0.029	0.71^{a} ±0.047	0.71^{a} ±0.047	0.71^{a} ±0.025	0.73^{a} ±0.029
1 hr	0.30^{d} ±0.0	0.77^{a} ± 0.029	0.59^{c} ±0.047	0.66^{b} ±0.025	0.68^{b} ±0.029	0.65^{b} ±0.040	0.64^{b} ±0.025	0.65^{b} ±0.040	0.66^{b} ±0.025
2hr	0.30^{f} ±0.0	0.80^{a} ± 0.050	0.53^{e} ±0.029	0.56^{ed} ±0.025	0.65^{b} ±0.0	0.64^{b} ±0.025	0.59^{cd} ±0.025	0.63^{cb} ±0.029	0.64^{b} ±0.025
3 hr	0.30^{e} ±0.0	0.80^{a} ± 0.050	0.49^{d} ±0.047	0.54^{c} ±0.025	0.58^{cb} ±0.029	0.56^{cb} ±0.025	0.59^{b} ±0.025	0.54^{c} ±0.025	0.55^{cb} ±0.0
4 hr	**0.30^{g}** ±0.0	**0.83^{a}** ± 0.029	**0.44^{f}** ±0.025	**0.49^{ed}** ±0.025	**0.54^{cb}** ±0.025	**0.53^{c}** ±0.029	**0.56^{b}** ±0.025	**0.46^{ef}** ±0.025	**0.51^{cd}** ±0.025

Data represent the mean ± S.D. Means of various litters within the same row are considerably different at ($P<0.05$).

Table 4. Percentage of inhibition:

Group		Initial paw volume	4 hr. (Cm)	Inhibition percentage
Control		0.30 ±0.0	0.83^{a} ±0.029	-----
Indomethacin (7 mg)		0.30±0.0	0.44^{f} ±0.025	73.58
ST	200	0.30±0.0	0.49^{ed} ±0.025	64.14
	400	0.30±0.0	0.54^{cb} ±0.025	54.72
FE	200	0.30±0.0	0.53^{c} ±0.029	56.60
	400	0.30±0.0	0.56^{b} ±0.025	50.94
Combination ST 50% + FE 50% 200mg		0.30±0.0	0.46^{ef} ±0.025	69.81
Combination ST 50% + FE 50% 400mg		0.30±0.0	0.51^{cd} ±0.025	60.38

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.

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الملخص العربى

قياس النشاط المضاد للالتهابات معملياً و داخل الانسجة الحية للمستخلصات الإيثانولي للاستفيا و الحلبة

مدحت إبراهيم احمد الطيب[1], جمال فخري عبد النعيم[2]، ياسر عبدالصبور محمد خليفه [1]، هريدي حسن هريدي[3]

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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 انسجة فئران التجارب الحية. في التجربة المخبرية أظهرت النتائج أن جميع المعاملات كانت انتقائية لإنزيم COX-2 وأظهرت نشاطًا قويًا مضادًا للالتهابات. كانت التوليفة المكونة من كلا المستخلصين (1: 1 ، 100 ميكروجرام) هي الأكثر فعالية لتثبيط COX-2 (83.64٪). كانت هذه النتيجة قابلة للمقارنة مع المعيار (CELECOXIB، 86.63٪ ، 100 ميكروجرام). بينما أظهر EESL أعلى نشاط مثبط لـ COX-1 (74.35٪ ، 100 ميكروجرام)، وهو أعلى من CELECOXIB القياسي (67.64٪ ، 100 ميكروجرام). تم إجراء النشاط المضاد للالتهابات في فئران ويستار بجرعات 200 و 400 ملجم / كجم من وزن الجسم باستخدام الوذمة التي يسببها الكاراجينان. أظهر مزيج (SLEE + FSEE ، كل 100 مجم) أعلى نشاط مضاد للالتهابات (EF 0.46، P <0.05) بعد 4 ساعات ، مقارنة مع الإندوميثاسين (F 0.44، P <0.05). أشارت النتائج إلى أن هذه النباتات قد يكون لها دور في اكتشاف عقاقير طبيعية جديدة مضادة للالتهابات. علاوة على ذلك، يمكن أن تخضع أوراق الاستيفيا وبذور الحلبة لمزيد من الدراسات كعوامل مضادة للسرطان.