doi.org/10.36721/PJPS.2021.34.4.REG.1445-1448.1

Anti-inflammatory and anti-nociceptive activities of polyphenols from *Feijoa* fruit and leaves

Mitra Mahmoudi[1], Sadaf Seifi[1], Barkat Ali Khan[2], Sultan M Alshahrani[3], Amirali Arimi[1], Alireza Allami[1,] Saad S. Alqahtani[4] and Mohammad Ali Ebrahimzadeh[1*]

[1]Pharmaceutical Sciences Research Center, Hemoglobinopathy Institute and Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[2]Faculty of Pharmacy, Gomal University, D.I Khan, Pakistan
[3]College of Pharmacy, King Khalid University, Abha, Saudi Arabia
Pharmacy Practice Research Unit, Department of Clinical Pharmacy, College of Pharmacy, Jazan University, Jazan, Saudi Arabia

Abstract: Many pharmacological activities have been reported from plants polyphenols. The aim of this study was to investigate anti inflammatory and antinociceptive activities of polyphenols from *Feijoa sellowiana* fruit and leaves. For the anti-inflammatory activity evaluation, inhibition of carrageenan induced edema was used. While for the evaluation of antinociceptive activity of the extract, writhing and hot plate tests in mice were used. Impairment in mouse coordination was evaluated by rota-rode test. Carrageenan induced edema was significantly inhibited by the extract at 50-400 mg kg^{-1} doses, when comparison was made with control group. The extract of leaf at the dose of 50 mg kg^{-1} i.p. the activity was equipotent with diclofenac (p>0.05). Extract reduced the writhing count in 50-400 mg kg^{-1} of doses. Fruit extract showed higher activity than diclofenac (p<0.001) at 400 mg kg^{-1} doses. In all tested doses, the extract significantly augmented the pain threshold in hot plate thermal test. No locomotor impairment in mice was induced by the extract at any tested doses. Extract was safe and didnot demonstrate any noxiousness up to 1 g kg^{-1}.This study indicates the potential therapeutic use of Feijoa as a potent anti-inflammatory and antinociceptive agent.

Keywords: Carrageenan, writhing, hot plate, locomotor impairment.

INTRODUCTION

Majority of the diseases are accompanied by inflammation. Inflammation is a part of the complex process, which is commonly associated with pain and increases vascular permeability, increases the denaturation of protein and alteration of cell membrane. When cells in the body are damaged by any chemical, physical or microbial object, the injury occurs in the form of stress. Inflammation of tissue is due to the response to stress. It is a defensive response that is characterized by redness, pain, heat and swelling and loss of function in the injured area (Tortora and Reynolds, 2009). When tissue cells become injured they release kinins, prostroglandins and histamine. These chemicals cause increased vasodilatation and permeability of the capillaries which leads to increased blood flow to the injured site. Current analgesia and anti inflammatory agents including drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial. This opens doors for new and better compounds (Mahmoudi *et al.,* 2016).

Oxidative stresses are believed to play an important role in the induction of cell adhesion molecules and pro-inflammatory cytokines involved in inflammatory processes. Antioxidant serves to protect against tissue injury (Lee *et al.,* 2013). Polyphenols are naturally occurring chemicals derived from plants. They are phytochemicals known for their biological antioxidative, neuroprotective and cognitive properties. It has been shown that different polyphenols can enhance learning and memory and reduce the risk of developing age related neurodegenerative diseases, possibly via a decrease in reactive oxygen species (ROS) production and inflammation in models of aging (Queen and Tollefsbol, 2010; Choi *et al.,* 2012). Polyphenols are highly attractive to researchers as a strategy for a cost-effective alternative to current pharmacologic therapeutics (Rabiei *et al.,* 2012).

Feijoa *(Feijoa sellowiana)* is belonging to family Myrtaceae being traditionally known as pineapple guava. Feijoa is considered native to the South America, and is extensively cultivated in Iran. Chemical compositions of this plant have clearly reported but its pharmacological studies have rarely carried out (Ebrahimzadeh *et al.,* 2008). It contains polyphenols, flavonoids and vitamin C (Vuotto *et al.,* 2000). A potent antimicrobial against *H. pylori* and anti-fungal activity was shown by Feijoa extract (Vuotto *et al.,* 2000). We have recently reported its good antioxidant and anti-toxoplasma effect (Ebrahimzadeh *et al.,* 2008), nephroprotective and antidepressant activities (Karami *et al.,* 2014; Mahmoudi *et al.,* 2015; Ebrahimzadeh *et al.,* 2017). Antibacterial, antifungal activities and strong inhibition of diabetes key

**Corresponding author:* e-mail: zadeh20@gmail.com

enzymes (α-amylase and α-glucosidase) were also reported from Feijoa, recently (Mosbah *et al.,* 2018). In addition, Feijoa has antitumor, gastroprotective and hepatoprotective activities (Weston, 2010; Monforte *et al.,* 2014).

Due to high antioxidant activities, this plant was selected for evaluating of anti-inflammatory and anti-nociceptive properties. To the best of our knowledge, no anti-inflammatory and antinociceptive activities of *F. sellowiana* fruit have been explored and reported until now.

MATERIALS AND METHODS

Plants materials and polyphenolic fraction preparation

The collection of fruit and leaves of Feijoa was done in autumn 2015 from Fajr citrus experimental institute, which were identified by Dr. B. Eslami. In Faculty of Pharmacy herbarium, a voucher specimen (No. 194, 195) had been deposited. Parts were dried and crushed coarsely. The extraction was performed two times at room temperature while using shaking incubator for 30 minutes. 100 g of each part were used. Solvents system used was methanol, acetone, and water (3.5/ 3.5/ 3) with 1% formic acid. For the removal of organic phase, the extracts were collected and evaporated under vacuum at 35-38°C. The extraction of aqueous phase was done for three times by using ethyl acetate. Over a rotary vacuum, organic solvent was evaporated. For complete dryness, the crude extract was freeze dried. The yields were 0.6 and 0.9 for fruit and leaf, respectively on dry weight basis (Rabiei*et al.,* 2012).

Animals

Male Swiss mice weighing 21±2g or Wistar rats weighing 180-200 g from Pasteur Institute were used to perform present experiments. Animals were housed at an ambient temperature and at relative humidity of 45-55%, with a 12 hrs. dark and 12 hrs. light cycle. The experimental animals were freely accessed to standard pellet, water and *libitum*. The experiments were conducted according to the norms NIH principles for Lab animals and Committee for the Purpose of Control and Supervision of Experiments in Animal. All animal experiments were achieved in accordance with the acts of the Ethical Committee of Mazandaran University of Medical Sciences (1395-83). Each animal was used once only. Seven mice were used in each experiment.

Writhing test/Antinociceptive assay

Seventy mice were divided into ten groups each containing seven mice. The induction of abdominal constrictions was obtained by using 0.3% acetic acid i.p. injection (Alemy *et al.,* 2012). Animals were pretreated with vehicle, extract (50-400 mg kg^{-1}, i.p.) 30 minutes before injection of acid. Diclofenac (50 mg kg^{-1} i.p.) was the reference drug. After challenges, the number of abdominal constrictions and stretches were cumulatively counted 8 minutes after acid injection in each mouse over a period of twenty minutes. Diclofenac sodium (50mg kg^{-1}, i.p.) was used as a positive control.

Hot plate test

Seventy seven mice were divided into eleven groups each containing seven mice. The extract was administered at single dose of 50-400mg kg^{-1}, i.p. to the animals. Mice were positioned on a hot plate apparatus (Harward, UK) which was controlled thermostatically. This apparatus was maintained at temperature of 52 ± 0.5 °C and the reaction time for kicking or licking of the hind or fore paws through was recorded. Those mice were excluded and discarded which didn't show any reaction after 15 seconds. Before and after 15, 30, 45 and 60 minutes of administration of extract, reaction time was recorded. To avoid tissue damage, 45 seconds of a cut-off time was imposed (Pourmorad *et al.,* 2007; Ahmadi *et al.,* 2011). Diclofenac sodium (50 mg kg^{-1}, i.p.) and morphine (5 mg kg^{-1}, i.p.) were used as positive controls.

Motor coordination by rota rod test

Rota rod apparatus (Harward, UK) was used to assess and measure the effect on motor coordination (Pourmorad *et al.,* 2007). Only those animals with capability to remain twice on the revolving rod for at least 45s were selected. A single dose (400 mg kg^{-1}) was used. The number of falls from the rod was counted for 45 seconds. The animals were observed before and 15, 30, 45 and 60 minutes after administration of extract. Seven mice were used in this experiment.

Anti-inflammatory activity

Anti-inflammatory activity in acute animal model was carried out as per the convenient reported method. Seventy rats were divided into ten groups each containing seven rats. Carageenan (50µL of 1% suspension, Sigma Chemicals Co. USA) was injected to each rat into the sub planar tissue of the right hind paw. Extract (50-400 mg kg^{-1}) or Diclofenac (50mg kg^{1}) was given (*i.p.)* and 1 hour before carageenan injection to the rats. Before and 3 hours after carageeenan injection, volume of edema was recorded. Degree of swelling was the ratio of the volume of hind paw before to after carageenan treatment (Ahmadi *et al.,* 2010). Diclofenac sodium (50mg kg^{-1}, i.p.) was used as a positive control.

STATISTICAL ANALYSIS

Results obtained are presented as means ± SD. One-way ANOVA (Analysis of variance) was used for writhing test. Similarly repeated-measures of one way analysis of variance (ANOVA) was used for rota-rod tests and hot plate tests, followed by Newman-Keuls multiple comparisons tests. The differences with $p<0.05$ were considered significant.

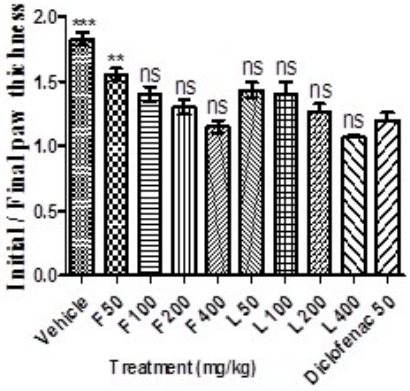
Table 1: Antinociceptive activity of polyphenol fraction of Feijoa fruit (F) and leaf (L) in mice (comparison was done with positive control)

Treatment	Dose (mg kg^{-1})	0	15	30	45	60
Control		6.2 ± 0.7	7.1 ± 1.5	6.8 ± 1.1	6.8 ± 0.7	7.2 ± 1.4
Fruit	50	7.3 ± 0.2	8.3 ± 2.8 ^{ns}	10.8 ± 0.6*	9.2 ± 0.6 ^{ns}	7.7 ± 0.9 ^{ns}
	100	5.7 ± 0.4	8.6 ± 0.8 ^{ns}	11.5 ± 0.7***	9.3 ± 0.9*	8.1 ± 1.2 ^{ns}
	200	5.3 ± 0.5	9.6 ± 0.8**	12.6 ± 0.6***	10.1 ± 0.9***	8.2 ± 0.8^{ns}
	400	6.3 ± 0.9	11.8 ± 3.1***	15.4 ± 1.6***	11.5 ± 3.3***	8.3 ± 1.5 ^{ns}
Leaf	50	5.5± 0.9	8.9 ± 1.0 ^{ns}	10.5 ± 1.5**	8.8 ± 0.6 ^{ns}	7.4 ± 0.8 ^{ns}
	100	5.9 ± 1.2	8.9 ± 1.3 ^{ns}	12.1 ± 1.7***	8.9 ± 1.7 ^{ns}	7.7 ± 0.7 ^{ns}
	200	6.0 ± 1.0	10.3 ± 2.7*	12.6 ± 1.5***	9.6 ± 1.3 ^{ns}	7.9 ± 1.3^{ns}
	400	6.5 ± 0.8	10.6 ± 3.6*	14.9 ± 2.9***	9.9 ± 1.7 ^{ns}	8.0 ± 0.8 ^{ns}
Morphine	5	6.2 ± 0.7	15 ± 2.5***	16.5 ± 1.2***	12.3 ± 1.4***	9 ± 1.9*
Diclofenac	50	5.8 ± 0.6	13 ± 0.6***	14 ± 0.9***	12.4 ± 0.4***	11.7 ± 1.5***

Data are expressed as mean ± *SD* (*n* =7). ***Groups were different from control group with $p<0.001$, **$p<0.01$, * $p<0.05$,^{ns}, not significant.

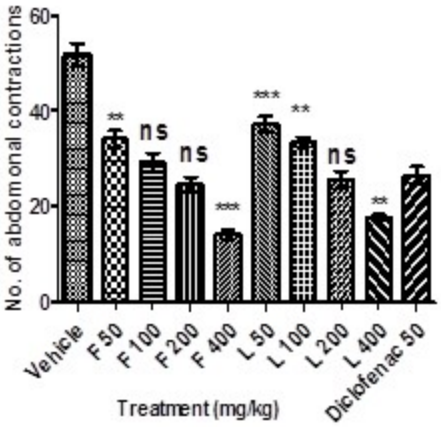
RESULTS

Carrageenan induced edema was significantly inhibited by the extract at all doses, when assessed and compared with the control groups as shown in fig.1 ($p<0.001$). The effect was dose-dependent. Extract of leaf was the most potent at 400mg kg^{-1} dose. No statistically significance was observed between extracts and standard (Diclofenac) ($p>0.05$). Even at 50 mg kg^{-1}(leaf extract), the activity with diclofenac was equipotent ($p>0.05$). Writhing count was significantly reduced by extract in all doses (50 to 400mg kg^{-1}) when compared with control group ($p<0.001$, fig. 2). Fruit extract (400mg kg^{-1}) was the most potent one and showed higher activity than diclofenac 50 mg kg^{-1}($p>0.001$). Extract augmented the pain threshold significantly in all tested doses (50 to 400 mg kg^{-1}) in hot plate thermal test (table 1). Both leaf and fruit extracts at 400 mg kg^{-1} showed the same activity of morphine and diclofenac sodium at 30th minutes. Differences between these positive controls and the highest dose of extracts were not significant ($p>0.05$).



Values are mean ± SD (n=7). All groups were different from control with $p<0.001$. **$p<0.05$, ns, not significant with respect to diclofenac.

Fig. 1: Antiinflammatory activity of polyphenol fraction of Feijoa fruit (F) and leaf (L) on carrageenan induced paw edema.



Values are mean ± SD. (n=7), ***$p<0.001$, ** $p<0.01$, ns, not significant with respect to diclofenac. All groups were different from control with $p<0.001$.

Fig. 2: Antinociceptive activity of polyphenol fraction of Feijoa fruit (F) and leaf (L) in mice (Writhing test).

DISCUSSION

Extract in all doses produced potent and significant inhibition of edema induced by carrageenan, when compared with control group (fig. 1). Extract of leaf produced the same action as that of diclofenac at 50 mg kg^{-1} dose ($p>0.05$). This finding indicates the potential therapeutic use of this plant as a potent anti-inflammatory agent. Since this effect was not antagonized by naloxone, therefore, it is concluded that mechanism underlying the anti inflammatory activity of extract might not be related to opioid system and other mechanisms such as interaction with prostaglandin (PG) biosynthesis or other mediators should be considered. There are several studies which have reported about the anti-inflammatory effect and activity of flavonoids. Feijoa contain flavonoids (Vuotto *et al.,* 2000; Ebrahimzadeh *et al.,* 2008), it is possible that these compounds are responsible compounds for antiinflammatory activity. The writhing method has been extensively used for evaluation and exploration of

peripheral antinociceptive activity. This method has the ability to find out the antinociceptive property of compounds at dose level. Other methods like tail flick test might appear inactive or does not show any effect. However, it is known that acetic acid induced constriction may be considered a non-selective antinociceptive model. Since, the release of endogenous mediators, which stimulates nociceptive neurons sensitive to NSAIDs are indirectly induced by acetic acid (Pourmorad *et al.,* 2007). Our study results showed that extracts, at all tested doses reduced the writhing count at a dose-dependent mode, and showed an extremely significant effect when comparison was made with control group. Remaining of treated animals on the rotating rod in all tested doses, indicate that extracts do not induce any deleterious effect on motor coordination and confirms that analgesic activity is not due to muscle relaxation or sedation.

CONCLUSIONS

These results introduced Feijoa as effortlessly available source of natural products. This study indicates the potential therapeutic use of this plant as a potent anti-inflammatory and antinociceptive agent. Elucidation of exact mechanism of action requires further investigations.

REFERENCES

Ahmadi A, Ebrahimzadeh MA, Ahmad-Ashrafi S, Karami M, Mahdavi MR and Saravi SS (2011). Hepatoprotective, antinociceptive and antioxidant activities of cimetidine, ranitidine and famotidine as histamine H_2 receptor antagonists. *Fund. Clin. Pharmacol.,* **25**(3): 72-79.

Alemy Sh, Karami M, Hossini E, Ebrahimzadeh MA and Shahbi Majd N (2012). Antinociceptive activity and effect of methanol extract of *Salvia limbata* on withdrawal syndrome in mice. *Eur. Rev. Med. Pharmacol. Sci.*, **16**(2): 38-42.

Choi DY, Lee YJ, Hong JT and Lee HJ (2012). Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. *Brain Res. Bull.,* **87**(4): 144-153.

Ebrahimzadeh MA, Hosseinimehr SJ, Hamidinia A and Jafari M (2008). Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves. *Pharmacologyonline* **1**(2): 7-14.

Ebrahimzadeh MA, Mohammad Taheri M, Ahmadpour E, Montazeri M, Sarvi Sh, Akbari M and Daryani A (2017). Anti-toxoplasma effects of methanol extracts of *Feijoa sellowiana*, quercus castaneifolia, and *Allium paradoxum*. *J. Pharmacopuncture,* **20**(3): 220-226.

Lee IT, Lin CC, Lee CY, Hsieh PW and Yang C (2013). Protective effects of (−)- pigallocatechin-3-gallate against TNF-α-induced lung inflammation via ROS-dependent ICAM-1 inhibition. *J. Nutr. Biochem.,* **24**(3): 124-136.

Karami M, KarimianNokabadi F, Ebrahimzadeh MA and Naghshvar F (2014). Nephroprotective effects of *Feijoa Sellowiana* leaves extract on renal injury induced by acute dose of ecstasy (MDMA) in mice. *Iran. J. Basic Med. Sci.*, **17**(4): 69-72.

Mahmoudi M, Ebrahimzadeh MA, Nabavi SF, Hafezi S, Nabavi SM and Eslami Sh (2010). Antiinflammatory and antioxidant activities of gum mastic. *Eur. Rev. Med. Pharmacol. Sci.,* **14**(3): 765-769.

Mahmoudi M, Ebrahimzadeh MA, Abdi M, Arimi Y and Fathi H (2015). Antidepressant activities of *Feijoa sellowiana* fruit. *Eur. Rev. Med. Pharmacol. Sci.,* **19**(2): 2510-2513.

Mahmoudi M, Hosseini M, Allami A and Ebrahimzadeh MA (2016). Anti-inflammatory activities of *Vicia canescence* and *Vicia hirsuta* aerial parts. *Pharmacologyonline*, **2**(5): 147-150.

Monforte MT, Lanuzza F, Mondello F, Naccari C, Pergolizzi S and Galati EM (2014). Phytochemical compositon and gastroprotective effect of *Feijoa sellowiana* Berg. fruit from Sicily. *J. Coast. Life Med.,* **2**(3): 14-21.

Mosbah H, Louati H, Boujbiha MA, Chahdoura H, Snoussi M, Flamini G, Ascrizzi R, Bouslema A, Achour L and Selmi B (2018). Phytochemical characterization, antioxidant, antimicrobial and pharmacological activities of *Feijoa sellowiana* leaves growing in Tunisia. *Ind. Crops Prod.,* **28**(4):521-31.

Pourmorad F, Ebrahimzadeh MA, Mahmoudi M, Karami M and Yasini S (2007). Antinociceptive activity of methanolic extract of *Epilobium Hirsutum*. *Pak. J. Biol. Sci.*, **10**(3): 2764-2767.

Queen BL and Tollefsbol TO (2010). Polyphenols and aging. *Curr. Aging Sci.*, **3**: 34-42.

Rabiei Kh, Bekhradnia S, Nabavi SM, Nabavi SF and Ebrahimzadeh MA (2012). Antioxidant activity of polyphenol and ultrasonic extracts from fruits of *Crataegus pentagyna* subsp. elburensis. *Nat. Prod. Res*., **26**(2): 2353-2357.

Tortora GJ and Reynolds S (2009). Principles of anatomy and physiology. 12nd ed., John Wiley and Sons, New York, p. 695.

Vuotto ML, Basile A, Moscatiello V, De Sole P, Castaldo-Cobianchi R, Laghi E and Ielpo MT (2000). Antimicrobial and antioxidant activities of *Feijoa sellowiana* fruit. *Int. J. Antimicrob. Agents,* **13**(3): 197-201.

Weston RJ (2010). Bioactive products from fruit of the feijoa (*Feijoa sellowiana*, Myrtaceae): A review. *Food Chem.,* **2**(3): 923-926.