Analgesic and Anti-Inflammatory Effects of *Phaseolus vulgaris* L. Fixed Oil in Rodents

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Abstract: The seeds of *Phaseolus vulgaris* are known as common beans or kidney beans. The dry seeds are eaten as pulse and are enriched with protein, fiber, starch, B vitamins (B1, B6, B9), iron, potassium and selenium. Beans also contain about 1-2 % of fixed oil. *Phaseolus vulgaris* is linked with anticancer, antihyperlipidemic, hypoglycemic and antioxidant actions. The fixed oil of *Phaseolus vulgaris* (PVFO) seeds is extracted with hexane and used in this study to assess acute oral toxicity, analgesic (by acetic acid induced writhing, hot plate and tail flick tests in mice) and anti-inflammatory (by carrageenan induced paw edema in rats) actions. Four groups were made (n=6): Group-I: Normal Saline Control (2ml/kg), Group-II: PVFO (2ml/kg), Group-III: PVFO (4ml/kg) and Group-IV: Standard Acetyl salicylic acid (ASA 300 mg/kg). PVFO in 2ml/kg and 4ml/kg dose demonstrated analgesic and anti-inflammatory activities but in hot plate results were unreliable as here significant activity started after 90 minutes. For toxicity test 5ml/kg dose was administered orally in mice and no toxicity symptoms were observed. It is therefore concluded that PVFO is safe for oral use up to 5ml/kg and may possess analgesic and anti-inflammatory actions.

Keywords: Analgesic, anti-inflammatory, fixed oil, kidney beans, *Phaseolus vulgaris.*

INTRODUCTION

Phaseolus vulgaris L. also known as kidney bean, is a popular specie of genus Phaseolus and belongs to family Fabaceae [1]. Asia, South America and Africa are massive producer of beans [2]. *Phaseolus vulgaris* Linn. is an ordinary vegetable that possess loads of healing and remedial properties. The plant is renowned for its antidiabetic activity. Kidney beans hold some biologically active elements with hypoglycemic effect [3,4]. *Phaseolus vulgaris* is commonly utilized as dry bean but consumption as fresh form is also feasible [5]. There is a high amount of protein present in bean as compared to other cereal grains [6]. It also has high quantity of dietry fiber, starch, minerals and some important vitamins [7,8]. Excluding that, it has several phytochemicals, free radical scavenging ability and an extensive group of flavonoids such as flavonols, isoflavones, anthocyanin, proanthocyanidins and phenolic acids [9-12].

There is only 2% of lipid content in beans with some important unsaturated fatty acids [13]. However, that little amount of lipid possess some important vitamins like E and K. Vitamin E has the ability to hunt free radicals and therefore act as an antioxidant and anti-inflammatory agent. Dry beans mainly contain 2 isomers of vitamin E i.e. α-tocopherol and γ - tocopherol. Vitamin K is also found in beans, it is needed for calcium binding in bones, that's why it is beneficial for bones. Lipids in dry beans are mainly composed of polyunsaturated fatty acids (PUFAs), which include linoleic acid (LA) and alpha linolenic acid (ALA). It has been revealed by epidemiologic studies that consumption of alpha linolenic acid has great impact on health of individuals suffering from coronary heart disease [14].

Most of the fatty acids are produced by human body whereas few important fatty acids such as ALA and LA are present naturally in vegetables and some seeds. These two PUFAs have a crucial role in body when utilized in food. After metabolism these PUFAs convert into longer-chain PUFAs. ALA changes into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas LA is converted into arachidonic acid. The PUFAs (n-3) are mostly found in green leafy vegetables, walnuts, fish and some seeds (grape, chia, flax), while PUFAs (n-6) are mostly present in plants' seeds and oils which include sunflower, corn,cottonseed and some others [15]. PUFAs are also involved in regulating certain processes in central nervous system, such as adjusting blood glucose and food consumption, as well as they take part in process of apoptosis, regulate emotional behavior, neurotransmission and neuroinflammation [16].

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Furthermore dietary PUFAs (n-3) control various neurotransmitter operations, as well as signal transmission, responsiveness, and phospholipid alteration [17]. PUFAs (n-3) are also connected with psychiatric syndromes and are required for mental development, avoidance of neuronal death, and the inhibition of neuroinflammation [18]. Fatty acids are source of energy and are the fundamental part of cell membrane. Research on plant lipids suggest that plant lipids are enriched in gammalinolenic acid (GLA). GLA has the ability to alter into di-homo-gamma linolenic acid (DGLA). Prostaglandin E_1 is formed from DGLA. It also exhibits anti-inflammatory and immunomodulatory effects. Arachidonic acid after metabolism converts into eicosanoids, such as prostaglandins, thromboxane, leukotrienes. These eicosanoids are the main triggers and promote the immunological and inflammatory responses[19]. Alpha linolenic acid (ALA) metabolizes into products which are anti-inflammatory and capable of inhibiting platelet aggregation. The latter mechanism suggest that ALA is helpful in preventing hypertension, CVD, diabetes type II and COPD [20].

The intake of beans may be helpful in treatment and avoidance of some metabolic disorders and chronic diseases such as diabetes, CVD and cancer [20]. Literature survey provided information that *Phaseolus vulgaris* Linn. seeds possess several biological activities like improvement of the immune system [21], antioxidant activity [22], prevent cancer [23], mimic the estrogen [24], relieves depression [25], antibacterial and antituberculosis effect [26], analgesic and anti-inflammatory activities [27].

Pain is characterized as a distressing feeling which is caused by intense stimuli. The management of pain is considered as a main clinical problem [28]. Pain is further described as acute or chronic. Acute pain specify injury and has a significant value as a sign of existing tissue lesion. Chronic or persistent pain is regarded as a condition that consequences from massive inflammatory reactions [29]. Analgesics are the synthetic drug molecules which are used for treatment of pain. Analgesic compounds are centrally or peripherally acting and reduce pain without causing any sedation. Analgesics or conventional pain relievers are more valuable when an individual can not get rid of injurious stimulus [30].

Inflammation is a process that occurs in response to different infectious or metabolic stimuli. Inflammatory process is basically a body defence system, that is activated to limit the proliferation of injurious agent. Management of inflammation is still a challenging task to the scientists despite the accessibility of variety of NSAIDs [31]. The reason is that NSAIDs not only manage inflammation and possess analgesic activity but are also associated with gastrointestinal complications. NSAIDs interfere with prostaglandins production by preventing both COX I and COX II. Pro drugs and modified formulations are being developed to overcome the side effects of NSAIDs [32]. Long term use of conventional analgesics bring about serious side effects which are unpreventable, therefore invention and detection of safe and effective analgesics is necessary [33]. As herbal medicines are comparatively safe from side effects for the treatment of different diseases. There is a rising tendency of research on plants and herbs. Plant oriented medicines have a great impact on individuals of developing countries and are also a source of developing novel drug molecule [34].

MATERIAL AND METHODS

Seed Collection and Oil Extraction

Dry seeds (2kg) of *Phaseolus vulgaris* were purchased from local market and sticks, dirt and stones were separated from seeds then crushed and extracted with 5 L hexane by using Soxhlet extractor with condenser (Wertlab Germany). Several cycles were run at 70° C until all oil contents were extracted. A clear light yellow color oil was obtained.

Animals

Healthy male and female swiss albino mice weighing between 20-25 g and wistar albino rats (male and female) of 180- 200 g were taken from animal house of Department of Pharmacology, University of Karachi. Animals were kept in propylene plastic cages at 22-25°C temperature, 12 hour light and 12 hour dark alternate cycle, 50-60% humidity and supplied with standard food and free access to water. Experiments were carried out in accordance with the international guidelines of animal ethics and study was submitted to and approved by institutional ethical committee, Department of Pharmacology in 2017 and Board of advanced studies (BASR), University of Karachi in 2015.

Acute Toxicity Test

To check the toxicity mice were given orally a single dose of 5ml/kg of oil [35]. No mortality was observed in mice after a period of 1 week. No signs of seizure,

sedation and unconsciousness were observed. Breathing and behavior were also normal.

Dosing for Analgesic and Anti-Inflammatory Tests

Four groups were made, each group had 6 mice or rats. Group-I: Normal Saline Control (2ml/kg), Group-II: PVFO (2ml/kg), Group-III: PVFO (4ml/kg), and Group-IV: Standard Acetyl salicylic acid (ASA 300 mg/kg). All drugs were administered orally.

Acetic Acid Induced Writhing Test

Writhing test previously suggested by Koster *et al.* was used [36]. After 30 minutes of dosing, acetic acid was injected intraperitoneally in mice of all groups and number of writhes were counted for a period of ten minutes.

Tail Flick Test

In this experiment water bath (51± 1 ℃) was used. After 30 minutes of dosing in each group, the reaction time was noted as the period between tail immersion and tail flicking [37].

Hot Plate Test

Hot plate was kept at 51± 1°C and mice were placed on it after 30 minutes of dosing in respective groups. Time taken by mice for paw licking or jumping was noted [38].

Carrageenan Induced Rat Paw Edema Test

Carrageenan was used to induce inflammation in rats' paw. After 30 minutes of drug administration paw volume was measured using plethysmometer (UGO Basile,Italy) [39].

Statistical Analysis

All data is expressed as mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by tukey hsd post hoc test. P<0.05 was considered statistically significant and P<0.01 was considered highly significant. SPSS 20 was used for the statistical analysis

RESULTS

Acute Oral Toxicity

No toxicity signs were observed during one week after 5ml/kg dose.

Writhing Test

PVFO at both doses i.e. 2ml/kg and 4ml/kg reduced writhing count (P≤0.01). Refer Table **1**.

Tail Flick Test

PVFO (2ml/kg and 4ml/kg) showed a marked rise in reaction time (P≤0.05). Refer Table **2**.

Hot Plate Test

Significant analgesic effect started in all groups after 120 minutes of dosing (P≤0.05). Refer Table **3**.

Carrageenan Induced Rat Paw Edema Test

PVFO reduced rat paw edema at both doses 2ml/kg and 4ml/kg (P≤0.01). Refer Table **4**.

Table 1: Acetic Acid Induced Writhing Test

Group	Dose (oral)	No of writhes
Control	2ml/kg	47.33±1.45
PVFO	2ml/kg	20.33±1.33**
PVFO	4ml/kg	21.33±3.48**
ASA	300mg/kg	15.66±0.66**

Values are expressed as mean ± SEM; N=6; PVFO = *Phaseolus vulgaris* fixed oil*;* ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at P <0.001 as compared to control; **= highly significant at P <0.01 as compared to control; * = significant at P <0.05 as compared to control.

Table 2: Tail Flick Test

Group	Dose (oral)	Reaction Time (sec)						
		0 min	30min	60min	90min	120min	150min	180min
Control	2ml/kg	0.7±0.08	0.9±0.1	0.8±0.05	0.9±0.08	0.9±0.04	0.8±0.03	0.8±0.05
PVFO	2ml/kg	0.5±0.04	3.6±0.7	5.0±0.7***	2.5±0.3*	2.5±0.3**	2.3±0.2**	2.4±0.4**
PVFO	4ml/kg	0.9±0.06	2.8±0.2	2.9±0.2*	2.9±0.2**	3.0±0.2***	3.2±0.4***	3.1±0.3***
ASA	300mg/kg	0.6±0.08	4.4±0.6**	4.5±0.5***	3.9±0.5***	3.4±0.2***	2.8±0.08***	2.4±0.1**

Values are expressed as mean ± SEM; N=6; PVFO = *Phaseolus vulgaris* fixed oil*;* ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at P <0.001 as compared to control; **= highly significant at P <0.01 as compared to control; *= significant at P <0.05 as compared to control.

Table 3: Hot Plate Test

Group	Dose (oral)	Reaction time (sec)						
		0 min	30 min	60 min	90 min	120 min	150min	180min
Control	2ml/kg	7.3±0.3	7.6±0.3	7.3±0.3	6.6±0.6	7.3±0.3	6.6±0.3	6.6±0.3
PVFO	2ml/kg	8.6±0.3	11.03±1.2	12.2±1.5	12.4±2.1	13.9±1.0**	13.5±0.7**	9.7±0.7
PVFO	4ml/kg	8.6±0.6	11.6±1.6	14.6±2.6*	11.6±2.9	11.6±0.8*	12.66±1.2**	10.6±1.2*
ASA	300mg/kg	7.6±0.3	8.6±0.6	12.0±0.5	13.3±0.8	14.3±1.2**	11.0±0.5*	9.3±0.3

Values are expressed as mean ± SEM; N=6; PVFO = *Phaseolus vulgaris* fixed oil; ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at P <0.001 as compared to control; **= highly significant at P <0.01 as compared to control*; = significant at P <0.05 as compared to control.

Table 4: Carrageenan Induced Rat Paw Edema Test

Group	Dose (oral)	Paw edema (ml)					
		0hr	1hr	2hr	3hr	4hr	5hr
Control	2ml/kg	1.5±0.06	2.9±0.05	3.8±0.06	4.5±0.05	5.3±0.12	5.7±0.09
PVFO	2ml/kg	1.6±0.02	2.5±0.11*	2.2±0.15***	2.3±0.11***	2.0±0.14***	1.9±0.14***
PVFO	4ml/kg	1.5±0.09	2.3±0.11**	2.2±0.13***	2.3±0.28***	2.1±0.36***	2.0±0.33***
ASA	300mg/kg	1.6±0.02	1.8±0.02***	1.8±0.05***	1.7±0.04***	1.7±0.03***	1.7±0.02***

Values are expressed as mean ± SEM; N=6; PVFO = *Phaseolus vulgaris* fixed oil; ASA = Acetyl salicylic acid; Control= Normal Saline; *** = very highly significant at P <0.001 as compared to control; **= highly significant at P <0.01 as compared to control; *= significant at P <0.05 as compared to control.

DISCUSSION

The oil extracted from seeds of *Phaseolus vulgaris* (PVFO) did not show any toxic effect in mice up to dose of 5ml/kg. This indicates that LD_{50} of PVFO will be more than 5ml/kg. This article mainly focuses on analgesic and anti-inflammatory effects of PVFO. Analgesics are class of drugs that act on central or peripheral nervous system to relieve pain selectively without altering consciousness [40]. The hot plate test excites supra-spinal neurons [41, 42]. The results of hot plate and tail flick test reveal that PVFO possess significant analgesic activity with both doses.

Peripheral nociception was investigated by acetic acid induced writhing test. Acetic acid triggers the pain through release of histamine, serotonin, bradykinins, prostaglandins (PGs) and substance P [43]. The results of writhing test suggest a peripheral effect of PVFO as analgesic activity in tail flick and hot plate tests was delayed.

Carrageenan induced edema is a biphasic phenomenon. In early phase serotonin (5-HT) and histamine liberate (0-2 hr), second quicken stage of swelling is due to PG release (>4hr) [44]. In the present study PVFO affected both phases as the anti-inflammatory effect started with in one hour and continued till 5 hour.

A high amount of polyunsaturated fatty acids (i.e. 71.1g/100g) such as linolenic acid is present in kidney beans [45, 46]. Linolenic acid decreases prostaglandins and leukotrienes [19] where as fatty acids such as palmitic acid are responsible for reducing heat perception in animal model [47].

It is concluded that fixed oil of *Phaseolus vulgaris* L. possess significant analgesic and anti-inflammatory activities. Further study is required on mechanism of action and chemical constituents of PVFO.

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