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Phytochemical screening and evaluation of analgesic and antiinflammatory activities of *Phaseolus vulgaris linn.*, seeds in rodents

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ABSTRACT

With an objective of developing some novel analgesic and antiinflammatory natural agents with fast acting and low toxicity profile here in, the different extracts of *Phaseolus vulgaris (Linn)* seeds were evaluated for analgesic and antiinflammatory activities using glacial acetic acid induced writhing and carrageenan induced rat paw oedema method respectively. For screening of the extracts for analgesic and antiinflammatory activities aspirin and diclofenac were used as standard drugs respectively. Petroleum ether extract exhibited significant analgesic and antiinflammatory activities. The petroleum ether extract can be considered as a potential candidate for analgesic and antiinflammatory activities. The presence of steroids and flavonoids in petroleum ether extract of *Phaseolus vulgaris Linn.*, seeds could be attributed for the analgesic and antiinflammatory activities.

INTRODUCTION

Inflammation is a body's defense mechanism in response to various infectious or metabolic stimuli. It is a body defence reaction in order to terminate or limit the spread of injurious agent as well as to remove consequent necrosed cells and tissues. Suppression of inflammation still continues to be a challenge to the scientists despite the availability of number of NSAID's. This is because NSAID's not only exhibit anti-inflammatory and analgesic activity but also cause gastrointestinal complications ranging from dyspepsia to upper GI tract bleeding and perforation. Efforts to improve adverse effect profile of current NSAID's have been focused on developing pro-drugs or modification of marketed formulations (Schenone *et al*., 2006).

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A recent approach is development of selective cyclooxygenase inhibitors (COX-2). Currently available NSAID's inhibit both COX-1 and COX-2. Inhibition of COX-1 reduces production of cytoprotective prostaglandins PGE2 and PGI2 and hence causes ulceration, while inhibition of COX-2 inhibits inflammation. Complete inhibition of COX-1 is therefore not preferred and drugs that selectively inhibit COX-2 are better anti-inflammatory agents. Another field of greater concern is synthesis of more effective analgesic agents. Pain is in fact a very serious problem associated in 90% of diseases. NSAID'S, which are used to treat pain, have several side effects. There is a great need to develop some natural agents (phytoconstituent), which are capable of treating both acute and chronic pain. Plants have payed a remarkable role in health care since the ancient times. Traditional plant based medicines still exert a great deal of importance to people living in developing countries and also lead to discovery of new drug candidates (Orhan *et al.*, 2007). *Phaseolus vulgaris (Linn)* seeds belong to the family of Fabaceae (Romero-Arenas *et al.*, 2013).

It is a sub-erect or twining annual herb, native of central and South America and is now grown extensively through out the warm regions of the world. Vernacular names of the plant *Phaseolus vulgaris* are in Kannada- Tingalavari, Hindi-Rajma, Gujarati-Fansi, Kashmiri-Fraa'sh bean, Marathi-Pharas bee and Punjabi-Fras bean. Literature survey revealed that *Phaseolus vulgaris (Linn)* seeds exhibit different biological activities like enhancement of the bifidogenic effect (Queiroz-Monici *et al.*, 2005*)*, antioxidant (Heimler *et al.*, 2005), anticarcinogenic (Hangen *et al*., 2002), estrogenic (Stephen *et al.*, 2011), antidepressant (Madhu *et a*l., 2014) and antibacterial and antitubercular (Pradeep Kumar *et al*., 2014) etc.

Phaseolus vulgaris (Linn) seeds have an important place in the folk medicine of the world and in the traditions of many cultures because of their pharmacotherapeutic effects (Mishra *et al*., 2010). However, *Phaseolus vulgaris (Linn)* has not been investigated for analgesic and antiinflammatory activities. Hence, this study was carried out to evaluate the potent bioactive constituents for analgesic and antiinflammatory activities in *Phaseolus vulgaris (Linn)* seeds.

Phaseolus vulgaris Linn seeds

MATERIALS AND METHODS

The Seeds of *Phaseolus vulgaris (Linn)* were collected from the local areas of Dharwad in Karnataka and were authenticated by Dr. S. S. Hebbar, Department of Botany, Government Pre-university College Dharwad. A voucher specimen (No- SETCPD/Pharmacog/Herb/2011/12) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T.'s College of Pharmacy, Dharwad, Karnataka.

The Seeds of *Phaseolus vulgaris (Linn)* were shade dried and finely powdered to particle size (#) 40. About 300 g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether, chloroform and ethanol (95%). Aqueous extract was also obtained by cold maceration of the drug (300 g) with 2% chloroform water. After the extraction, the extracts were filtered and concentrated under reduced pressure using a rota evaporator. The yield of petroleum ether, chloroform, ethanol and aqueous extract was found to be 8.36 g (3.12 % w/w), 6.285 g (1.95 % w/w), 24 g (7 % w/w) and 11 g (4.01 % w/w), respectively. All the extracts were kept in a dessicator for drying.

Pharmacological activity

The pharmacological activities of synthesized compounds were investigated in albino rats and mice. Rats were used for acute anti-inflammatory and chronic anti-inflammatory activities. Mice were used for analgesic activity. All animal experiments were approved by institutional animal ethical committee. All the animals were stabilized to laboratory conditions before starting the experiments.

Acute toxicity

The acute toxicity test was carried out according to the Organization for Economic Co operation and Development (OECD) guidelines (OECD/ OCDC, 2000) to establish the effective dose of all the synthesized and extracted compounds.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the well known Carageenan induced rat paw oedema model (Winter *et al*., 1962) using groups of six animals each. A freshly prepared aqueous suspension of carrageenan (1% w/v, 0.1 ml) was injected in the sub planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pre-treated with the test extract at a dose of 200 mg/ kg body weight of rat, 1 h before the carageenan treatment. The volume was measured before and after carageenan treatment at the 30 min. interval with the help of digital plethysmometer. Standard drug used was diclofenac at a dose of 200 mg/kg body weight of rat.

Analgesic activity

Twenty four hours prior to actual testing a large number of mice (20-25 gm) received intraperitoneally (i.p) 10 ml/kg of 0.6 % glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received compounds half an hour prior to glacial acetic acid challenge (Koster *et al*., 1959). Extracts were given at a dose of 200 mg/kg orally. Standard drug used was aspirin at a dose of 30 mg/kg body weight of mice.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of steroids, flavonoids (Milan, 2011) in the petroleum ether extract of seeds of *Phaseolus vulgaris (Linn)*. The results of the physico-chemical parameters and preliminary phytochemical screening of *Phaseolus vulgaris* Linn seeds is given in Table 1 and Table 2 respectively. Hence, the presence of flavonoids and steroids in petroleum extract could be attributed for observed significant analgesic (Jain *et al.*, 2011; Parveen *et al.*, 2007) and antiinflammatory activities (Sakat *et al.*, 2010; Nagore *et al.*,

2010). However, research work is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principle for the claimed analgesic and antiinflammatory activities.

Table. 1: Physico-chemical parameters of *Phaseolus vulgaris* Linn seeds.

Sl.No.	Parameter	Determined Value %w/w
A	Extractive values	
1	Alcohol soluble extractive value	2.1
2	Ether soluble extractive value	0.6
3	Water soluble extractive value	6.0
B	Ash Values	
1	Total ash	8.0
2	Acid insoluble ash	1.2
3	Water soluble ash	1.7
4	Sulfated ash	1.8
C	Moisture content	2 .2

Table 2: Preliminary phytochemical analysis of various extracts of *Phaseolus vulgaris* Linn seeds.

Phytoconstituents	Successive extraction fractions			
	Petroleum ether	Chloroform	Alcohol	Aqueous
Alkaloids	-ve	+ve	+ve	-ve
Steroids	+ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	+ve
Phenolic	-ve	-ve	-ve	+ve
Flavonoids	+ve	+ve	-ve	+ve
Glycoside	-ve	-ve	-ve	+ve
Triterpenoid	-ve	+ve	-ve	-ve
Tannins	+ve	-ve	+ve	+ve

+ve = Present; –ve = Absent

Table 3: Antiinflammatory activity screening of *Phaseolus vulgaris* Linn seeds.

Extract/ Compound	Percentage inhibition of Paw oedema		
	1hr	3hr	5hr
Control	-	-	-
Aqueous Extract	22.06	31.43	26.26
Alcohol Extract	36.10	64.38	55.95
Chloroform Extract	26.20	48.53*	42.63*
Pet-ether Extract	39.89	71.38**	69.14**
Diclofenac	42.12	79.53	76.68

Animals were dosed 200 mg/kg. Std. drug diclofenac at a dose of 5 mg/kg bod weight. Data were analysed by ANOVA. Followed by Dunnet's test. **P<0.001. P < 0.05. Values are mean ± SEM, of six animals in each group.

Table 4: Analgesic activity screening of *Phaseolus vulgaris* Linn seeds.

Extract/ Compound	No. of wriths in 15 min.	± SEM	% Protection
Control	44	1.713	--
Aspirin	13	1.317	70.45
Aqueous Extract	32	1.163	27.28
Alcohol Extract	24	1.392	45.46*
Chloroform Extract	28	1.065	36.37
Pet-ether Extract	16	1.447	63.64**

Animals were dosed 200 mg/kg. Standard drug aspirin at 30 mg/ kg body weight. Data were analysed by ANOVA. Followed by Dunnet's test.
**P<0.001. P<0.05. Values are mean ± SEM, of six animals in each group.

CONCLUSION

The present study provides an evidence for the analgesic and antiinflammatory activities of *Phaseolus vulgaris (Linn)* seeds. Aspirin used as standard drug for screening the analgesic activity which act by obtunding of peripheral pain receptors and prevention of PG- mediated sensitization of nerve endings (Tripathi, 2013) and the diclofenac which is used as standard drug for screening the antiinflammatory activity that acts by inhibiting the prostaglandin synthesis and specially it is COX-2 selective (Tripathi, 2013). As the activity results (petroleum ether extract) are closer to those of diclofenac and aspirin, the bioactive principles present in the extract may be having the mechanism of action similar to that of the tested standard drugs. However research is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed analgesic and antiinflammatory activities. The present study may form the basis for the selection of plant species for further investigation in potent bioactive compounds for analgesic and antiinflammatory activities.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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