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Phytopharmacological Evaluation of Ethanolic Extract of the Seeds of *Abrus precatorius* Linn

Rashmi Arora, Naresh Singh Gill, Sukhwinder Kaur and Ajay Deep Jain
Rayat School of Pharmacy, Near Railmajra, Punjab Technical University, Ropar-144533, India

Corresponding Author: Rashmi Arora, Rayat School of Pharmacy, Near Railmajra, Punjab Technical University, Ropar-144533, India

ABSTRACT

Abrus precatorius Linn. is a leguminous plant of the fabaceae family whose stem, bark, leave and roots are widely used for medicinal purposes in tropical and subtropical regions of the world. The plant parts are purgative, emetic, tonic, anti-phlogistic, aphrodisiac and anti-ophthalmic agents. The purpose of the present investigation was to study antioxidant, anti-inflammatory and analgesic activity of *Abrus precatorius* Linn. ethanolic seed extracts by soxhlet method. The free radical scavenging activity of extract was carried out by 1,1-diphenyl-2-picrylhydrazyl method and anti-inflammatory activity by carrageenan induced rat paw edema and analgesic activity carried out by tail flick and tail immersion method. The extract showed significant activity i.e., 80.1±0.34% at 300 μg mL^{-1}. The extract was evaluated for its anti-inflammatory activity it showed significant anti-inflammatory activity i.e., 62.68% at 375 mg kg^{-1} by carrageen induced method. Further, the extract was evaluated for analgesic activity and the extract showed significant activity at 300 mg kg^{-1} after 90 min interval by tail flick and tail immersion method. As ethanolic extract of *Abrus precatorius* Linn. was found to have potent antioxidant, anti-inflammatory and analgesic potential. The present study concludes that seeds of *Abrus precatorius* Linn. can be used as good natural antioxidant to treat free radical induced disease.

Key words: *Abrus precatorius* Linn., antioxidant, anti-inflammatory, analgesic, carrageenan

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all aliments of mankind (Kokate *et al.*, 2002). Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Pain, inflammation and fever are very common complications in human beings. Several plants and their products are proved to posses analgesic and antipyretic property (Nanda *et al.,* 2009). Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic efficacy. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Gill *et al.*, 2011a). Plant extracts as well as their primary and secondary metabolites have important therapeutic role in the treatment of many human diseases (Gill *et al.*, 2011b; Sood *et al.*, 2009). Medicinal plants have a traditional medicine or folk medicine practice based on the use of plants and plant extracts. According to World Health Organization more than 21000 plants are being in use as medicinal purpose in around the world (Karim *et al.*, 2011). Many herbs synthesize substances that are useful for the maintenance of human health (Tapsell *et al.*, 2006).

Natural antioxidants are the compounds obtained from secondary metabolites. Antioxidants are the substances which protect our body from Reactive oxygen and nitrogen species. Oxidation reactions can produce free radical which starts chain reactions that damage cell. Free radical production is actually a normal part of life (Gavani and Paarakh, 2008). Reactive oxygen and nitrogen species are natural and physiological modulators of cellular redox reactions. Despite of the multiline antioxidant system, the level of reactive oxygen and nitrogen species generation can exceed the capability of defense network, leading to oxidative stress (Askew, 2002). Oxidative stress result from an imbalance between the generations of oxygen derived radicals. Environmental agents also initiate free radical generation leads to different complication in body (Londonkar and Kamble, 2009). It is generally assumed that increases in aerobic metabolism or hyperoxia easily increased level of reactive oxygen and nitrogen species which lead to oxidative damage to lipids, proteins, carbohydrates and DNA (Gill *et al*., 2010). Inflammation is a disorder involving localized increase in the number of leucocytes and variety of complex mediators (Jothimanivannan *et al*., 2010). Drugs that are currently used for the management of pain are non-steroidal anti-inflammatory drugs and corticosteroids (Vittalrao *et al*., 2011). Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Nonciception is the mechanism by which pain information is passed to the central nervous system (Asmawi *et al*., 2011).

The plant *Abrus precatorius* Linn. belongs to the family Fabaceae commonly known as rosary pea and ratti, is a medicinal herb used for various diseases. The plant parts are purgative, emetic, tonic, anti-phlogistic, aphrodisiac and anti-ophthalmic (Manoharan *et al*., 2010). The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form, upon long refrigerated storage (Khare, 2004; Vaidyarathnam and Varier, 1995). However, cooking destroys the poison so that the seeds may be eaten (Neal, 1965). In the present study the ethanolic seed extract of *Abrus precatorius* Linn. was evaluated for antioxidant, anti-inflammatory and analgesic activity.

MATERIALS AND METHODS

Plant material: *Abrus precatorius* Linn. seeds were collected in the month of July 2010. Seeds were purchased from the local grain market, Ropar (India). The seeds were authenticated and the voucher specimen No. 0398 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar. The seeds were cleaned, washed, dried and carefully powdered in the grinder at room temperature and were kept in tight containers to protect them from light.

Extraction: The fresh seeds were selected and powdered coarsely. About 300 g of air dried powdered material was extracted with ethanol in a soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The concentrate filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on water bath. The yield of the extract was calculated. The ethanolic extract yielded a brown sticky mass. The crude extracts were used for further investigation (Kokate *et al*., 1999).

Phytochemical screening: *Abrus precatorius* Linn. seed extract was subjected to qualitative chemical examination for the presence of alkaloids, carbohydrates, flavonoids, saponins,

phytosterols, triterpenoids, coumarin glycosides and tannins according to standard procedures (Rahman *et al.*, 2011).

Drugs and chemicals: 1,1-diphenyl-2-picrylhydrazyl was obtained from Hi-media. Carrageenan, ascorbic acid and diclofenac sodium were procured from Jackson Laboratories, Amritsar (Punjab). Physician sample of aspirin was obtained from Ranbaxy, Ropar (Punjab) and the solvents like hexane, chloroform, ethyl acetate and methanol were of analytical grade and purchased from SD Fine Chemical.

Animals: The antioxidant, anti-inflammatory and analgesic activity was carried out from 1th December (2010) to 30th May 2011. Thirty wistar rats each weighing 160-180 g were divided in five groups each containing six rats in and 60 Swiss albino mice each 20-30 g were collected from Sanjay Biologicals, Amritsar (PB). Animals were maintained standard environmental condition temperature (24.0±1.0°C), relative humidity: 55-65% and 12 h light/dark cycle and had free access to feed and water. The animals were acclimatized to laboratory condition for one week prior to experimentation. All protocol for experiment was approved by Institutional animal research Ethical Committee.

Antioxidant activity

Qualitative evaluation of the 1,1-diphenyl-2-picrylhydrazyl scavenging activity: The qualitative assay was performed according to reported method by Gill *et al.* (2011c).

Quantitative evaluation of the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity: Quantitative evaluation of the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity was done by method prescribed by Sood *et al.* (2009). All, the readings are taken in triplicate and their mean value taken in consideration. Inhibition of 1,1-diphenyl-2-picrylhydrazyl radical was calculated using equation.

$$\text{Scavenging activity}(\%) = 100 \times (A_0 - A_s) / A_0$$

where, A_0 is the absorbance of the control and A_S is the absorbance of the test sample.

Pharmacological evaluation

Experimental design: The animals of either sex were divided into five groups each groups consists of six animals (n = 6).

Group I (Control group)	:	Carboxy methyl cellulose (0.5% CMC, p.o.) + Carrageenan treated group
Group II (Standard group)	:	Diclofenac sodium (10 mg kg^{-1}, p.o.) + Carrageenan treated group
Group III (Ethanolic extract 100)	:	Ethanolic extract at the dose of (125 mg kg^{-1}, p.o.) + Carrageenan treated group
Group IV (Ethanolic extract 200)	:	Ethanolic extract at the dose of (250 mg kg^{-1}, p.o.) + Carrageenan treated group
Group V (Ethanolic extract 300)	:	Ethanolic extract at the dose of (375 mg kg^{-1}, p.o.) + Carrageenan treated group

Carrageenan method: The inflammation was assessed by the method described by Winter *et al.* (1962). The paw volume was measured at intervals of 1, 2 and 3 h by the mercury displacement method using a plethysmometer. Diclofenac sodium (10 mg kg^{-1}, p.o.) was used as standard drug. The percentage inhibition of edema was calculated as:

$$\text{Percentage inhibition of edema} = \frac{C_C - V_T}{V_C} \times 100$$

where, V_c is the inflammatory increase in paw volume of control group of animals and V_t is the inflammatory increase in paw volume of drug treated animals.

Methodology for analgesic activity: In present study the analgesic effect of the ethanolic extract of *Abrus precatorius* Linn. seed was checked out by two methods i.e., tail immersion and tail flick methods. The albino mice were divided into five groups of six animals in each group.

Group I (Control group)	:	Gum acacia suspension (2% w/v gum acacia, p.o.) treated group
Group II (Standard group)	:	Aspirin (100 mg kg^{-1})
Group III (Ethanolic extract 100)	:	Ethanolic extract at dose of 100 mg kg^{-1}, p.o. treated group
Group IV (Ethanolic extract 200)	:	Ethanolic extract at dose of 200 mg kg^{-1}, p.o. treated group
Group V (Ethanolic extract 300)	:	Ethanolic extract at dose of 300 mg $kg,^{-1}$ p.o. treated group

Tail immersion method: Analgesia was assessed according to the tail immersion method (Patil *et al.*, 2010). The withdrawal latency was recorded at 0, 30, 60, 90, and 120 min after the administration of drug and extract.

Tail flick method: In this test method the tail withdrawal latency was recorded by using analgesiometer at 0, 30, 60, 90, and 120 min after the drug administration (Shanmugasundaram and Venkataraman, 2005).

Statistical analysis: Descriptive statistics and comparisons of differences between each data set were calculated by the use of Sigma Stat 3.5 trial version software. The data were expressed as Mean±SEM, and analyzed by two ways ANOVA in each experiment. Statistical significance was accepted at the level of $p<0.05$.

RESULTS

Phytochemical screening: Preliminary phytochemical screening of *Abrus precatorius* Linn. seeds was done. The extract showed presence of various chemical constituents such as proteins, amino acids, carbohydrates, sterols, tannins, Polyphenolic compounds, triterpenoid and steroid as shown in Table 1.

The qualitative *in vitro* antioxidant activity of ethanolic extract of *Abrus precatorius* Linn. was carried out by 1,1-diphenyl-2-picrylhydrazyl method. The extract showed change in the colour from purple to yellow on the TLC plate and ethanolic extract showed the maximum change in the colour.

Table 1: Phytochemical investigation of Ethanolic extracts of *Abrus precatorius* seeds

Phytochemical constituents	Ethanolic extract of *Abrus precatorius* seeds
Carbohydrates	+
Alkaloids	++
Steroids and sterols	+
Glycosides	-
Triterpenoids	+++
Flavanoids	++
Tannins and phenolic compound	+
Proteins and Amino acids	+
Fixed oil	+
Anthraquinone	-

Phytochemical screening of *Abrus precatorius* Linn. extract. + Indicates the Presence of compound, - indicates the absence of compound, ++ and +++ indicates maximum presence of compound

Table 2: Percentage scavenging activity of DPPH radical by Ethanolic extract of *Abrus precatorius* Linn

	Percentage scavenging of DPPH radical	
Conc. of extract (µg mL^{-1})	Ethanolic extract	Ascorbic acid
100	54.4±0.36	60.020±0.56
200	62.4±0.42	70.034±0.46
300	80.1±0.34	82.020±0.64

Values are the average of triplicate experiments and represented as Mean±S.E.M. The ethanolic extract shows maximum antioxidant activity (80.1±0.34%) at concentration 300 µg mL^{-1}as compared to standard (Ascorbic acid)

Table 3: Effect of EEAB (Ethanolic extract of *Abrus precatorius* Linn. on carrageenan induced paw edema) in rats

		Mean paw volume (mL)			
Treatment groups	Dose (mg kg^{-1}) Orally	1 h	2 h	3 h	Percentage inhibition of edema
Control	0.5%	0.52±0.003^{a}	0.60±0.002^{a}	0.67±0.001	-
Diclofenac	10	0.40±0.002	0.30±0.004^{a}	0.20±0.003^{a}	70
EEAB	125	0.48±0.002	0.56±0.005^{a}	0.55±0.006^{b}	17
EEAB	250	0.45±0.009^{a,b}	0.43±0.006^{b}	0.40±0.013^{a,b}	40.29
EEAB	375	0.44±0.007^{b}	0.42±0.015^{a,b}	0.25±0.008^{b}	62.68

EEAB abbreviate ethanolic extract of *Abrus precatorius* Linn. The values are Mean±SEM of animals. ^{a}p<0.05 compared with disease control group, ^{b}p<0.05 compared with disease control group

The quantitative antioxidant activity was evaluated for ethanolic extract of *Abrus precatorius* Linn. Seeds. The extract showed maximum antioxidant activity i.e., 80.1±0.34% at 300 µg mL^{-1} as compared to standard (ascorbic acid) as shown in Table 2.

Anti-inflammatory activity was evaluated by Carrageenan-induced rat paw edema model. The extract exhibited statistically significant inhibition of paw volume i.e., 17, 40.29 and 62.68% at dose levels of 125, 275 and 375 mg kg^{-1} as compared to diclofenac sodium (standard) as shown in Table 3.

Further, the extract showed dose dependent analgesic activity tail immersion and Tail flick method against conduction of heat induced analgesia in mice at 200, 300 mg kg^{-1} doses. The extract showed significant analgesic activity i.e., 6.52±0.23% at 90 min interval at concentration 300 mg kg^{-1} as compared to control group by tail immersion method as shown in Table 4. Ethanolic extract showed maximum analgesic effect i.e., 5.05±0.08% observed was at 90 min at 300 mg kg^{-1} doses by Tail flick method as compared to control group shown in Table 5.

Table 4: Analgesic activity by tail immersion method

		Tail immersion latency (time in sec)				
Treatment groups	Dose (mg kg^{-1})	0	30	60	90	120
Control	2% w/v	2.65±0.01[a]	2.82±0.23[a]	2.47±0.14	2.51±0.04	2.67±0.14[a,b]
Aspirin	100	2.54±0.03	5.63±0.13[a,b]	7.32±0.03	9.85±0.04	8.32±0.01
EEAB	100	2.47±0.01	2.91±0.04	3.82±0.12[b]	4.73±0.05	4.12±0.10[b]
EEAB	200	2.74±0.03	3.43±0.15	5.26±0.09[b]	6.52±0.23[b]	5.52±0.30
EEAB	300	2.86±0.15	4.31±0.05	6.34±0.01	8.67±0.09	6.64±0.06

EEAB abbrebiate ethanolic extract of *Abrus precatorius* linn. Analgesic effect of the ethanolic extract of *Abrus precatorius* Linn. seeds by tail immersion test. Values are Mean±SEM of 6 animals in each group, [a]p<0.05 vs. control, [b]p<0.05 vs. Aspirin

Table 5: Analgesic activity by tail flick method

		Tail flick latency time (sec)				
Treatment group	Dose mg kg^{-1}	0	30	60	90	120
Control	0.2%	1.97±0.03	2.44±0.08[b]	2.43±0.07	2.37±0.08[b]	2.35±0.06[b]
Aspirin	100	2.15±0.12[a,b]	4.76±0.10[a]	6.13±0.05	9.16±0.06	7.20±0.01
EEAB	100	2.04±0.01	2.56±0.01	2.75±0.04	3.50±0.11[a]	3.41±0.08[a]
EEAB	200	2.06±0.02	2.88±0.06[b]	3.01±0.10[a]	5.05±0.08[a]	3.70±0.07[a]
EEAB	300	2.03±0.04	3.80±0.12[a,b]	5.71±0.08[a]	8.01±0.05	6.80±0.09[b]

EEAB abbreviate ethanolic extract of *Abrus precatorius* linn. Analgesic effect of the ethanolic extract of *Abrus precatorius* Linn. seeds by tail flick test. Values are Mean±SEM of 6 animals in each group, [a]p<0.05 vs. control, [b]p<0.05 vs. Aspirin

DISCUSSION

The ethanolic extract of *Abrus precatorius* Linn. (EEAB) was evaluated for its *in vitro* antioxidant activity which exhibited significant scavenging potential. There is no reported study related to analgesic, anti-inflammatory and antioxidant activity on seeds of *Abrus precatorius* Linn. which gave basis to this present research.

Antioxidants are used to treat free radical produced disease. Antiradical activity assay is based on the reduction of 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH). Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm (Lata and Ahuja, 2003). The ethanolic extract of *Abrus precatorius* Linn. showed maximum antioxidant activity i.e. (80.1%). The other plants of leguminosae family *Acacia confuse* (bark, heartwood) and *Carob* (Leaves) has been reported for antioxidant activity (Chang *et al.*, 2001; Nagib *et al.*, 2010).

Further the extract was evaluated for its anti-inflammatory and analgesic potential. Anti-inflammatory activity was evaluated by carrageenan induced rat paw edema. Carrageenan is a strong chemical for the release of inflammatory and pro-inflammatory mediators (Crunkhorn and Meacock, 1971) and also several ROS are released during such inflammation (Koblyakov, 2001). Inflammation is believed to be biphasic of which the first phase is mediated by release of histamine and serotonin in the early phase followed by kinin release and then prostaglandin in the later phase (Garg and Paliwal, 2011). The extract showed significant anti-inflammatory activity i.e., 62.68%. Similar studies conducted on the isolated triterpenoid saponins obtained from aerial parts of *Abrus precatorius* shows potent anti-inflammatory activity (Anam, 2001). Anti-infertility activity has been done on seeds of *Abrus precatorius* (Sarwat *et al.*, 2009). However, free radical scavenging activity can be responsible for the reduction of inflammation in the Carrageenan induced paw edema in rats (Cuzzocrea *et al.*, 2001).

Furthermore, Analgesic activity was evaluated by tail flick and tail immersion method. *Abrus precatorius* Linn. ethanolic seed extract showed significant analgesic activity which was evidenced of increase in tail withdrawal latency as compared to the control group. The other plant of family Mimosaceae, *Albizia lebbeck* has been reported for analgesic activity (Saha and Ahmed, 2009). Drugs that are currently used for the management of pain are opioids or nonopioids Pain induced by local release or formation of various autacoids. Thermal nociceptive tests are more sensitive to opioid μ receptors and nonthermal tests are to opioid Kappa receptors (Paul *et al.*, 2007). Aspirin is used as a standard drug in both the methods and its ability to suppress the production of prostaglandins and throm boxanes is due to its irreversible inactivation of the cyclooxygenase (COX) enzyme (Sachdev *et al.*, 2011). The ethanolic seed extract shows optimum antioxidant, anti-inflammatory and analgesic activity and it is responsible to cure of all health ailments.

CONCLUSION

As ethanolic extract of *Abrus precatorius Linn.* was found to have potent antioxidant, anti-inflammatory and analgesic potential. The present study conclude that seeds of *Abrus precatorius* Linn. can be used as good natural antioxidant to treat free radical induced disease.

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