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Evaluation of Crude Ethanolic Extract of *Qurcus incana* Fruit for Analgesic and Gastrointestinal Motility Profile

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Abstract: The crude ethanolic extract of *Quercus incana* fruit was tested for its gastrointestinal and analgesic effect. The analgesic effect was assessed in mice, using acetic acid induced writhing test at a dose of 50, 100 and 200 mg/kg; and the effect on GIT motility was tested in mice using charcoal as a marker, as due to its black color the distance traveled in intestine was easy to observe. The plant showed dose dependent analgesic effect in compression with standard drug (diclofenac sodium 10 mg/kg); the plant extracts significantly ($P<0.05$) inhibited the writhing at 100 and 200 mg/kg, while the analgesic effect was non-significant at the dose of 50 mg/kg. The analgesic effect of the standard drug was higher than the extract. The ethanolic extract of the plant decreased the intestinal motility at dose dependent manner and produced a significant effect at 100 and 200 mg/kg. It was concluded that the ethanolic extracts should be used in high dose for analgesic effect and can also be used as anti-diarrheal in higher doses.

Key words: *Quercus incana* · Analgesic and Gastrointestinal motility

INTRODUCTION

It is reported that different plant extracts are used in different diseases [1-3] and especially in gastrointestinal transit disorders [4-6]. *Quercus incana*, Rox is a species of oak commonly known as bluejack oak or cinnamon oak. It belongs to the plant family Fagaceae. A large evergreen tree, bark dark gray; male spikes slender, drooping; female flower axillary, sessile; fruit nut [7]. The wood is employed for building and especially for making agricultural implements such as plough, handles and beams. The branches are used in roof making. It is also used as a fuel for making fire and charcoal. The acorns are given as a diuretic, also used as astringent in indigestion, used in asthma, the decoction of the bark is used in diarrhea and dysentery [8]. Before being administered, they are usually buried in the earth to remove their bitter principle. It is found in Balakot, Sangar, Hangrai, Kawai, Paras, Manur, Kaghan [9]. It is also found in Dir and is locally known as Serie. The leaves contain phenolics [1], condensed tannins [2] proanthocyanidins [3] and flavan-4-ols [4, 10]. In this study we tested the crude ethanolic extract of the fruit of *Quercus incana* for its analgesic and GIT motility effect.

MATERIALS AND METHODS

Plant Material: Fresh fruit of the plant *Quercus incana* was collected from Dir, Pakistan in June 2011. After the botanical authentication, the plant material was washed under running tap water to remove adhering dust. The plant was air dried under shade and powdered into small frictions. The resulting powder material was subjected to extraction with ethanol as reported earlier [11, 12].

Preparation of Extracts: The plant's ethanolic extract was prepared using the well recommended methods [13, 14]. About 100 g of powdered material of the plant was taken in a large, clean beaker and soaked in 500ml of 90%

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ethanol. The beaker was sealed and kept for a period of one week accompanying occasional shaking and stirring. The solution was then filtered through muslin cloth. The filtrate (ethanol extract) obtained was evaporated by using rotary evaporator. The gummy brown crude extract was obtained which was screened for their pharmacological studies.

Chemicals: Diclofenac sodium, castor oil, activated charcoal, acetic acid and sterile normal saline was used in all the experiments as control while extracts were prepared in normal saline.

Animals: Albino mice of either sex (18 to 22 g) were used. Animals were purchased from the Pharmacology Section of the Department of Pharmacy, University of Peshawar. The animals were maintained in standard Laboratory conditions (25°C and light/dark cycles that is 12/12 hr and were fed with standard food and water. The experimental protocol was recommended from the university ethical committee (ECUOP) following the well established procedures.

Acetic Acid Induced Writhing Test: All animal (18 to 22 g) were fasted for 2 hr before starting experiment. Animals were divided into five groups. Group I was injected with normal saline (10 ml/kg i.p) as control while Group II was injected with standard drug diclofenac sodium (10 mg/ kg i.p) and the remaining three groups were injected with 50, 100 and 200 mg/kg i.p. of ethanolic extract. After 30 min of saline, diclofenac sodium and plant extract injection, the animals were treated i.p. with 1% acetic acid. The writhing was counted after 5 min of acetic acid injection. The number of abdominal constrictions (writhing) was counted for 10 min [15, 16].

The percent analgesia was calculated using the following formula:

Percent analgesia = 100 - Number of writhing in tested animals/ number of writhing in control animals ×100.

Gastrointestinal Motility Test: For this purpose, the mice of either sex (25 to 35 g) were fasted 18 to 24 hr before starting the experiment. Animals were divided in five groups each of six animals. First group were given normal saline (10 ml/kg) i.p, Group II were treated with castor oil (0.1 ml/kg) as standard drug, remaining three groups were treated with ethanolic plant extract (50, 100 and 200 mg/kg i.p), after 30 min of injecting saline, castor oil and extract 10% charcoal suspension in 5% gum acacia was administer (5 mg/kg p.o). After 15 min of administering charcoal, the animal was killed by cervical dislocation and dissected out. The dissected animals were placed on clean surface and the distance travelled by charcoal was measured. Then GIT motility was calculated for all groups [17]. The percent motility was calculated using the following formula:

Percent Motility = 100 - Distance covered / total length of intestine × 100.

Statistical Analysis: The results were articulated as mean ± SEM of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnetts test for multiple comparisons. Effects were considered to be significant at the $P < 0.05$ level.

RESULTS

The analgesic effect of the ethanolic extract was tested in mice of either sex in the dose of 50, 100 and 200 mg/kg body weight. The dose dependent analgesia was noticed, the percent analgesia was 17.40, 26, 43 and 17 for 50, 100, 200 and diclofenac sodium respectively (Table 1). The plant extract decreased the intestinal motility in dose dependent manner. The percent motility is shown in Table 2. The highest activity was observed at 200 mg/kg in comparison with castor oil.

Values (mean ± SEM) present the writhing after treatment with diclofanec sodium (10mg/kg and 50mg/kg) and extract (50, 100 and 200 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P<0.01.

Table 1: Analgesic activity of the fruit of *Quercus incana*

Treatment	Dose	No, of writhing (mean)
Control	10ml/kg	65±3.46
Diclofenace sodium	10mg/kg	17.6**±1.14
Ethanolic extract	50mg/kg	43.2±1.48
	100 mg/kg	26.6±1.95
	200 mg/kg	17.4±2.7

Table 2: GIT motility activity the fruit of *Quercus incana*

Treatment	Dose	Total length of intestine	Distance covered by charcoal
Control	10ml/kg	59.33±0.07	15.17±0.27
Castor oil	10mg/kg	55.75±0.10	33.25**±0.17
	50mg/kg	53.6±2.07	42.67±0.58*
Ethanolic extract	50 mg/kg	45.57±0.58	14.8±4.15
	100 mg/kg	47±2.65	8.2±6.76
	200mg/kg	47±2.65	4.8±6.38

DISCUSSION

The analgesic effects of the crude ethanolic extract of fruit of *Quercus incana* were investigated in this study. Acetic acid writhing test was used for the analgesic effect, because of its sensitivity that could give different grades of injurious stimuli in chemically induced tissue damage [18]. Similarly, the acetic acid induced writhing has been used to evaluate analgesic effects of drugs and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways [19]. The acetic acid induced writhing allows the acid to act via central mechanisms and motor performance of the animal. Therefore, the crude ethanolic extract of *Quercus incana* has a significant inhibition in the duration of the writhing in each mouse [20, 21]. The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins. Increase level of protanoids as well as lipoxygenase products have been found in the peritoneal fluid after the injection of the acetic acid. The analgesic effect of any plant extract may therefore be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful message [22].

The charcoal meal paradimn is one of the well established methods for finding the GIT motility in animal models [23, 24]. The significant GIT motility was decreased with increasing the dose of the plant extract. The plant is good constipating as it decreased the intestinal motility. In conclusion the crude extract from the fruit of this plant can be used as an analgesic and anti-diarrheal in traditional medicine and the isolation of such compounds can help in management of pain and constipation.

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