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RESEARCH ARTICLE

Neuropharmacological Effect of the Rhizome of *Drynaria quercifolia* in Mice

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ABSTRACT

The neuropharmacological effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of *Drynaria quercifolia* J. Smith were studied in mice by intraperitoneal administration. The tests used were determination of effect on duration of diazepam-induced sleep, determination of effect on nikethamide-induced toxicity, light-dark test and force swimming test. The duration of diazepam-induced sleep was extended by administration of these fractions. Nikethamide at high dose cause death of mice and time to cause death of mice was delayed by administration of these fractions. In light-dark test and force swimming test these fractions were given diazepam type effect. The changes in doses (50-200 mg/kg) showed changes in efficiency of these effects. These results suggest that both these fractions of *D. quercifolia* rhizome have dose dependent depressant effect.

Keywords: *Drynaria quercifolia, Rhizome, CNS depression, Diazepam, Nikethamide*

Drynaria quercifolia J. Smith (syn. *Polypodium quercifolium*, Fam. Polypodiaceae), locally known as Gurar, is a parasitic fern [1, 2] that is widely distributed in Bangladesh, India and Thailand [2-4]. The rhizomes of the plant are used traditionally for the treatment of cough, and fever [1, 2]. The rhizomes also have antibacterial properties [1]. In different region of Lakshmipur district of Bangladesh, the rhizome of this plant is used by local people in the treatment of excited mental disorder (information gathered from local herbalist Mr. Monto herbalist, Lakshmipur, Bangladesh). By mixing with the plant *Asparagus racemosus* (Liliaceae) when applied on the head, calm the head and reduce hair loss [3].

ASEAN Centre for Biodiversity mentioned in their Checklist of Medicinal Plant in Southeast Asia that rhizome decoction or drink of D. quercifolia rhizome uses as antipyretic preparation [4]. Although the plant is widely used for remission of several ailments related to central nervous system, its CNS potential has not been explored yet. Therefore, in the present study an attempt was made to establish the CNS effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of D. quercifolia.

Drugs acting in the central nervous system are still the most widely used group of pharmacological agents [5]. Commonly used CNS depressants are barbiturates (e.g. secobarbital, pentobarbital, phentobarbital, thiopental etc), benzodiazepines (e.g. diazepam, flurazepam, temazepam etc) and ethanol. Both barbiturates and benzodiazepines give their CNS effect by interaction with postsynaptic gamma amino butyric acid receptor ($GABA_A$ receptor) [6]. The most serious drawback of barbiturates as depressant related to their narrow margin of safety and only 10 times of their therapeutic dose may be lethal [7]. Moreover barbiturates can produce both psychological and physiological dependence [8, 9]. Benzodiazepines are the most commonly used CNS depressant lead to tolerance and physical dependence for example diazepam typically produce sedation at dose of 5 to 10 mg in a first-time user, but those who repeatedly use it may become tolerant to a doses of several hundreds of mg [10]. Ethanol gives its depressant action by changing membrane fluidity and interaction with the GABA system [7, 11], also give tolerance and physical dependence effect [10]. Alcohol addiction in American society is 5% to 10% for men and 3% to 5% for women [12]. A natural CNS depressant with reduced or no toxicity is therefore, essential. As rhizome of *D. quercifolia* is an old medicaments used in ailments related CNS [3], so it will be a cost effective alternative approach to study this plant for the evaluation of its CNS potential.

MATERIALS AND METHODS

Plant materials

The rhizome of the plant was collected from various part of Lakshmipur district of Bangladesh and identified by Prof. A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh where its voucher specimen (No. 1939) was deposited. The rhizome were cut, air-dried and ground into powder.

Preparation of petroleum ether and ethyl acetate fractions of ethanol extract

The powder materials (600 g) were extracted with ethanol (3 L) in a Soxhlet apparatus (Quickfit, England) at 65^0 C for 72 h [13]. The extract was filtered through filter paper. The filtrate was concentrated under reduced pressure at 50°C in a rotary vacuum evaporator to afford a blackish green mass (25.4 g). This green mass was further extracted with petroleum ether (3 x 50 ml), ethyl acetate (3 x 50 ml) and methanol (3 x 50 ml) and dried under reduce pressure to afford petroleum ether (15 g), ethyl acetate (5.5 g) and methanol fractions (0.5 g), respectively [14, 15]. Because of lack in amount of methanol fraction, experiments with this fraction were avoided.

Preparation of sample solutions

The working solutions of different fractions of plant extract were prepared by dispersing each dried fraction of plant extract in distilled water with few drops of Tween 80. Preparation of solution was made in such a way so that each 0.2 ml solution contain 1.107 mg dried fraction of plant extract (used to maintain the dose 50 mg/kg body weight). Solution of another concentration also prepared in such a way so that each 0.2 ml solution contain 2.213 mg dried fraction of plant extract (used to maintain the dose 100 mg/kg body weight). To maintain the dose 200 mg/kg body weight 0.4 ml of second solution was used. These solutions were kept frozen until use [16].

Standards and their solutions

Diazepam as Sedil injection (contain 10 mg diazepam) was collected from local market of Square Pharmaceutical Ltd, Bangladesh to use as known central nervous system (CNS) depressant. Its solution was prepared by dilution in distilled water. Dilution was made in such a way so that each 0.2 ml solution contains 0.12 mg diazepam (used to maintain the dose 5 mg/kg body weight) and 0.045 mg diazepam (used to maintain the dose 2 mg/kg body weight).

Nikethamide as Nikethamide injection (contain 500 mg nikethamide) was collected from local market of Jayson Pharmaceutical Ltd, Bangladesh to use as known CNS stimulant. Its solution was prepared by dilution in distilled water. Dilution was made in such a way so that each 0.2 ml solution contains 6.7 mg nikethamide (used to maintain the dose 300 mg/kg body weight) and 0.45 mg nikethamide (used to maintain the dose 20 mg/kg body weight). All these solutions were kept frozen until use [16].

Animals

The experiment was carried out on Albino mice (Swiss strain). They were 2-3 months old of both sexes weighing between 20 -27 g (average weight was 22.13 g). They were collected from the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B). The animals were housed in iron cages (considering group) under temperature and light controlled condition [17]. They were fed a balanced diet [18] and tap water. The animals were maintained in this condition for 25 days before experiment to adjust with food and environment. Food and water were withdrawn 2 h prior to the experiment [19]. Number of mice in each group was six and for all administration intraperitoneal route was used.

Acute toxicity study

Acute toxicity study was carried out by using graded doses of each fraction in albino mice. Both petroleum ether and ethyl acetate fractions were administered intraperitoneally in graded doses (200 to 1000 mg/kg body weight). They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality [20].

Treatment protocol to determine effect on duration of diazepam-induced sleep

Total eight groups of animals were used for this test. The animals were subjected to pretreatment and treatment [21, 22]. Pretreatment was carried out 30 min prior to treatment [21]. In pretreatment 0.2 ml distilled water was given to each animal of a control group and 0.4 ml distilled water was administered to each animal of another control group. For each fraction, sample solutions were given to animals of three remaining groups with doses 50, 100, 200 mg/kg body weight, respectively (Table 1).

In treatment, 0.2 ml diazepam solution having dose 5 mg/kg body weight was given to each animal of all groups. Each mouse was observed and duration of sleep was recorded. Sleeping time in all cases was measured as the time interval between the loss and regaining of righting reflex [21].

Treatment protocol to determine effect on nikethamide-induced toxicity

Total eight groups of animals were used for this test. The animals were subjected to pretreatment and treatment [21, 23]. Pretreatment was carried out 30 min prior to treatment [21]. In pretreatment 0.2 ml distilled water was administered to each animal of a control group and each animal of remaining control group was administered with 0.4 ml distilled water. For each fraction, sample solutions were given to animals of three remaining groups with doses 50, 100, 200 mg/kg body weight, respectively (Table 2).

In treatment, 0.2 ml nikethamide solution having dose 300 mg/kg body weight was given to each animal of all groups. Each mouse was observed and time to cause death was recorded. Time to cause death in all

Table 1. Effect of petroleum ether and ethyl acetate fractions of ethanol extract of the rhizome of *D. quercifolia* on duration of diazepam-induced sleep of mice

Pretreatment	Treatment	Duration of sleep (Min) Mean ± S.E.M.
Distilled water 0.2 ml	Diazepam 5 mg/kg	56.42 ± 1.56
Distilled water 0.4 ml	Diazepam 5 mg/kg	55.54 ± 0.29
Petroleum ether fraction 50 mg/kg	Diazepam 5 mg/kg	63.52 ± 1.47
Petroleum ether fraction 100 mg/kg	Diazepam 5 mg/kg	77.24 ± 0.38*
Petroleum ether fraction 200 mg/kg	Diazepam 5 mg/kg	85.40 ± 1.27*
Ethyl acetate fraction 50 mg/kg	Diazepam 5 mg/kg	61.15 ± 2.26
Ethyl acetate fraction 100 mg/kg	Diazepam 5 mg/kg	73.20 ± 1.51*
Ethyl acetate fraction 200 mg/kg	Diazepam 5 mg/kg	81.32 ± 0.47*

*$p < 0.05$ significant compared to control (solvent).

Table 2. Effect of petroleum ether and ethyl acetate fractions of ethanol extract of the rhizome of *D. quercifolia* on nikethamide-induced toxicity of mice

Pretreatment	Treatment	Latency of Death (Min) Mean ± S.E.M.
Distilled water 0.2 ml	Nikethamide 300 mg/kg	48.43 ± 2.02
Distilled water 0.4 ml	Nikethamide 300 mg/kg	49.12 ± 1.38
Petroleum ether fraction 50 mg/kg	Nikethamide 300 mg/kg	55.21 ± 1.45
Petroleum ether fraction 100 mg/kg	Nikethamide 300 mg/kg	63.20 ± 2.20*
Petroleum ether fraction 200 mg/kg	Nikethamide 300 mg/kg	69.27 ± 1.46*
Ethyl acetate fraction 50 mg/kg	Nikethamide 300 mg/kg	53.31 ± 0.48
Ethyl acetate fraction 100 mg/kg	Nikethamide 300 mg/kg	61.00 ± 1.32*
Ethyl acetate fraction 200 mg/kg	Nikethamide 300 mg/kg	65.15 ± 1.40*

*$p < 0.05$ significant compared to control (solvent)

cases, was measured as the time interval between the treatment and death [16, 23].

Treatment protocol for light-dark test

Crawley and Goodwin's method was used in this determination [24]. Total nine groups of animals were used for this test. Each animal of control group 0.2 ml distilled water, each animal of diazepam group 0.2 ml diazepam solution having dose 2 mg/kg body and each animal of nikethamide group 0.2 ml nikethamide solution having dose 20 mg/kg body weight were given. For each fraction, sample solutions were given to animals of three remaining groups with doses 50, 100, 200 mg/kg body weight, respectively (Fig. 1).

The apparatus used consisted of a Plexiglas box with two compartments (20 x 20 cm each). One of them was illuminated with white light and other stayed in dark [24]. After 1 h of drug administration, each animal was placed at the center of the illuminated compartment, facing toward dark area. The time spent in the illuminated and dark area was recorded for 5 min [24].

Treatment protocol for force swimming test

The experiment was carried out using Porsolt *et al.* method [25]. Total nine groups of animals were used for this test. Each animal of control group 0.2 ml distilled water, each animal of diazepam group 0.2 ml diazepam solution having dose 2 mg/kg body and each animal of

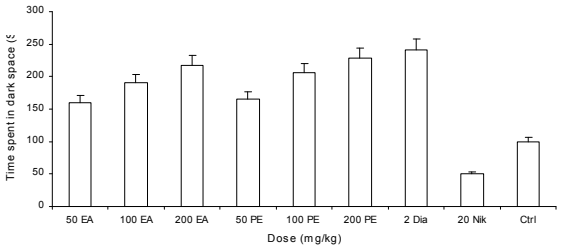

Fig 1. Effect of petroleum ether fraction, ethyl acetate fraction, diazepam and nikethamide in light-dark test. Mean ± S.E.M. of each group expressed in fig. PE = petroleum ether fraction, EA = ethyl acetate fraction, Dia = diazepam, Nik = nikethamide and ctrl = control. * $p < 0.05$ significant compared to control (solvent). Like diazepam group, mice administered with ethyl acetate and petroleum ether fractions spent most of their time in dark place and their living tendency increase with increasing dose of both ethyl acetate and petroleum ether fractions. Nikethamide group mice spent less time in dark place indicating more time spent under light.

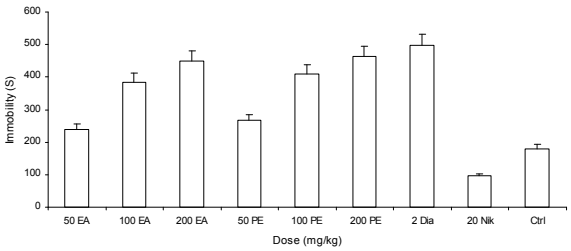

Fig 2. Effect of petroleum ether fraction, ethyl acetate fraction, diazepam and nikethamide in force swimming test. Mean ± S.E.M. of each group expressed in fig. PE = petroleum ether fraction, EA = ethyl acetate fraction, Dia = diazepam, Nik = nikethamide and ctrl = control. * $p < 0.05$ significant compared to control (solvent). Like diazepam group, mice administered with petroleum ether and ethyl acetate fractions showed more immobility (i.e. reduced swimming tendency) and their swimming tendency more reduced (i.e. immobility increase) by increasing dose of fractions. Nikethamide group mice showed more swimming tendency.

nikethamide group 0.2 ml nikethamide solution having dose 20 mg/kg body weight were given. For each fraction, sample solutions were given to animals of three remaining groups with doses 50, 100, 200 mg/kg body weight, respectively (Fig. 2).

The apparatus used consisted of a clear Plexiglas cylinder (20 cm high x 12 cm diameter) filled to a 15 cm depth with water (24 ± 1° C) [25]. After 1 h of drug administration, every animal was placed individually into the cylinder for 15 min and observed for climbing behavior (upward movement along the side of the swim chamber), swimming behavior (movement through out the swimming chamber) and immobility (by keeping head above water mice made no further attempts to escape) [25].

Statistical analysis

The results were presented as mean ± standard error mean (Mean ± SEM) where n = 6. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test. The analysis was performed using SAS statistical software. $p<0.05$ was considered to be statistically significant.

RESULTS

Acute toxicity study

In acute toxicity study, both petroleum ether and ethyl acetate fractions were found to be safe and no mortality was observed to a dose as high as 800 mg/kg.

Effect on diazepam-induced sleep

At doses 50, 100 and 200 mg/kg body weight, both petroleum ether and ethyl acetate fractions were extended the duration of diazepam-induced sleep and it was also observed that for higher doses the duration of sleep was more extended (Table 1). These indicate dose dependent CNS depressant effect of the rhizome of *D. quercifolia*.

Effect on nikethamide-induced toxicity

At doses 50, 100 and 200 mg/kg body weight, both petroleum ether and ethyl acetate fractions were delayed the latency of death caused by nikethamide toxicity and it was also observed that for higher doses the latency of death were more delayed (Table 2). These indicate dose dependent CNS depressant effect of the rhizome of *D. quercifolia* interfere with CNS stimulant effect of nikethamide and made delay to cause death.

Effect of light-dark test

Mice administered with nikethamide spent most of their time in light illuminated space. Mice administered with petroleum ether fraction, ethyl acetate fraction and diazepam were moved toward dark space and spent most of their time in this space (Fig. 1). These indicate the diazepam type effect of petroleum ether and ethyl acetate fractions of ethanol extract of the plant.

Result of force swimming test

Decreased times of immobility were observed for mice administered with nikethamide. Increased times of immobility were observed for remaining mice (Fig. 2). These indicate the diazepam type effect of petroleum ether and ethyl acetate fractions of ethanol extract of the plant.

DISCUSSION

The acute toxicity result reveals that this plant might be considered as a broad non-toxic one. The data presented in table 1, table 2, fig 1 and fig 2 were suggested that both petroleum ether and ethyl acetate fractions of ethanol extract of rhizome of *D. quercifolia* (Syn. *Polypodium quecifolium*) contain psychoactive substances, which are CNS depressant in nature. In all experiment it was observed that petroleum ether fraction is more active than ethyl acetate fraction. At same doses petroleum ether fraction more extended the duration of

diazepam-induced sleep and latency of death caused by nikethamide toxicity. In light-dark test, it was also observed that for similar doses mice administered with pet ether fraction spent more time in dark area than mice administered with ethyl acetate fraction. In force swimming test, mice administered with petroleum ether fraction were showed more immobility than mice administered with ethyl acetate fraction. It was also reported by Raintree Nutrition in their Samambaia Plant Database file that related species *Polypodium decumanum* in CNS induce hypokinesia, decrease cytokines IL-1beta and IL-2 in frontoparietal cortices [26]. The results of Raintree Nutrition indicate toward the CNS depression effect of *Polypodium decumanum*. Isolation of naringin from rhizome of *D. quercifolia* [27] and CNS depressant effect of naringin on mice [28] was reported.

Therefore, the ethnopharmacological report as well as our experimental pharmacological data supports the traditional use of this plant as CNS depressant. However, to know the exact mechanism of action of *D. quercifolia* rhizome extract further study with purified fractions/bioactive compounds are warranted.

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