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Original article

Potential antidepressant-like activity of silymarin in the acute restraint stress in mice: Modulation of corticosterone and oxidative stress response in cerebral cortex and hippocampus

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ABSTRACT

Background: Silymarin is a polyphenolic flavanoid of Silybum marianum, elicited neuroprotection and antidepressant like activity in stressed model. It was found to increase 5-hydroxytryptamine (5-HT) levels in the cortex and dopamine (DA) and norepinephrine (NE) in the cerebellum in normal mice. The aim of the present study was to investigate the potential antidepressant-like activity of silymarin in the acute restraint stress (ARS) in mice.
Methods: The ARS was induced by immobilizing the mice for a period of 7 h using rodent restraint device preventing them for any physical movement. One hour prior to ARS, silymarin was administered at doses of 100 mg/kg and 200 mg/kg per oral to non stressed and ARS mice. Various behavioral parameters like immobility time in force swim test, locomotor activity in open field test, and biochemical alterations, serum corticosterone, 5-HT, DA, NE level, malondialdehyde (MDA), and antioxidant enzymes (GSH, CAT and SOD) in hippocampus and cerebral cortex in non stressed and ARS subjected mice were investigated.
Results: Experimental findings reveals mice subjected to ARS exhibited significant increase immobility time, serum corticosterone, MDA formation and impaired SOD and CAT activities in hippocampus and cerebral cortex as compared to non stressed mice. Silymarin treatment (100 mg/kg and 200 mg/kg) significantly attenuated immobility time, corticosterone and restored the antioxidant enzymes after ARS.
Conclusion: The present experimental findings indicate that silymarin exhibits antidepressant like activity probably either through alleviating oxidative stress by modulation of corticosterone response, and antioxidant defense system in hippocampus and cerebral cortex in ARS mice.

Introduction

Depression is one of the most common neuropsychiatric disorders characterized by low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being. The appearance of these symptoms might be due to impaired monoamineric neurotransmission and/or oxidant-antioxidant system [1–3]. Stressful life experiences are considered to be the major culprit in the development of neuropsychiatric diseases [4] including depression. Various documented findings demonstrated the relationship between stressful life, and subsequent depressive like symptoms [5,6]. Further, consequences of production of reactive oxygen species (ROS) due to oxidative stress and impaired endogenous antioxidant system leads to depressive like behavior [7,8]. Continue stressful situation can be responsible for activation of hypothalamic-pituitary-adrenal (HPA) axis which subsequently results into the development of anxiety and depression. The stimuli for activation of the HPA axis is hypothalamic corticotrophin releasing factor (CRF) which results into production of adrenocorticotrophic hormone (ACTH) from pituitary gland and subsequently release of cortisol and corticosterone from the adrenal cortex [9–11]. Numerous documented studies reveal that the rodents subjected to acute restraint stress induced significant depressive like symptoms [2,3].

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); ACTH, adrenocorticotrophic hormone; ARS, acute restraint stress; CAT, catalase; CRF, corticotrophin releasing factor; ELISA, enzyme-linked immunosorbent assay; FST, forced swimming test; GSH, reduced glutathione; HPA, Hypothalamic-pituitary-adrenal; MDA, malondialdehyde; PBS, phosphate buffer solution; SOD, superoxide dismutase.

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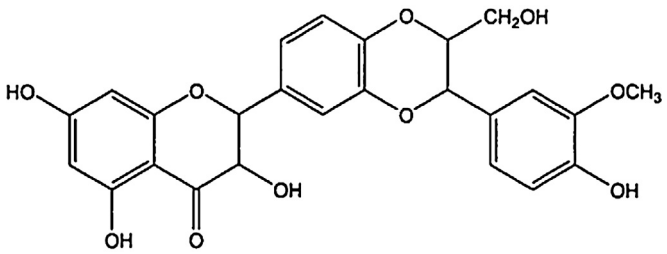

Fig. 1. Structure of silymarin.

Silymarin (Fig. 1) is a polyphenolic flavanoid of *Silybum marianum* used clinically in the management of hepatic disorders [12,13]. Silymarin crosses blood brain barrier [14], improves glutathione in brain due to oxidative stress, aged-related and pathological degenerative processes [15,16]. In our earlier experiments, silymarin exhibited improvement in behavioral, biochemical and histopathological alterations in both focal and global ischemic rats [17,18]. Furthermore, silymarin showed increase 5-hydroxy tryptamine or serotonin (5-HT) level in the cortex and increased DA and NE levels in the cerebellum [19]. In addition, silymarin (100 mg/kg) preserved dopaminergic neurons in the substantia nigra by 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine induced histopathological alterations. It also attenuated the rotational behavior in 6-hydroxydopamine lesioned rats and protected the neurons of substantia nigra pars compacta [20,21] partly through modulation of oxidative stress. Moreover, silymarin (50 and 100 mg/kg) doses significantly displayed antidepressant like activity probably mediated at least in part through nitric oxide system [22]. Although, silymarin improves neurotransmitters level, modulate oxidative stress in brain areas, its effects on depressive behavior are scientifically unknown. Hence, the present study was undertaken to investigate the potential antidepressant-like activity of silymarin and associated mechanism(s).

Materials and methods

Mice

Swiss albino mice weighing 30–35 g (70–80 days old) of either sex were procured from Serum Institute of India, Pune, India. Mice were housed separately in polycarbonate cages under standard laboratory conditions with Food and water *ad libitum*, and lights were on from 07:00 to 19:00 h. Experimental protocol was reviewed and approved by the institutional animal ethics committee (SIPS/IAEC/2014-15/01) and conformed to the Indian National Science Academy guidelines for the use and care of experimental animals in research.

Drugs and Chemicals

Silymarin was obtained as gift sample from Serum Institute of India, Pune, India. Fluoxetine was purchased from Sigma Co., St. Louis, USA; mouse corticosterone enzyme-linked immunosorbent assay (ELISA) kit was purchased from Arbor Assays, USA. The other reagents used were of analytical grade and procured from local suppliers.

Treatment schedule

The mice were divided in to various groups (n = 8) and they were treated as shown in Table 1.

The doses of silymarin as 100 and 200 mg/kg *per oral* (*po*) were selected based on findings of Khoshnoodi et al. [22], Muley et al. [17,18] and conducting pilot experiments (dose-effective relationship). The documented reports of Khoshnoodi et al. [22] and Sharma et al. [23] demonstrated silymarin at 50, 100, 250 mg/kg and at 250 and 300 mg/kg, exhibit significant antidepressant like activity in mice [22,23] and improves behavioral and biochemical alterations in focal ischemic rat model at 100 and 200 mg/kg dose level [17,18].

Table 1
Experimental groups and treatments in mice.

Groups	Mice condition as non stressed/ARS	Treatment and dose (mg/kg *po*)
I	Non-stressed	Vehicle (10 ml/kg)
II	Non-stressed	Fluoxetine (20)
III	Non-stressed	Silymarin (100)
IV	Non-stressed	Silymarin (200)
V	ARS	Vehicle (10 ml/kg)
VI	ARS	Fluoxetine (20)
VII	ARS	Silymarin (100)
VIII	ARS	Silymarin (200)

ARS—Acute restraint stress.

Silymarin was prepared in 1% (w/v) carboxy methyl cellulose, fluoxetine in normal saline solution. All the mice from groups (V–VIII) were subjected to acute restraint stress (ARS) procedure.

ARS procedure in mice

The mice were restraint for stress as per the procedure described by Freitas et al. [3] and Kumar and Goyal [24]. Briefly, the mice were immobilized for a period of 7 h using an individual rodent restraint device made of Plexiglas fenestrate, restraining all physical movement without causing pain. The silymarin (100 and 200 mg/kg) and fluoxetine (20 mg/kg) were administered *po* 1 h prior to ARS procedure. Forced swimming test and Open-field test were performed 8 hours 40 minutes after silymarin administration simultaneously group wise.

Behavioral evaluation

Forced swimming test (FST)

Mice were individually forced to swim in an open cylindrical container (10 cm × 25 cm), containing 19 cm of water (25 °C) and the immobility time was recorded, as per the method described previously [25,26]. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, producing only those movements necessary to keep its head above water.

Open-field test (OFT)

After the FST, the same mice were evaluated in the open-field paradigm as per the procedure previously described by Rodrigues et al. [27] and Freitas et al. [3] In this, the number of squares crossed with all paws (crossings) of mouse was counted and recorded in a 6 min session.

Biochemical investigation

Thirty minutes after the behavioral evaluation, blood was collected between 8.30-9.30 am from direct cardiac puncture and serum was separated and stored (−20 °C) until use. After blood withdrawal, mice were sacrificed by decapitation, the hippocampus and cerebral cortex were identified and isolated, washed with 10% cold sucrose solution and homogenized (1:10 w/v) in phosphate buffer solution (PBS). The tissue homogenates were centrifuged (REMI, USA) at 16,000 × *g*, at 4 °C for 15 min and resultant supernatants were used for neurochemical and biochemical analysis.