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Original Article

COMBINED EFFECT OF SELECTIVE BIOACTIVE COMPOUNDS FROM PLANT ORIGIN IN AN ANIMAL MODEL OF ANXIETY

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ABSTRACT

Objective: Evidence is emerging that specific combinations of bioactive compounds may be far more effective in protecting against several diseases as compared to the effect of a single compound. The present study was aimed to investigate the interactive effect of Diosgenin and Silymarin, the bioactive compounds from plant origin in an animal model of anxiety.

Methods: Albino wistar rats of either sex were divided into five groups and treated for 5 d. Group I and II served as control and standard and test groups were treated with Diosgenin (100 mg/kg, p. o.), Silymarin (100 mg/kg, p. o.) and combination of Diosgenin (50 mg/kg, p. o.)+Silymarin (50 mg/kg, p. o.), respectively. Diazepam (1 mg/kg, i. p.) was used as a standard for the study. Anxiolytic effects were studied in the Elevated plus-maze, Hole-board test and Light/Dark model.

Results: The results suggested that Diosgenin when given alone at a dose of 100 mg/kg, does not shown significant anxiolytic effect when as compared with control. Whereas, the compound Silymarin (100 mg/kg) shown significant anti-anxiety effect ($P<0.01$), independently. The same two bioactive compounds, given in combination at a dose of 50 mg/kg, (each), exhibited significant anxiolytic-like effect, potentially.

Conclusion: It can be concluded that Diosgenin has got a synergistic effect on anti-anxiety action when given in combination with Silymarin.

Keywords: Bioactive compounds, Anxiety, Diosgenin, Silymarin, Diazepam, Elevated plus-maze, Hole-board test, Light-Dark model

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INTRODUCTION

Anxiety is the most common neuropsychiatric disorder of this new era associated with disability and premature death [1] and is accompanied by overactivity of autonomic system leading to tachycardia, tremors, sweating, loss of confidence and so on [2]. Many numbers of anxiolytic drugs including benzodiazepines are now clinically available. However, these drugs have their limitations like amnesia, drug dependence, and sedation including cardiovascular toxicity, sexual dysfunction, weight gain and Drug-Drug interactions [3].

Many numbers of bioactive compounds from medicinal herbs may exhibit potent pharmacological actions and are found effective in the treatment of several disorders, traditionally. Flavonoids, alkaloids, steroids, terpenoids, saponins, and phenolics are examples of phytoconstituents and are present in abundant in a variety of plant components such as leaves, stems, roots, flowers, fruits and seeds. These bioactive compounds from medicinal plants, with significant potency and lesser side effects, are found to be an alternative therapeutic option to overcome the adverse effects of synthetic benzodiazepines. These compounds with varying chemical structures may act at different drug targets through multiple mechanisms and produces more beneficial effects as compared to the effect of single phyto-compound. They may act as multi-targeted drugs even at low doses, synergistically [4].

Diosgenin (DI), is a steroidal saponin present naturally in a variety of plants like fenugreek (*Trigonella foenum graecum*) and roots of wild yam (*Dioscorea villosa*) [5]. It has numerous biological effects such as anticancer, hypolipidemic, anti-oxidant and anti-inflammatory properties as well [6]. Similarly, Silymarin (SY), a flavanolignan from Milk thistle (*Silybum marianum*), is a polyphenolic flavonoid, comprised of seven flavanolignans namely Silibin A, Silibin B, Isosilibin A, Isosilibin B, Silichristin, Isosilichristin, Silidirmin with Silibinin being the main active ingredient. Silymarin also contains Taxifolin, the most effective antioxidant flavanoid [7]. Silymarin is used for liver diseases. It possesses anti-cancer and antiapoptotic effects along with renoprotective and cardioprotective effects. It is also a potent antioxidant agent that crosses the blood brain barrier and exerts a neuroprotective effect in various disorders like Parkinson's disease, stroke and ageing [8].

The present work was aimed to investigate the interactive effects of Diosgenin and Silymarin both individually and in combinations against anxiety using various experimental models in rats.

MATERIALS AND METHODS

Drugs and chemicals

Diosgenin and Silymarin were procured from Vital Herbs, New Delhi. Diazepam was obtained from Vulcan Laboratories Pvt Ltd, Kolkata. All other chemicals were of analytical grade and purchased commercially. Water used was double distilled throughout the experiment.

Dose selection of drugs

Diosgenin

Studies assessing steroidal saponins for toxicity have shown that they did not show any sign of toxicity up to an oral dose of 562.5 mg/kg. Therefore, in the present study, the dose of Diosgenin 50 and 100 mg/kg has been fixed. They were given by oral route using oral gavage [9].

Silymarin

Silymarin was found to be non-toxic and symptom-free with maximum doses of 2500 and 5000 mg/kg. It has also been demonstrated that it is not a teratogen and had no post-mortem toxicity [10].

Animals

Albino wistar rats of either sex, weighing between 150-200 g were used throughout the study. They were housed for at least one week in the laboratory room under standard environmental conditions of

temperature (25±2 ° C), humidity 30-70 % and 12 h light and 12 h dark cycles. The animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (SBCP/2019-20/CPCSEA/ IAEC/I(4)/F16/69).

Preparation of drug suspensions

Diosgenin and Silymarin suspensions were prepared in 0.5 % w/v carboxymethylcellulose (CMC) in distilled water prior to oral administration to animals [11]. Fresh preparations were used for each and every experiment.

Sample size and experimental groups

The tests for anxiety were done in 5 different groups (n=6/group) of rats. The vehicle and the three test groups were administered with 0.5 % CMC (10 ml/kg, p. o.), Diosgenin (100 mg/kg, p. o.), Siymarin (100 mg/kg, p. o.) and Diosgenin (50 mg/kg, p. o.)+Silymarin (50 mg/kg, p. o.), respectively, one hour prior to the experiment. The standard group was treated with Diazepam (1 mg/kg, i. p.) 30 min before the commencement of the experiment.

- Group I: Control: 0.5 % CMC (10 ml/kg, p. o.)
- Group II: Standard: Diazepam (1 mg/kg, i. p.)
- Group III: Test 1: Diosgenin (100 mg/kg, p. o.)
- Group IV: Test 2: Silymarin (100 mg/kg, p. o.)
- Group V: Test 3: Diosgenin (50 mg/kg, p. o.)+Silymarin (50 mg/kg, p. o.)

Experimental models

Elevated plus-maze model (EPM)

The apparatus consisted of two open arms (35 × 5 cm) crossed with two closed arms (35 × 5 × 20 cm) extended from a common central platform (5 × 5 cm), elevated to the height of 25 cm. The two arms of each type were opposite to each other. Each animal was placed at the centre of the maze facing one of the open arms. The number of open arm and closed arm entries plus the time spent in open and enclosed arms were recorded for 10 min of test period [12]. Entry into an arm was noted as the point when the animal places all the four paws within the arm. An increase in the proportion of time spent and the number of entries in the open arm is indicative of the anti-anxiety effect.

Hole-board test (HBT)

The apparatus consisted of a wooden box measured 40 × 40 × 20 cm with 16 holes, each of 3 cm in diameter that are evenly distributed on the box. The apparatus was elevated to a height of 25 cm. Each rat was placed at the centre of the box and allowed to move freely in the box for 5 min during which the number of head dips into the holes was recorded. Dipping the at least to the eye level was considered. However, these effects were reversed at high doses of diazepam, which induced sleep [13].

Light-dark model (LDM)

The light-dark box test apparatus consisted of a wooden box (44 cm length × width 21 cm × height 21 cm) with two compartments, one-third comprises dark area and two-third of the box makes the brightly illuminated white area. Rats were placed individually in the center of the illuminated bright compartment, facing the light area away from the dark section. The number of entries and time spent in light and dark box was observed for the next 10 min. Entrance into the lightbox was regarded as an index of less anxiety [14].

Statistical analysis

Data were calculated as mean±SEM values. One-way ANOVA followed by Dunnett's multiple comparison tests was performed using Graphpad Prism. P<0.05 was considered as significant with respect to control and Diazepam treatment (mentioned in the caption of results).

RESULTS

Elevated plus-maze model

The result in table 1 indicates that the rats treated with test drugs, Diosgenin (100 mg/kg, p. o.) and Silymarin (100 mg/kg, p. o.) shown an increase in the number of open arm entries (P<0.05 and P<0.001) and time spent in open arms, significantly, (P<0.01 and P<0.001) as compared against a control group of animals. They also showed a reduction in number of entries and time spent in closed arms. Group of rats treated with Diosgenin shown lesser effect than Silymarin treated animals. Eventually, the animals treated with a combined dose of Diosgenin and Silymarin (50 mg/kg, p. o., each) shown much more significant effect (P<0.0001), when compared to that elicited by either treatment alone. The standard drug, diazepam (1 mg/kg, i. p.) treated rats also shown an increase in the number of open arm entries and time spent in open arms, significantly, (P<0.0001).

Table 1: Combined effect of diosgenin and silymarin on elevated puls maze model

Groups	Mean no of entries		Mean time spent (s)	
	Open arm	Closed arm	Open arm	Closed arm
Control (Vehicle)	3.500±0.6191	8.833±0.5426	64.17±8.060	236.0±11.52
Diazepam (1 mg/Kg)	14.00±0.6831****	4.000±0.5774****	232.3±7.288****	88.33±2.603****
Diosgenin (100 mg/Kg)	7.333±1.430*	6.500±0.7638*	107.5±9.507**	205.7±4.077**
Silymarin (100 mg/Kg)	8.500±0.8851***	5.667±0.5578**	180.2±8.750***	155.0±3.376***
Diosgenin (50 mg/kg)+Silymarin (50 mg/kg)	10.50±1.088****	4.833±0.5426****	221.8±8.643****	109.3±2.603****

Data represent mean±SEM of six rats. Comparisons were made by using a one-way ANOVA followed by Dunnett's test as the post hoc. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared with control.

Hole-board test

In hole board test, the test doses of Diosgenin (100 mg/kg, p. o.) and Silymarin (100 mg/kg, p. o.) shown a significant increase in a number of head dips (P<0.05 and P<0.001) when compared with vehicle-treated rats. The number of head dips was found to be increased by Diazepam (1 mg/kg, i. p.) treated animals and the animals coadministered with Diosgenin (50 mg/kg, p. o.) and Silymarin (50 mg/kg, p. o.), significantly with P value less than 0.0001. The result indicates the synergistic activity of Diosgenin with Silymarin coadministration (fig. 1).

Light-dark model

The data summarized in table 2 states that Diosgenin (100 mg/kg, p. o.) and Silymarin (100 mg/kg, p. o.) treated rats shown a significant increase in a number of entries (P<0.05, P<0.01) and time spent in light box (P<0.01, P<0.001) than the dark box when compared with the control group of rats. Diazepam (1 mg/kg, i. p.) treated rats have spent increased time in a lightbox, significantly (P<0.0001) than the dark box. In the same manner, coadministration of Diosgenin (50 mg/kg, p. o) and Silymarin (50 mg/kg, p. o.) was also found to increase the number of entries and time spent in a lightbox, with P value less than 0.0001, the effect of which is more or less equivalent to that of the standard drug.

Table 2: Combined effect of diosgenin and silymarin on the light-dark model in rats

Groups	Mean no of entries		Mean time spent (s)	
	Light box	Dark box	Light box	Dark box
Control (Vehicle)	4.000±0.5774	13.67±1.202	70.50±4.639	259.2±7.167
Diazepam (1 mg/Kg)	14.67±1.453****	4.667±0.8819****	252.8±10.22****	87.50±4.349****
Diosgenin (100 mg/Kg)	8.833±0.9804*	9.500±1.088*	113.2±6.215**	221.0±7.633**
Silymarin (100 mg/Kg)	10.67±1.333**	7.000±1.155***	167.7±11.16***	144.7±8.285***
Diosgenin (50 mg/kg)+Silymarin (50 mg/kg)	12.33±1.801***	5.833±0.9098****	228.7±6.510****	101.3±2.801****

Data represent mean±SEM of six rats. Comparisons were made by using a one-way ANOVA followed by Dunnett's test as the post hoc. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared with control.

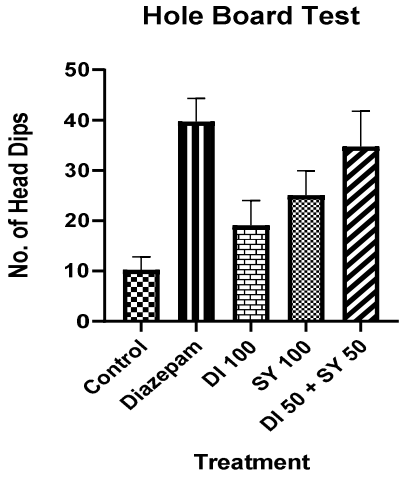

Fig. 1: Combined effect of diosgenin and silymarin on hole board test in rats

DISCUSSION

This study investigated the anxiolytic activity of the bioactive compounds, Diosgenin and Silymarin, both individually and in combination using Elevated plus-maze Model, Hole-board Test and Light-Dark Model, respectively. Anxiety occurs as a result of disturbances in the impulse transmission and other regulatory functions of various neurotransmitters such as gamma amino-butyric acid (GABA), nor adrenaline, serotonin, dopamine, opioid peptides, endocannabinoids, neuropeptide Y and oxytocin in the central nervous system [15].

Elevated plus-maze is a valid animal model of anxiety to screen several psychoactive substances. The natural stimuli such as fear of balance on a narrow raised platform and fear of a bright light open space were used to study the behavioral changes in an animal. It is well known that antianxiety drugs may increase the number of entries and time spent in open arms when compared with the control [16]. In our study, the test drugs DI and SY increased the frequency of entries and time spent by the animals in open arms, both independently and in combination on oral administration. Diazepam injected through the intraperitoneal route also exhibited the effect similar to that of the combined effect of phytocompounds.

Hole board test is a simple method used to measure the response of animals in an unfamiliar environment. It is one of the widely used models to study the effect of several anxiolytic agents including the bioactive compounds that are obtained from different sources. It is believed that the changes in the emotional state of an animal could be reflected in the head-dipping behaviour and the anxiolytic drugs may increase the number of head dips [17]. The test compounds, Diosgenin and Silymarin upon oral administration, increased the number of head dip counts, both independently and in combination. The combined effect was found to be greater than their individual anxiolytic effect.

The light/dark model is another model to study the anxiolytic or anxiogenic-like effect of test compounds with prominent CNS activity. Similar to an elevated plus-maze method, in light/dark model, Diosgenin (100 mg/kg) and Silymarin (100 mg/kg) treated animals shown a significant increase in a number of entries and time spent in the light box than the dark one. Their dose in combination, 50 mg/kg, each, was more significant like the standard drug, Diazepam (1 mg/kg) as compared against the vehicle-treated group.

In all the three anxiety models studied above, the combined effect of two bioactive compounds, namely, Diosgenin and Silymarin was found to produce a greater effect than their independent anxiolytic effect. The result of the present study reveals that Diosgenin could be able to produce an increased anti-anxiety-like effect when given along with Silymarin rather than independent administration, thereby, proving the synergistic effect of Diosgenin and Silymarin against anxiety disorder.

The molecular mechanisms that is responsible for their anti-anxiety activity have yet to be determined. The current findings, for the first time, to best of our knowledge demonstrated the combined effect of DI and SY on anxiety using experimental animal models.

CONCLUSION

The overall results of this study indicated that the combination of two Bioactive Compounds, Diosgenin and Silymarin has clinical benefits in the management of anxiety at low doses rather than their individual effect that requires a higher dose. Application of these compounds in combination for anxiety may also reduce the risk of adverse effects that arises because of the use of certain synthetic psychopharmacological drugs. Further investigations of the mechanism are under progress.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest for the current study.

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