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RESEARCH ARTICLE

Antidepressant Activity of Selective Bioactive Compounds-Diosgenin and Silymarin in an Experimental Animal Model

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Abstract

The present study was aimed to investigate the combined effect of bioactive compounds, Diosgenin and Silymarin in an animal model of depression. Wistar albino rats of either sex were randomly divided into five groups and treated for 7 days. Groups I and II were served as a control and a standard and groups III, IV and V 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. Imipramine (25 mg/kg, p.o.) was used as a standard for all antidepressant models and the activity was screened by using Forced swim test and Tail suspension test. The results suggested that Diosgenin and Silymarin, independently, at a dose of 100 mg/kg, shown anti-depressant like effect with P value less than 0.05 and 0.01, respectively. The compounds when given in combination at a dose of 50 mg/kg, each, shown potentiating antidepressant like activity as compared to their individual effect. It can be concluded that Diosgenin has got a synergistic effect on antidepressant action when given in combination with Silymarin.

Keywords: *Bioactive compounds, Depression, Diosgenin, Silymarin, Imipramine, Forced swim test, Tail suspension test.*

Introduction

Medicinal plants and their bioactive compounds are available in abundant in nature. In spite of their efficacy, the bioactive compounds are most powerful in the treatment of variety of psychiatric disorders with lesser side effects [1]. There arises a need for the researchers to become familiar with the bioactive compounds in the development of pharmacotherapies for diseases affecting central nervous system (CNS).

Diosgenin (DI), is a highly potential bioactive compound present in several plant species, such as *Trigonella foenum-graecum* (Fenugreek) and *Dioscorea villosa* (Wild Yam), *Costus speciosus*, *Smilax menispermoidea* and several species of *Trillium* [2, 3]. Diosgenin, a steroidal sapogenin, was first discovered by Fujii and Matsukawa from Dioscorea tokoro Makino in 1935 [4]. Cholesterol was found to be a precursor in the biosynthesis of Diosgenin. Cholesterol is obtained from lanosterol by the catalytic action of the enzyme cytochrome P450 system. The other route of synthesis was believed to be from squalene-2,3-oxide in two ways, one from cycloartenol through formation of sitosterol [5] and the other from lanosterol by the formation of cholesterol [6]. It is obvious from several studies that DI has been used in traditional medicine for its anti-diabetic, anti-hyperlipidemic, anti-inflammatory, immunomodulatory, anti-infectious, anti-inflammatory, anti-hyperglycemic and anti-oxidant properties [7].

Diosgenin, was founded to induce apoptosis in a variety of cancer cell lines like, colorectal, hepatocellular, breast cancer, osteosarcoma and leukemia [8-10]. It acts by modulating multiple cell signalling pathways, thereby plays an important role in cell-cycle regulation, differentiation and apoptosis [11].

Diosgenin is a precursor for synthesising many steroidal drugs in pharmaceutical companies and is also found useful in many functional fool and cosmetic industries [12]. In addition, DI was proven to have some neuroprotective effects and was found to improve certain memory impairments. Antioxidant nature of diosgenin favours its aid the treatment of many neurodegenerative disorders like Alzheimer [11]. These findings support the present concept of assessing Diosgenin as an antidepressive phytochemical. Silymarin (SY) is a lipophilic polyphenol flavonoid, isolated from the seeds and fruits of Milk Thistle, Silubum marianum L. Geartn which belongs to the family Aster of Asteraceae or Compositae [13].

Silymarin, the active phytocompound of milk thistle is a mixture of seven flavanolignans with silibinin (or silibin) as the main bioactive principle. Silibinin comprises of two diastereomers, Silibin A and Silibin B in 1:1 proportion. The other flavonolignans of Silymarin includes Isosilibin, Dehydrosilibin, Silichristin, Isosilichristin, Silydianin and Taxifolin, a flavonoid with antioxidant potential [14, 15].

Silymarin is mainly used for its anti-oxidative, antidiabetic, anti-inflammatory, antifibrotic and cytoprotective properties [16]. At present, Silybin is clinically used as a hepatoprotector to treat liver diseases [17]. Silymarin also act as an immunomodulator and an iron chelating and membrane stabilizing agent [18]. SY is effective against certain drug-induced hepatic injuries and it has been recommended by The German Commission E to treat dyspeptic complaints, toxin-induced liver damage and hepatic cirrhosis and SY is prescribed as a supportive therapy for chronic inflammatory liver disorders [19].

The evidence suggested that SY exerts neuroprotective effect in various disorders of central nervous system including Alzheimer's disease and cerebral ischaemia [20]. In this context, our research work was designed to evaluate the antidepressant activity of SY along with DI. Depression is one of the major public health problem concerns worldwide and is found to be most common among the various stress-related mental disorders that are responsible for disability and early death. More than 20% of adult population is suffering from depression during their life at present [21]. Depression is manifested as negative thoughts, sadness of mood, loss of interest; laziness in routine work, low or high appetite, insomnia and severe suicidal tendency. Likewise, anxiety is accompanied by over activity of autonomic system leading to tachycardia, tremors, sweating, loss of confidence and so on [22].

Many numbers of experimental models have been used to test the antidepressant activities of medicinal plants and their phytoconstituents. The reserpine effects reversal test was the first one that demonstrated the efficacy of imipramine and its metabolite desipramine [23]. The commonly used experimental models to screen antidepressant drugs in India, includes Despair swim test, Tail suspension test, Diazepam-induced sleeping time, Locomotor activity using actophotometer, Learned helplessness in rats, Muricide behaviour in rats, compulsive gnawing in mice and so on [24].

We used Forced swim test (FST) and Tail Suspension test (TST) in our study to screen the pharmacological activity of the two bioactive compounds, Diosgenin and Silymarin, both individually and in combination against depression.

Materials and Methods

Drugs and Chemicals

The chemicals used were of analytical grade. Diosgenin and Silymarin were purchased from Vital Herbs, New Delhi and others from local suppliers.

Preparation of Drug Suspensions

Suspensions of Diosgenin and Silymarin were prepared using 0.5 % w/v of carboxy methyl cellulose (CMC) in distilled water prior to oral administration to animals [25]. Fresh preparations were used for each and every experiment.

Experimental Animals

Wistar albino rats of either sex, weighing between 150-200 g were selected randomly and used for the study. The animals were placed in group of six in polypropylene cages with paddy husk as bedding and were housed at a temperature of 25±2^{o} C, humidity 30 - 70 % with 12 h light-dark cycles.

The animals had free access to water and were fed with standard rodent pellet diet. The experimental protocol was approved by the Institutional Animal Ethics Committee of department (SBCP / 2019-20 / CPCSEA / IAEC/I (4) / F16/69). Each animal was tested only once and the observations were recorded in a noiseless diffusely illuminated room.

Experimental Design

The study was done in 5 different groups (n=6/group) of rats and the groups were divided as follows.

- Group I: Control: 0.5 % CMC (10 ml/kg, p.o.)
- Group II: Standard: Imipramine (25 mg/kg, p.o.)
- 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

Forced Swim Test (FST)

This test was done based on the method of Porsolt et al. (1977). Rats were forced to swim individually in a glass cylinder (20 cm × 14 cm) containing fresh water up to a height of 10 cm at 25±1° C. Animals were trained in 15 min session individually, constituted the "pre-test session" a day before. Twenty four hours later, in a "test session", rats were forced to swim for a 6 min period and the total duration of immobility was recorded for the last 4 min period, manually using a stop watch. Animal was considered immobile when it floats in water without struggling and making only those movements that is necessary to keep its head above the water. The animals were allowed to dry for 15 min following their swimming session. Decrease in immobility was an indicative of anti-depressant effect [26].

Tail Suspension Test (TST)

This test was performed as described earlier (Steru et.al., 1985). Animal was suspended individually from the side of the table/shelf at 50 cm height from the floor with the aid of adhesive tape placed approximately 1 cm from the tip of the tail.

The duration of immobility was recorded during the final 4 min of the total 6 min test period, excluding the initial 2 min of struggling. Animals were considered to be immobile when they hung passively or remained completely motionless [27].

Statistical Analysis

The Data were statistically analysed by using One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests in Graphpad Prism software and the values were expressed as mean± SEM. $P<0.05$ was considered as significant with respect to control and Standard treatment (mentioned in the caption of results).

Results and Discussion

Forced Swim Test

Oral administration of Diosgenin and Silymarin, independently, at a dose of 100 mg/kg, p.o., each, elicited significant decrease in immobility time of rats with P value less than 0.05 and 0.01, respectively, during the 5 minute of test session. The same compounds when given in combination at a dose of 50 mg/kg, p.o., each, also decreased the immobility time of rats subjected to Forced Swimming, significantly ($P<0.001$) (Table 1).

The standard drug, Imipramine (25 mg/kg, p.o.) reduced the immobility time, significantly ($P<0.001$), when compared with control group of rats. FST is a common behavioural paradigm used to evaluate the antidepressant-like activity of many test compounds in rodents [28]. This test is based on the observations that the rodents, such as rats and mice, when constrained to swim in a restricted space from which there is no possibility of an escape, eventually cease the animal to struggle and brings them to a state of despair or lowered mood.

This behaviour is either increased or decreased by test drugs [29, 30]. The parameter that is observed in this test is termed immobility time and the shorter immobility time denotes antidepressant-like activity of a drug while the extended immobility time indicates its CNS depressant-like effect [31].

It has been reported in several studies that swimming is sensitive to serotoninergic compounds mainly the selective serotonin reuptake inhibitor such as fluoxetine,

whereas the climbing behaviour is sensitive to tricyclic antidepressants and others with selective catecholamine transmission effects [32]. Imipramine, the tricyclic antidepressant, exerts its action by blocking the reuptake mechanism of norepinephrine (NE) and 5-hydroxytrytamine (5-HT) into their neurons, respectively [33], thereby, decreases the immobility frequency and increases the escape-type behaviour of climbing [34]. Taking this into an account, the results obtained in this study, strongly suggested that Diosgenin and Silymarin, exerted their antidepressant-like effect through the mechanism of serotoninergic and catecholaminergic neurotransmission, similar to that of the standard drug, imipramine.

Table 1: Combined Effect of Diosgenin and Silymarin on Forced Swim Test

Group	Dose (mg/kg)	Duration of Immobility (Sec)
Control	-	146.3±4.998
Imipramine	25 mg/kg, p.o.	51.50±2.363***
Diosgenin	100 mg/kg, p.o.	117.3±4.462*
Silymarin	100 mg/kg, p.o.	113.2±10.48**
Diosgenin + Silymarin	50 mg/kg, p.o. + 50 mg/kg, p.o.	93.5±6.850***

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 compared with Control group of rats

Tail Suspension Test

Diosgenin and Silymarin, when administered individually, at a dose of 100 mg/kg, p.o., each and in combination of Diosgenin (50 mg/kg, p.o.) and Silymarin (50 mg/kg, p.o.), decreased the mean duration of immobility of rats subjected to Tail Suspension Test significantly with P value less than 0.05 (Figure 1). The standard drug, Imipramine (25 mg/kg, p.o.) also decreased the duration of immobility, significantly (P<0.001), when compared with control group of rats. Like FST, the TST is also a validated model to determine the efficacy of many antidepressant drugs. In this test, the rats are placed in an unpreventable distressing condition, similar to that of FST, but in slight moderate. Lack of escaping behaviour is termed to be immobility [35]. In contrast to FST, there is no submersion of animal in water in TST; hence the animal is safe from hypothermia. The sensitivity and reproducibility of FST and TST also differs [36, 37]. The result demonstrated that, the combined antidepressant activity of phytocompounds, Diosgenin and Silymarin was found to be greater than their individual effect.

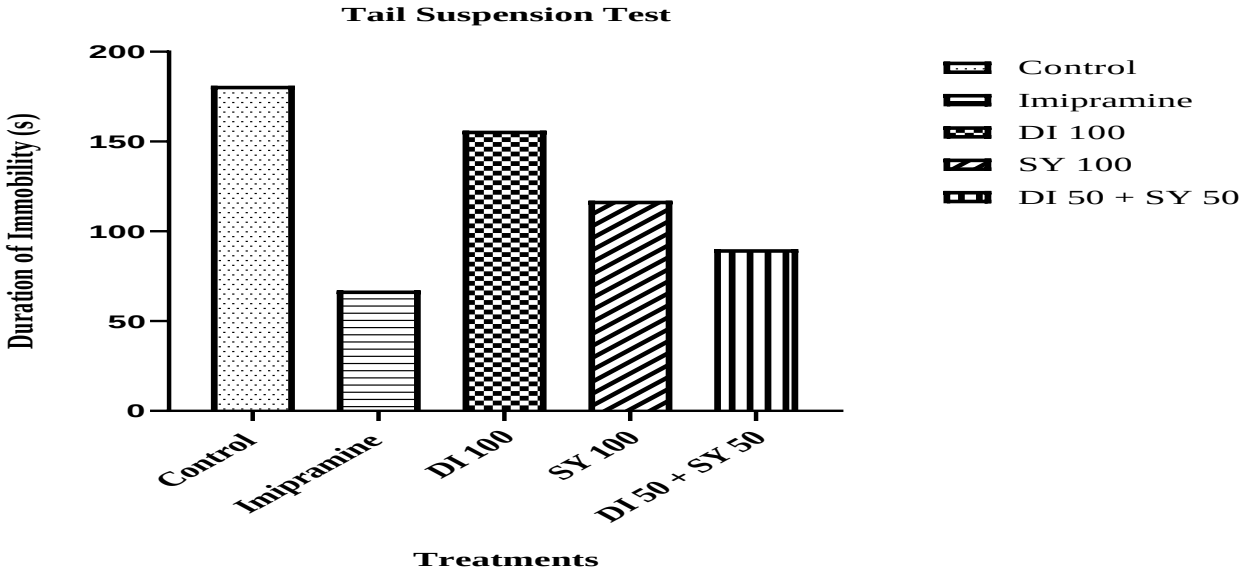

Fig. 2: Combined Effect of Diosgenin and Silymarin on Tail Suspension Test in Rats

The forced swimming and the tail suspension tests are behavioural despair tests used to study the mechanism of depression and are sensitive to all major classes of antidepressant drugs such as tricyclic antidepressants, atypical antidepressants, monoamine oxidase inhibitors and serotonin reuptake inhibitors. The behavioural

responses observed in both the tests were duration of immobility time in seconds, reflecting behavioural despair similar to that of human depression [38].

The results obtained in this study indicated that Diosgenin and Silymarin, given orally were found to be effective both in independent and in combination against depression. The antidepressant activity of DI and SY, when given in combination at a dose of 50 mg/kg, p.o., each, was found to be more or less significant to that of the standard drug, imipramine (25 mg/kg, p.o.) rather than when given individually.

Conclusion

The anti-depressant studies revealed that the combined dose of Diosgenin and Silymarin required to produce a 90% reduction in depression were lower than the doses of either drug alone necessary to produce the same effect. Application of these bioactive compounds in combination for depression has an advantage of reduced risk of adverse effects which are found to be more in case of certain synthetic antidepressant medications. Further investigations are recommended to determine the exact biomedical pathway of these phytocompounds.

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