ANTIDEPRESSANT AND ANXIOLYTIC ACTIVITIES OF BIO FLAVONOID-GOSSYPIN

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Summary

Gossypin is a bioflavonoid compound and is found in various herbs, especially in *Malvaceae* family. The pharmacological properties of Gossypin have been rarely reported with the exception of its antioxidant and anti-inflammatory activity. The purpose of this study was to characterize the putative anxiolytic-like properties and antidepressant activity using an elevated plus maze (EPM), Light –Dark test forced swim test, chronic mild stress (CMS) and sedative property by pentobarbitone induced sleeping time. Control mice were treated with an equal volume of vehicle (Saline 0.9% w/v), and positive control mice were treated with diazepam (1 mg/kg). All the test drugs were administered orally. Gossypin (20mg/kg) significantly increase the time spent in open arms of EPM test ($p< 0.01$). In the light-dark test, Gossypin significantly increased the time spent in light space at 20mg/kg ($p<0.01$). The Antidepressant effect was supported by the forced swim test, Gossypin (20mg/kg) significantly shortened the immobility time and the effect was comparable to Imipramine (20mg/kg) ($p<0.01$). In the CMS test, mice were subjected to CMS exhibited as reduction in sucrose intake, the reversal of the intake has been shown by Gossypin (20mg/kg) in 21 days treatment, which was comparable to Amytriptaline (20mg/kg) ($p<0.01$). At higher doses, a sedative effect produced by acute administration of Gossypin (20mg/kg) was indicated by the potentiation of pentobarbital induced sleep. The present results shows the anxiolytic profile and antidepressant effect of Gossypin.

Key words; Gossypin, Anxiety, Antidepressant and Bio-flavonoid.

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Introduction

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different naturally accuring flavonoids have been described. The flavonoids shows various biological activities including antioxidant, anti-inflammatory activity, activity on coronary heart diseases and cytotoxic-antitumour activity. However the anxiolytic properties of these flavonoids have been rarely investigated. Previously, the anxiolytic-like effects of simple flavone, chrysin (5,7-dihydroyflovone) have been reported, which behaves as a competitive ligand of the benzodiazepine receptors[1]. Apigenin has been reported to show similar activity in mice with only slight sedative effect[2].

Gossypin is a bioflavanoid (Gossypin-8-0 glucoside; 3,5,7,3,4-pentahydroxy-8-0-glucosylflavone), which is naturally occurring in various plants belonging to the family of *Malvaceae*. The pharmacological activity of Gossypin has not been previously examined. In the present study, we investigated the anxiolytic-like activity of Gossypin, in an elevated plus maze test (EPM) and light-dark test. The Forced swim test and Chronic mild stress used to asses antidepressant effect of Gossypin. The sedative property of the gossypin also investigated by pentobarbitione induced sleeping time in mice.

Materials and Methods

Drugs & Chemicals

Gossypin was purchased from Sigma Chemical Company, St. Louis, Missouri, USA. All other chemicals used were in analytical grade.

Animals

Albino mice weighing between 18 – 22 gm were used for this study. The animals were obtained from animal house, IRT Medical College, Perundurai, Erode. After the arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat/mice chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Anxiolytic Activity

Elevated Plus Maze: [3,4]

The apparatus comprised of two open arm (25 cm × 10cm) and two closed arms (25cm×10cm×20cm) that extended from a common central platform (10cm×10cm). The entire maze was elevated to a height of 90cm above the floor level.

The mice were grouped into five with six animals each. Group I, animals were administered with saline (10ml/kg) as solvent control, Group II received Diazepam (1mg/kg) as standard drug and Group III-V received, Gossypin 5, 10 and 20mg/kg

respectively. The drugs were administered orally by dissolving in saline 30 minutes before the commencement of experiment. Then the mice were submitted to elevated plus maze. The number of open and closed arm entries and time spent on open and closed arms was registered.

Light - Dark Test: [5,6,7]

The testing apparatus consist of the box (48×28×27) is divided into two compartments with one third of the box. The box constructed from black plexiglass with bright fluorescent lighting. Mice can transit between compartments through an opening (7.5×7.5cm) located at floor level in the centre of the partition between compartments.

The mice were grouped into five with six animals each. Group I, animals were administered with saline (10ml/kg) as solvent control, Group II received Diazepam (1mg/kg) as standard drug and Group III-V received, Gossypin 5, 10 and 20mg/kg respectively. The drugs were administered orally by dissolving in saline 30 minutes before the commencement of experiment. The testing is initiated by placing the mouse in the white, aversive compartment to increase aversion to the light compartment and to increase the sensitivity of measuring anxiety behavior and the time spent in the compartments were monitored for 5 minutes experimental time.

Antidepressant Activity

Forced Swim Test:[8,9,10,11]

The apparatus consisted of a clear plexiglass cylinder (20gm high × 12cm diameter) filled to 15 cm depth with water (24±1^{o}C).

The mice were grouped into five groups of six animals each. Group I, animals were administered with saline (10ml/kg) as solvent control, Group II received Imipramine (20mg/kg) as standard drug and Group III-V received, Gossypin 5, 10 and 20mg/kg respectively. The drugs were administered orally by dissolving in saline 30 minutes before the commencement of experiment.

A pretest was conducted by placing the animals individually into the cylinder for the period of 15 min, 24hrs prior to the commencement of the actual test session. During the 5 minutes test session the following behavior response were recorded, climbing behavior, swimming behavior and immobility time.

Chronic Mild Stress:[12,13,14,15,16]

The mice were grouped into six of six animals each. Group I, animals were served as non-stress control, Group II served as stress control, were administered with saline (10ml/kg), Group III received Amytriptalin (10mg/kg) as standard drug and Group IV-VI received, Gossypin 5, 10 and 20mg/kg respectively. The drugs were administered orally by dissolving in saline 30 minutes before the commencement of experiment.

The chronic mild stress consisted in the exposure to the following stressors; 3-5h periods of food and water deprivation, immediately prior to sucrose test, one additional 16h period of water deprivation and two periods of continuous overnight illumination; two periods (7 and 17h) of 45 degree cage tile; one 17h period in a soil cage (100 ml water in a sawdust bedding); Two periods (3and 5h) of intermittent sand. These stressors were scheduled throughout the 21 days in a similar manner. The normal mice were housed under identical conditions in a separate room, and had no contact with the stressed animals. They were deprived of food and water for 3hr before each sucrose in take test.

At the start of the experiment, the animals were first trained to consume 2% sucrose solution. Sucrose consumption was monitored through out the experiment. After one week period of adaptation, sucrose solution intake baseline tests were performed (two tests per 6 days) over a period of 21 for all subjects. These test involved a 3hour period of food and water deprivation, followed by the offering of a sucrose solution for 1hour. Intake was determined by measuring the volume of sucrose solution at the beginning and at the end of each test.

Sedative & Hypnotic Activity

Pentobarbitone Induced Sleeping Time:[17,18,19]*:*

The mice were grouped into five groups of six animals each. Group I, animals were administered with saline (10ml/kg) as solvent control, Group II received Diazepam (1mg/kg) as standard drug and Group III-V received, Gossypin 5, 10 and 20mg/kg respectively. The drugs were administered orally by dissolving in saline 30 minutes after the administration of pentobarbitone (40mg/kg) intraperitoneally. After 30 minutes of test drugs, the duration of sleeping time in minutes was recorded.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

Results

Anxiolytic Activity

Effect of Gossypin on Elevated Plus Maze

The effect of Gossypin on Elevated plus maze was shown in figure 1a & 1b. Diazepam significantly ($p<0.01$) increased the number of entries and the time spent by mice in the open arms. The administration of Gossypin 10 and 20mg/kg , significantly increased the number of entries ($p<0.01$) and the time spent on the open arms ($p<0.01$) as compared to the control group.

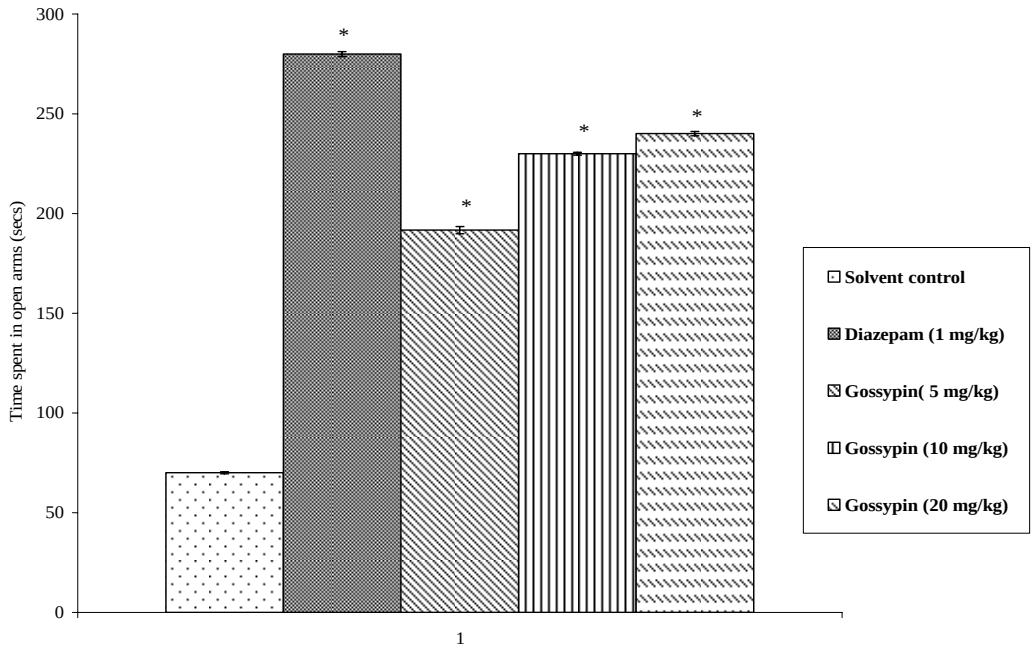

Figure 1a.

Effect of Gossypin on time spent by mice in open arms of elevated plus maze.
Values are in mean±SEM (n=6) $^{\#}$P<0.05, *P<0.01 and **P<0.001 Vs Solvent Control

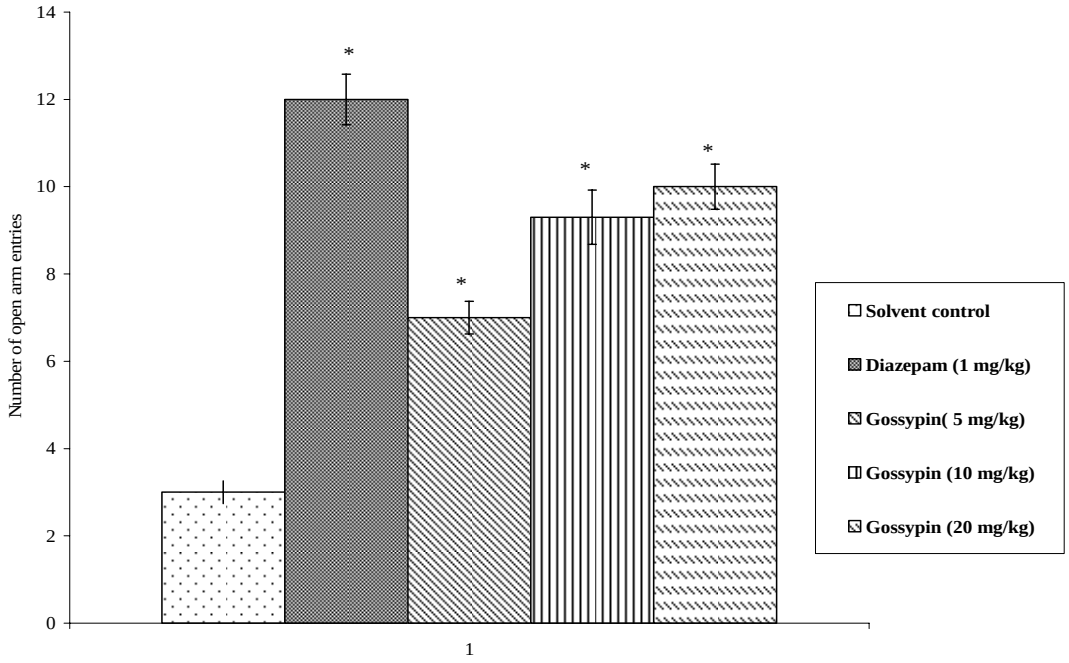

Figure 1b.

Effect of Gossypin on number of open arm entries in elevated plus maze.
Values are in mean±SEM (n=6) $^{\#}$P<0.05, *P<0.01 and **P<0.001 Vs Solvent Control

Effect of Gossypin on Light –Dark Test

The effect of Gossypin on Light –Dark test was shown in figure 2. The administration of different doses of Gossypin in mice induced a significant increment of the time spent by mice on the illuminated side of the light-Dark apparatus (Vehicle: 110±93; Gossypin 5, 10 and 20mg/kg were 140±2.89, 167±0.82 and 170±1.65 respectively) All the doses of Gossypin shows significant ($p<0.01$) increase in the time spent in light space and comparable with the effect produced by Diazepam (180± 2.89).

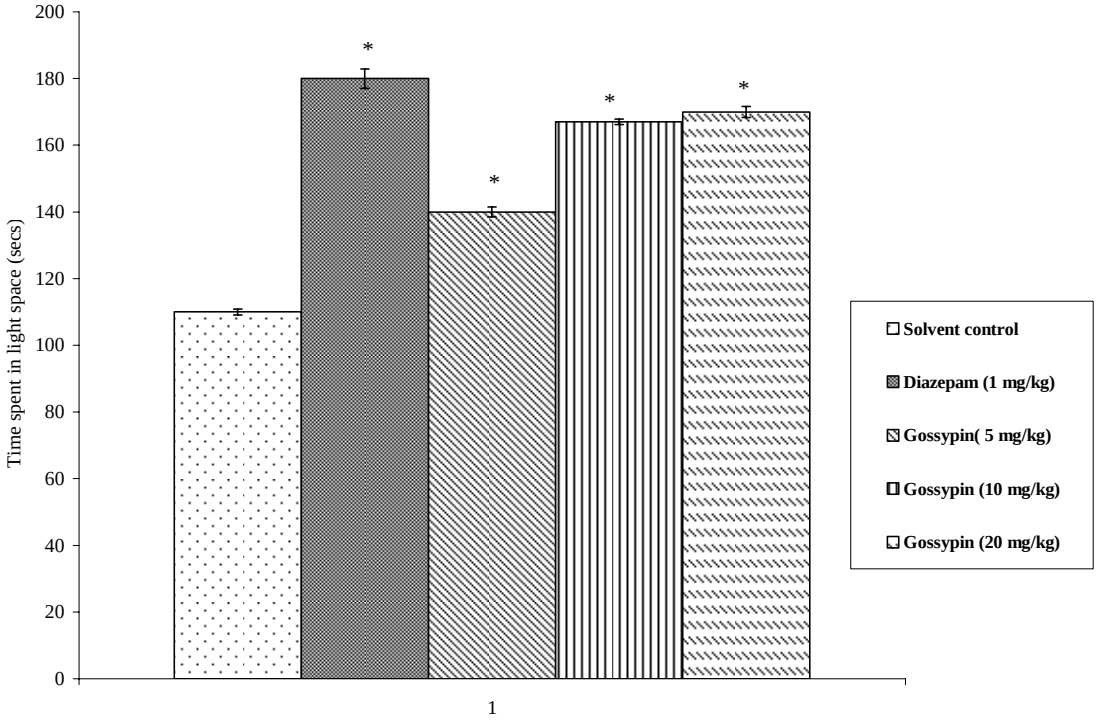

Figure 2.

Effect of Gossypin on time sent by mice in light space of Light-Dark test
Values are in mean±SEM (n=6) $^{\#}P<0.05$, $*P<0.01$ and $**P<0.001$ Vs Solvent Control

Antidepressant Activity

Effect of Gossypin on Forced Swim Test

The effect of Gossypin on Forced swim test was shown in figure 3. All the doses of Gossypin administrated in mice provoked a significant (vehicle: 240± 1.32; Gossypin 5, 10 and 20mg/kg were, 210± 1.13, 200±1.59 and 197± 0.58 respectively) ($p<0.01$) diminution of immobility time when the animals were exposed to the forced swim test. All the doses of Gossypin shows similar effect as that of Imipramine, (178.83±1.17)

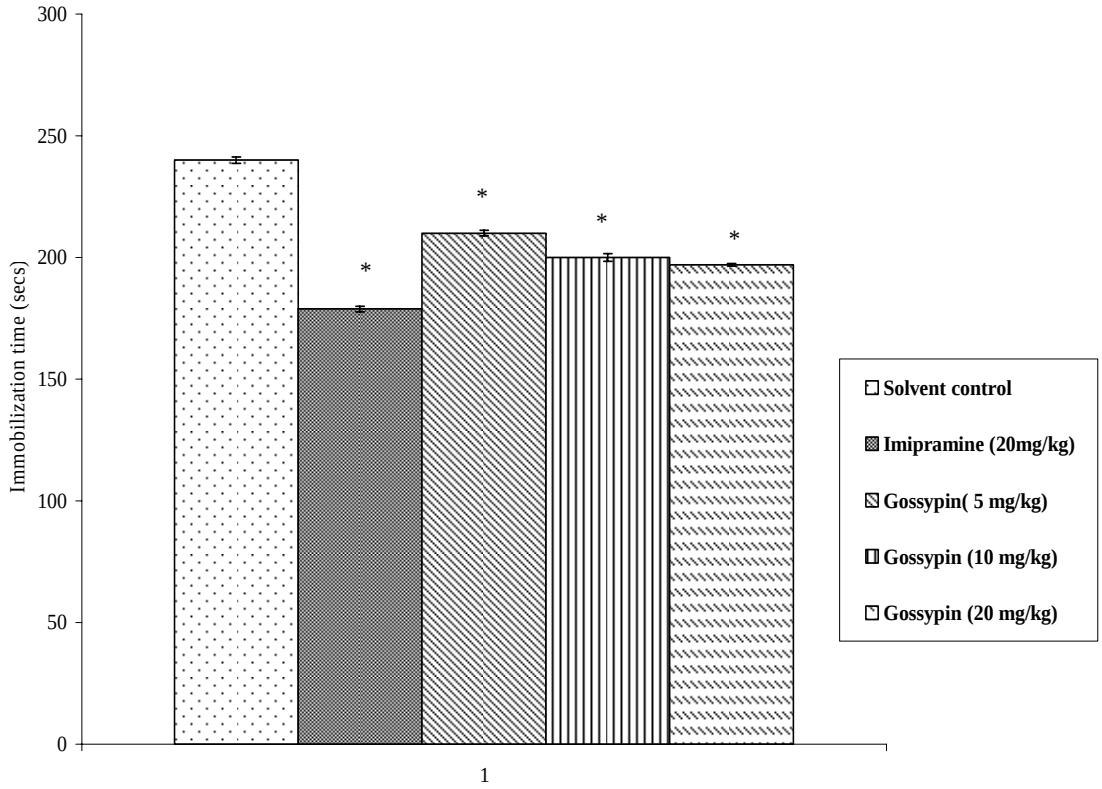

Figure 3.
Effect of Gossypin on immobilization time by mice in Forced swim test.
Values are in mean±SEM (n=6) $^{\#}P<0.05$, *P<0.01 and **P<0.001 Vs Solvent Control

Effect of Gossypin on Chronic Mild Stress

The effect of Gossypin on Chronic mild stress was shown in table 1. Chronic mild stress caused a decreased intake of 2% sucrose solution, until the beginning of drug treatment. In stressed mice after 3 days of treatment with Gossypin at 20mg/kg, sucrose intake was significantly ($p<0.01$) increased, and elevated consumption continued for 21 days, similar results were observed with 10 mg/kg of Gossypin treatment ($p<0.01$). This effect did not last in any significant way until the Chronic mild stress was over. The similar effects ($p<0.01$) was observed with Amytrptaline 10mg/kg treatment for 21 days.

Table 1. Shows the effect of Gossypin on 2 % sucrose intake by mice in chronic mild stress. Values are in mean ±SEM, #P<0.05, *P<0.01, **P<0.001 Vs Stress control

S.NO	Drug Treatment	2% Sucrose Consumption (ml/hr)				
		0 day	*3rd day*	*9th day*	*15th day*	*21st day*
1	Non stress Control	17±0.37*	18±0.37 *	16±0.58*	16.7±0.33*	18.7±0.56*
2	Stress Control	10±0.58	11±0.58	10±0.58	10±0.58	8±0.37
3	Amytriptaline (10mg/kg)	14±0.37*	14.2±0.18*	17±0.37*	14±0.37*	13±0.37*
4	Gossypin (5mg/kg)	11±0.58*	11.5±0.18 *	15±0.37*	13.5±0.18*	12.5±0.22*
5	Gossypin (10mg/kg)	12±0.37*	13±0.58*	16±0.58*	13.5±0.22*	13±0.37*
6	Gossypin (20mg/kg)	12±0.37*	14±0.37*	16.5±0.22*	14±0.37*	14±0.42*

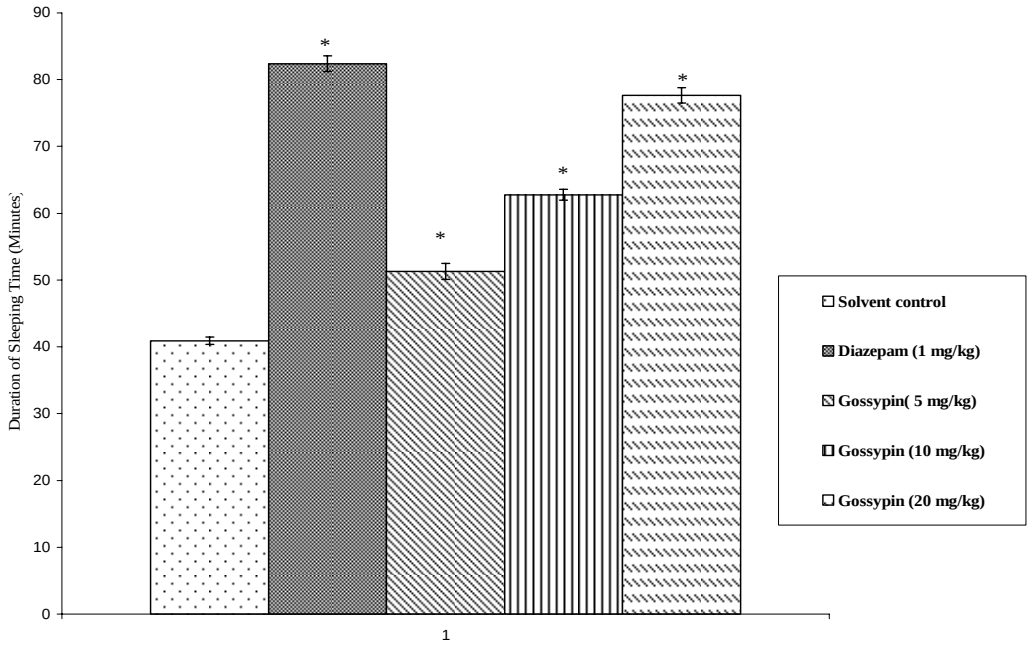

Figure 4.
Effect of Gossypin on duration of sleeping time in pentobarbital induced sleeping time in mice. Values are in mean±SEM (n=6) #P<0.05, *P<0.01 and **P<0.001 Vs Solvent Control

Sedative & Hypnotic Activity

Effect of Gossypin on Pentobarbital Induced Sleeping Time

The effect of Gossypin on pentobarbital induced sleeping time was shown in Figure 4. The Gossypin 5, 10mg and 20mg/kg produced a prolongation of pentobarbital-induced sleeping time in mice (vehicle; 40.90± 0.55, Gossypin 5, 10 and 20mg/kg were 51.30±.19; 62.77± 0.82 and 77.68± 1.15 respectively).Gossypin showed dose dependent increase in sleeping time. All the doses of Gossypin showed significant ($p<0.01$) increase in sleeping time when compare to control. Gossypin shows similar effect as that of Diazepam.

Discussion

This animal model is considered one of the most widely validated tests for assessing sedative and anxiolytic substances such as the benzodiazepines[20]. An increase of the most important variables of the elevated plus maze test was found that, the time spent by the mice in open arm. In our study the Gossypin 10 and 20mg/kg, shows the anxiolytic activity which was comparable with time spent by mice in open arms of animals treated with diazepam.

The Light-Dark test is widely used for rodents as a model for screening anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminate areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is a novel environment and light. It has been reported that, the measurement of time spent in the light area, but not the number of transfers, is most consistent and useful parameter for assessing an anxiolytic action,[21] . The present study showed that all the three doses of Gossypin could increase the time in light area, suggesting again that Gossypin possesses anxiolytic properties.

Rodents immersed in a vessel of water, develop an immobile posture after initial struggling. Most antidepressants, administrated acutely before the test, reverse the immobility of promote struggling [22]. Results showed that, all the doses of Gossypin produced diminution of immobility time of mice exposed to the forced swimming test. These behavioral effects of Gossypin, were similar to that of Imipramine[23] .

Chronic mild stress involves relatively prolonged exposure to various mild stressors leads to induce an anhedonia-like state[24], inferred with reduced intake of 2% sucrose solution. The above mentioned state can be reversed by chronic anti-depressant treatment. In our study the Gossypin 20mg/kg shows the higher intake of 2% sucrose indicate the antidepressant activity. This effect was very much comparable with amytrptaline.

The Righting reflux is the indications for the measurement of sedative effect of sedative and hypnotics[25]. The sedative effect has been observed by the Gossypin at all the doses used, in pentobarbital induced sleeping time in mice. Gossypin dose dependently increased the pentobarbital induced sleeping time in mice.

The effect of Gossypin in various CNS functions was studied by employing anxiolytic, antidepressant and sedative& hypnotics models in animals. The results of our study, concludes that the Gossypin produced anti-depressant effect in dose dependent manner and it can be consider as a novel anti depressant drug in the category of bio-flavonoid.

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