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Research Article

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Anxiolytic and antidepressant-like effect of the ethanolic extract of *Cassia tora* seed in Swiss mice

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ABSTRACT

Background: *Cassia tora* is a traditional herbal medicine has been used as an antihyperlipidemic, anti-inflammatory, anti-diabetic, expectorant, antioxidant effect, etc.
Objectives: The aim of this study was to evaluate the anxiolytic and antidepressant effect of hydro-alcohol extract of *Cassia tora* in Swiss mice.
Methods: Hydro-alcohol extract of *Cassia tora* was used for the evaluation of antidepressant, anxiolytic, biochemical estimation, skeletal muscle relaxant, and anoxic tolerance test.
Results: The study showed that**,** in Locomotor activity, there was significant increase in no. of mobility count by *Cassia tora* extract as compared to negative control and diazepam treatment group ($p<0.05$, $p<0.01$). In forced swim test, there was a significant reduction in immobility at different time intervals with 400 mg/kg of *Cassia tora* extract as compared to negative control and was similar with imipramine treatment group ($p<0.01$). In a dose of 200 and 400 mg/kg of *C. tora*, there was a significant reduction in convulsion. At a dose of 400mg/kg, In Elevated Plus Maze test, the mean time spent in open arm increases and in close arm decreases compared to negative control group.
Conclusion: Results indicated that *Cassia tora* extract has antidepressant, anxiolytic and anticonvulsant effect in adult Swiss mice.

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Cassia tora, antidepressant, anxiolytic, Elevated Plus Maze, Forced swim test.

Introduction

Depression is a chronic neuropsychological disorder, nearly about 350 million peoples are affected worldwide. It is one of the most common disease with a tendency to become the world 2nd ranked cause of premature death in the year 2020, stress is the key factor to develop the depression in human and rodents [1, 2] .

Recent study shown that the cost of treatment is about one trillion dollars annually, generally conventional drugs are used to treat anxiety (specially benzodiazepine like drugs) but the adverse drug reaction (ADR) are very common with benzodiazepines these effects are dependent like about 8% people are hospitalized due to their ADR by their consumption in United States [3]. The monoamine hypothesis suggests that depression is caused by a functional defects of norepinephrine (NE) and /or serotonin (5-HT) (or dopamine DA) at the certain site of brain this theory is based on the ability of the NE and 5-HT neurotransmission and to act as effective antidepressant drugs [4]. Reserpine reduce the level of 5–HT and NE and tryptophan which increase the level of 5-HT and NE, therefore depression must be associated with decrease the NE/5-HT neurotransmission [5]. Biochemical studies on depressed patients do not entirely support this hypothesis, there are various group of antidepressant drugs to treat the depression like selective serotonin reuptake inhibitors (SSRIs), selective serotonin norepinephrine reuptake inhibitor (SNRIs), tricyclic antidepressant (TCA), monoamine oxidase (MAO) inhibitor, etc. [6].

Medicinal plants have important role in the primary health care, about 80% of the world population is characterized by less ADR. Better compliance and good efficacy in the prevention and treatment of depression [7]. *Cassia tora* is originally find in Nepal, India, Bangladesh and Pakistan popularly known as Tovara is an herbaceous about 1-2 meters in height. It is considered as important medicinal plant.

The *Cassia tora* seed has variety of bioactive compounds including alkaloid, flavonoid, saponin and tannins [8] known for their antihyperlipidemic [9], anti-inflammatory [10], antibacterial [11], antioxidant [11]

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etc. there is no scientific investigation that demonstrate the efficacy of *C. tora* in treatment of anxiety and depression. So, the present study aimed to investigate the hydro-alcohol extract of *C. tora* and its pharmacological potential in the treatment of this pathology.

Material and Methods

Plant Material and authentication

The seed of *Cassia tora* were collected from local area of Bhairahawa, Rupandehi District, Nepal in the month of October 2018. The plant was authenticated and reconfirmed by Mr. Subodh Khanal, Assistant professor, of medicinal and aromatic plants, Department of soil and environmental science, Institute of Agriculture and Animal Science (IAAS) Paklihawa, Rupandehi reference no. 2073/074.

Chemicals

Carboxyl methyl cellulose (CMC) (SD fine chem ltd, India.), Tween 80 (Merk Millipore USA), Diethyl ether (Merk Millipore USA), Ethanol (Merk Millipore USA), Ascorbic Acid (SD fine chem ltd, India.), Quercetin (Kemphasol pvt.ltd., India), Follin-ciocalteu reagent (Sd fine - chem Ltd, India), Sodium Hydroxide (Sd fine - chem Ltd, India), etc. all chemicals are laboratory grade.

Preparation of Ethanolic extract

Collected plant material was washed they were shade dried (at room temperature) and grinded into coarse powdered form. Thus, weighed quantities of coarsely powdered *Cassia tora* seed were placed in maceration flask and added with sufficient quantity 300 g of 700 ml of hydro-alcohol (30:70) and placed at 4º C. The complete maceration was done for about 7 days. During the maceration process, occasional shaking was done at 10 to 4 pm then it was kept at 4º C overnight. After the completion of maceration process, the menstrum was collected, evaporated by rotary evaporator at 40ºC and the extract was obtained. The percentage yield of extract (weight of dried extract divided by weight of plant material taken expressed in terms of percentage) was calculated and crude extracts were stored in air tight container (inside refrigerator) for further studies. The dried extract was mixed with tween 80 and used for various experimental purposes [12].

Preliminary Phytochemical screening

Qualitative phytochemical screening tests were carried out based on the methods used to determine the presence or absence of alkaloids, tannins, flavonoids, saponins, phenols, terpenoids, steroids, glycosides, and carbohydrate in hydro-alcohol seed extract of *Cassia tora.*

Animals

The animal albino Swiss mice of either sex weighing about 25 – 30 g were divided into 6 group n=5. The animals had free access to standard food and water *ad libitum* and were kept in room maintains 25ºC±2 in a 12 hrs. light and dark cycle. The animals were used after acclimatization period 7 days to the laboratory environment. This experimental study was conducted in Department of Pharmacology, Universal College of Medical Sciences (UCMS), Bhairahawa, Nepal in the year 2019. The study was approved by Intestinal Review Committee Reg. No UCMS/IRC/013/18.

Acute toxicity

Acute toxicity was previously studied Vijayalaxmi A. *et al* 2014 briefly described the mice were divided into six group n=5 in each group the animals were treated orally with Ethanolic extract at different dose 2000 mg/kg and 500 mg/kg body weight. The animal were observed mortality and behavioral changes for a period of 15 days [13].

Induction of Disease

Mice were randomly divided into 6 group of five animals in each group. The control group were receiving vehicle only and standard group receive diazepam (2 mg/kg) and imipramine (10 mg/kg) and test-1 and test -2 group receive different concentration of hydro-alcohol extract of *Cassia tora*

Table 1: represent the group of experimental animals with different treatment.

S.N.	Group	Treatment	Dose	Reference
1	Normal Control	CMC	2 ml/kg	[14]
2	Negative control	vehicle and induction of disease	2 ml/kg	[14]
3	Standard 1	Imipramine	10 mg/kg p.o.	[15]
4	Standard 2	Diazepam	2 mg/kg i.p.	[16]
5	Test 1	*Cassia tora*	200 mg/kg p.o.	[17]
6	Test 2	*Cassia tora*	400 mg/kg p.o.	[18]

Forced swim test

Sharma VK. et al 2009 previously describe The cylindrical tank with flat bottom (30 cm height x 20 cm diameter) was required for the forced swim test. The tank was filled with tap water up to 75% of total height of tank and temperature was maintained at 25ºC±2ºC. The forced swim test was carried out after 1 hr. administration of *Cassia tora* extract and 30 min after administration of imipramine (10 mg/kg) drug. The mice was placed in the tank and record the immobility time for 6 min [19].

Tail Suspension test

Shinde V *et al* 2015 previously explain the tail suspension test for antidepressant activity. The mice were suspended above 58 cm above from floor by adhesive tape placed approximately 1 cm from the tip of the tail. The mice were considered completely immobile only hung passively. The total duration of immobility of tail suspension test was recorded for 5 min each mice was performed four conjugative trials [20].

Elevated Plus Maze

The EPM test has been widely validated to measure the anxiety in mice *SA. Onasanwo et al*. 2018, Pillow S et al 1985 this apparatus was made up of wood and consisted two open arm and two close arm (30cm×5 cm) with 25 cm walls. The arm extends from the central platform. The maze was elevated 38 cm from the room floor. Mice were treated with *Cassia tora* extract 200 and 400 mg/kg p.o. and diazepam 2 mg/kg i.p. 1 hr. prior the experiment. Each of the animals was placed in the center of the instrument and record the number of entry and time spent in open and close arm. The latency time is 5 min [21].

Anoxic tolerance test

This test was based on previously describe by (Aluko O et al 2015) the animals were individually placed in air tight container of 250 ml capacity. The time taken for the mice of first clonic convulsion was taken at the end point. The animals were removed immediately from the jar for recovery [22].

Locomotor Activity

The Locomotor behavior of animal was monitored by actophotometer the animal was placed in actophotomer and basal movement activity was recorded individually of each rat for 10 minutes. Each animal was treated respective drugs *Cassia tora* extract (200 and 400 mg/kg p.o.) and Diazepam (2 mg/kg i.p.) and activity was recorded after 30 min. and 1 hr. [23].

Rota rod test

Bhosale U et al 2011 suggested that neurological depression in mice could be evaluated by using Rota rod apparatus. Animals were placed on Rota rod apparatus at one time with 20 – 25 rpm speed. Only the mice which remain on rod for 5 min. tanning session were used in this study. Fall of time were recorded in all groups of animals [23].

Statically Analysis

The data were analyzed by Graph Pad Prism 5.0 and presented as mean ± SEM stastical test were used one-way Analysis of Variance (ANOVA) followed by turkey test.

Results

Preliminary phytochemical screening

Preliminary phytochemical screening was done to identify the different phytochemical constituents by standard screening protocol which was present in *Cassia tora* seeds it showed that many of effective constituents are present in this plant.

Table 2: represent different phytochemical constituent present in hydro-alcohol extract of *Cassia tora* seeds.

S.N.	Constituents	Present/Absent (+,-)
1	Alkaloid	+
2	Glycoside	-
3	Flavonoids	+
4	Tannins	+
5	Saponin	+

Forced swim test

Forced swim test (FST) was significantly increase the immobility time as compared to normal control mice. Standard drug imipramine (10 mg/kg p.o) was significantly reduced the immobility time as compared to negative control group. Whereas hydro-alcohol extract of *Cassia tora* 200 and 400 mg/kg p.o. was significantly reduced the immobility time after 14 days' treatment, 400 mg/kg of *C. tora* was more effective (Table 3).

Anoxic tolerance test

The effect of Cassia tora on the latency of Anoxic tolerance test induced convulsion in Swiss mice showed in table 4. The result showed that Diazepam treatment mice was significantly increase the appearance of convulsion and

similarly the cassia tora extract 200 and 400 mg/kg was also increase the appearance time but the aqueous extract of Cassia tora 400 mg/kg was showed more effective as compared to 200 mg/kg.

Table 3: Result expressed as Mean ± SEM (n=5) significant at *P<0.05, **P<0.01, *** P< 0.001 vs Negative control group by one way ANOVA followed by post Tukey test.

S.N.	Group	Dose	Immobility time		
			0th day	**7th day**	**14th day**
1	Negative control	CMC (2 mL/kg)	184.10±8.06	184±8.04	191.66±6.18
2	Imipramine treatment	10 mg/kg	136.43±4.28***	132.3±3.29***	124.33±3.85***
3	*Cassia tora* treatment	200 mg/kg	171.27±6.75*	160±3.55*	151.66±2.86*
4	*Cassia tora* treatment	400 mg/kg	150.77±5.81**	141±2.94**	134.33±0.94**

Abbreviation: CMC (Carboxyl methyl cellulose), ANOVA (Analysis of Variance), SEM (Standard Error Mean), i.p. (Intraperitoneal), mg. (Milligram), kg. (Kilogram). FST (Forced Swim Test), p.o. (per oral)

Table 4: Result expressed as Mean ± SEM (n=5) significant at, *** P< 0.001 vs Negative control group by one way ANOVA followed by post Tukey test.

S.N.	Group	Dose	Appearance of Convulsion
1	Normal control	CMC (2 mL/kg)	1.63±0.02
2	Diazepam treatment	2 mg/kg i.p.	3.28±0.11***
3	*Cassia tora* treatment	200 mg/kg p.o.	1.76±0.10
4	*Cassia tora* treatment	400 mg/kg p.o.	2.48±0.12***

Abbreviation: CMC (Carboxyl methyl cellulose), ANOVA (Analysis of Variance), SEM (Standard Error Mean), i.p. (Intraperitoneal), mg. (Milligram), kg. (Kilogram).

Tail Suspension Test

As shows in table 5 the result indicates that hydro-alcohol extract of *C. tora* (200 and 400 mg/kg) seed reduce the immobility time in albino Swiss mice in tail suspension test. The obtained results were found significantly (*P<0.05, **P<0.01) respectively.

Table 5: Result expressed as Mean ± SEM (n=5) significant at *P<0.05, **P<0.01, Vs Negative control group by one way ANOVA followed by post Tukey test.

S.N.	Groups	Immobility time		
		0 day	**2nd day**	**4th day**
1	Negative control (CMC 2 mL/kg)	3.73±0.53	4.01± 0.50	3.95±0.39
2	Imipramine (10 mg/kg)	2.59±0.44**	2.8±0.43**	2.05±0.55**
3	*Cassia tora* (200 mg/kg)	2.73±0.44*	3.25±0.15*	2.87±0.34*
4	*Cassia tora* (400 mg/kg)	2.38±0.79**	2.91±0.37**	2.34±0.17**

Abbreviation: CMC (Carboxyl methyl cellulose), ANOVA (Analysis of Variance), SEM (Standard Error Mean), i.p. (Intraperitoneal), mg. (Milligram), kg. (Kilogram), SD (Standard deviation).

Elevated plus maze test

The effect of *Cassia tora* on the time latency of elevated plus maze test induced anxiety in Swiss mice showed in table 6. The result showed that Diazepam 2 mg/kg treatment mice was increase the time latency in open arm and similarly the cassia tora extract 200 and 400 mg/kg was also increase the latency time but the hydro-alcohol extract of *Cassia tora* 400 mg/kg was showed more effective as compared to 200 mg/kg.

Table 6: Effect of CTEE in open arm elevated plus maze test induced anxiety in Swiss mice

S.N.	Groups	Time spent in open arm		
		0 day	**2nd day**	**4th day**
1	Negative control (CMC 2 mL/kg)	1.57±0.34	0.87±0.42	0.41±0.13
2	Diazepam (2 mg/kg)	0.96±0.32	2.51±0.52	2.2925±0.50
3	*Cassia tora* (200 mg/kg)	0.88±0.66	1.9625±0.38	2.17±0.49
4	*Cassia tora* (400 mg/kg)	0.765±0.53	1.7775±0.20	2.34±0.46

Note: Result expressed as Mean ± SD (n=5) there is no significant Vs Negative control group.
Abbreviation: CMC (Carboxyl methyl cellulose), mg. (Milligram), kg. (Kilogram), SD (Standard deviation).

Table 7: Effect of *Cassia tora* ethanol extract in close arm elevated plus maze test induced anxiety in Swiss mice

The effect of *Cassia tora* on the latency of elevated plus maze test induced anxiety in Swiss mice showed in table 6. The result showed that Diazepam 2 mg/kg treatment mice was decrease the time latency in close arm and similarly the *Cassia tora* extract 200 and 400 mg/kg was also decrease the latency time but the hydro-alcohol extract of *Cassia tora* 400 mg/kg was showed more effective as compared to 200 mg/kg (Table 7).

S.N.	Groups	Time spent in close arm		
		0 day	**2nd day**	**4th day**
1	Negative control (CMC 2 mL/kg)	3.87±0.24	4.37±0.36	4.87±0.31
2	Diazepam (2 mg/kg)	4.07±0.44	4.90±0.42	3.95±0.52
3	*Cassia tora* (200 mg/kg)	3.92±0.59	4.79±0.32	3.33±0.59
4	*Cassia tora* (400 mg/kg)	4.06±0.65	4.87±0.39	3.78±0.34

Note: result expressed as Mean ± SD (n=5) there is no significant Vs Negative control group.
Abbreviation: CMC (Carboxyl methyl cellulose), mg. (Milligram), kg. (Kilogram), SD (Standard deviation).

Table 8: Effects of *Cassia tora* ethanol extract on locomotor activity/muscle relaxant in restraint stress test

S.N.	Group	Treatment	No. of count in actophotometer	Fall of time in Rotarod
1	Normal control	CMC 2 mL/kg	66.50±2.59	67±1.76
2	Negative control	CMC 2 mL/kg	45.67±2.16	44.27±1.32
3	Diazepam treatment	2 mg/kg	57.17±3.45**	60±1.80***
4	*Cassia tora*	200 mg/kg	53.17±3.54	57±1.81
5	*Cassia tora*	400 mg/kg	63.30±4.19*	57.17±1.45**

Note: Result expressed as Mean ± SEM (n=6) significant at *p<0.05. ** P<0.01, ***P< 0.001 vs control group by one-way ANOVA followed by Post Tukey test.
Abbreviation: CMC (Carboxyl methyl cellulose), mg. (Milligram), kg. (Kilogram), SD (Standard deviation).

Table 9: Effect of *Cassia tora* ethanol extract on lymphocytes, monocytes, eosinophil, RBC, and glucose level in Anoxic stress model

S.N.	Group	Biochemical estimation				
		Lymphocytes %	**Monocytes %**	**Eosinophil %**	**RBC (million/cmm)**	**Glucose (µg/dL)**
1	Normal control (CMC 2 mL/kg)	56.93±1.14	1.58±0.22	1.14±0.05	10.26±0.17	92.50±1.37
2	Negative control (CMC 2 mL/kg)	70.29±1.03	6.53±0.20	4.37±0.23	18.08±0.57	142.61±8.7
3	Diazepam 2 mg/kg	62.13±2.22**	1.90±0.11	1.15±0.11	12.85±0.53*	110.51±6.43
4	*Cassia tora* 200 mg/kg	63.93±1.65	4.07±0.17	2.18±0.27	16.83±0.59	123.42±1.49
5	*Cassia tora* 400 mg/kg	64.07±1.80	3.64±0.14	2.25±0.14	12.25±0.62	122.93±4.52

Note: Result expressed as MEAN±SEM (n=6) significant at*P< 0.05,**P<0.01, vs negative control group by one way test.
Abbreviation: CMC (Carboxyl methyl cellulose), mg. (Milligram), kg. (Kilogram), SD (Standard deviation), mL (milliliter) % (percentage), RBC (Red Blood Cells), µg (micro gram).

Table 10: Effect of CTEE on cholesterol, glucose, triglycerides and protein level in Anoxic stress model

S.N.	Groups	Biochemical estimation			
		Cholesterol	**Glucose**	**Triglycerides**	**Protein**
1	Normal control (CMC 2 mL/kg)	70.68±1.73	92.50±6.15	62.93±9.20	3.39±0.24
2	Negative control (CMC 2 mL/kg)	96.16±5.15	118.51±11.46	93.13±5.47	6.35±1.43
3	Diazepam 2 mg/kg	82.63±1.70**	113.50±2.18	82.15±1.54	5.16±1.08***
4	*Cassia tora* 200 mg/kg	72.70±3.10	115.46±6.64	80.35±3.25	5.90±1.37
5	*Cassia tora* 400 mg/kg	78.18±2.89	121.35±5.69	81.78±1.78	5.85±1.33

Note: Result expressed as Mean± SEM (n=6) significant at **P<0.01, ***p<0.001 vs Control group by one-way ANOVA followed by post tukey test.
Abbreviation: CMC (Carboxyl methyl cellulose), mg. (Milligram), kg. (Kilogram), SD (Standard deviation), mL (milliliter)

Discussion

Anxiety and depression are a very common disorder in the world population; out of four, one adult has some anxiety at the same point in their life [24]. Depression affects the mood, lack of interest, and confidence, poor concentration, disturbed sleep and unusual negative thought [25]. Ayurveda provides a lot of medicinal plants to that the different diseases and counteracts the adverse effect [26]. *Cassia tora* is one of the most common types of medicinal plants used to treat different kinds of illnesses. In this study, we use the Hydro-alcohol extract of *Cassia tora* as an antidepressant effect in Swiss mice. There is various model are available to screen the antidepressant activity in Swiss mice. There is various model are available to screen the antidepressant activity.

Forced swim and tail suspension test is the most widely used models for antidepressant screening. The plant extract of *Cassia tora* was used for in vivo antidepressant activity. The dose was selected based on acute toxicity study from the literature [13]. In forced swim test the result showed that oral administration of *Cassia tora* 200 and 400 mg/kg compared to negative control and imipramine (10 mg/kg p.o.) the 400 mg/kg (10.77±5.81, 141±2.94, 134.33±0.94) of *Cassia tora* showed the potent effect to decrease the immobility time as compared to

negative control (184±8.06, 184±8.04, 191.66±6.18) and similar to 10 mg/kg imipramine (136.43±4.28, 132.3±3.29, 124.33±3.85) at different time intervals (table 3). to evaluate the anxiolytic activity of *Cassia tora* extract of elevated plus maze (EPM) test was applied. It is a well-known model that has been use to determine the fear of rodents. The result showed that in EPM of the open arm, the *Cassia tora* extract increased the time period in open arm and similarly also increase the time period in the close arm as compared to the negative control and 2 mg/kg of diazepam (table 6 &7). Evaluating the Locomotor activity by using actophotomer showed that ethanol extract of *Cassia tora* at the dose of 200 (53.17±3.54) and 400 mg/kg (63.30±4.19) significantly increase the number of mobility count in actophotometer as compared to the negative control and diazepam (2 mg/kg) treatment (Table 8). Rotarod test the diazepam is effective skeletal muscle relaxant (SKM) 2 mg/kg diazepam was administered in Swiss mice and 200 and 400 mg/kg of *Cassia tora* extract the result showed that the mean fall of time was decreased in the negative control (44.27±1.32) as compared to diazepam (60±1.80) similarly *Cassia tora* extract of different dose 200 mg/kg (57±1.81) and 400 mg/kg (57.17±1.45) was significantly increase the fall of time and the dose of 400 mg/kg was more significant as compared to 200 mg/kg. Biochemical estimation of Swiss mice showed that the standard, and test group was improved their blood components quantities (lymphocytes, monocytes, eosinophil, RBC) and reduced the cholesterol, triglyceride, and blood glucose level (table 9 and 10).

Conclusion

We concluded that the present study was provided evidence for antidepressant and anxiolytic activity of hydro-alcohol extract of *Cassia tora* comparable with the standard group. Terpenoids and flavonoid are the main active phytochemical constituents which are responsible the activity. However, further study must be carried out to detect and synthesize the principal active phyto-consituents to show this activity.

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Conflict of interest

The authors declare that there is no conflict of interest.

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