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Anxiolytic and antidepressant potential of extracts of *Duchesnea Indica* in animal models

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ABSTRACT: THIS study was designed to investigate the anxiolytic and antidepressant activity of *Duchesnea Indica*. The methanolic extract and *n*-hexane fraction of plant were tested in albino mice (20-30 g of 6-8 week). Safety profile of each extract was determined at different doses. Anxiolytic effect of plant was determined by Elevated Plus Maze and Light and Dark Model at different doses of methanolic and *n*-hexane extract. Antidepressant activities were assisted by Tail Suspension and Forced Swim Model at different doses. Result illustrated that *D. Indica* had a significant ($P < 0.05$) potential of reducing anxiety and depression. The methanolic extract at 100 mg and 200 mg significantly increased the time spend in light region of light and dark model and more time spent in open arm of Elevated Plus Maze model. *n*-hexane extract at dose of 5 mg and 10 mg/kg significantly increases the time spend in light region of Light and dark model and more time spend in open arm of Elevated Plus Maze model as compare to Control group. *Methanolic* extract of *D. Indica* significantly reduced the time of immobility in Tail Suspension and Force Swim Model at Dose of 100 mg and 200mg/kg. n-hexane extract also exhibit anti-depressant effect by reducing the time of immobility in Force swim and tail suspension Model at 100 mg and 200mg/kg dose as compare to control group. From the above observations, it could be assumed that the plant has marked anxiolytic and antidepressant potential. However further additional studies will be necessary to determine the underlying exact mechanism and clinical uses.

Keywords: *Duchesnea Indica*; crude extract; hexane fraction; Anxiolytic and antidepressant effects.

1. Introduction

ACCORDING to world health organization report 2013, approximately 450 million people suffer from mental and behavioral disease and this is almost 12.3 % to be claimed to rise up further in future (Herrera-Ruiz et al., 2006). Anxiety is life threatening and uncontrollable fear with marked number of causes. WHO has defined mental health as "a state of well-being in which every person realizes his or her own

potential, can work fruitfully and productively, and is able to make contribution to his or her community and can cope with the normal stresses of life"(Galderisi et al., 2015).

Anxiety has negative impact on quality of life and is known to increase morbidity and mortality (da Silva Oliveira et al., 2014). Anxiety may interfere with memory, intelligence and psychomotor functions (Pine et al., 1999). Depression is common and chronic mental disease characterized by change in behavior, mood , thought, physical changes , physical and psychological impairment (Fekadu et al., 2017) . In both developing and developed countries anxiety and depression is associated with chronic pain and therefore need strategy based on natural product for effective management (Fedotova et al., 2017).

Different pharmacotherapeutic agents are uses to treat anxiety and depression but due to their potential side effects, narrow safety margin and slow action leads to search for new drugs which less side effects, wider safety margin and fast action (Sartori and Singewald, 2019). *Duchesnea Indica* also named Indian *Mock Strawberry* herb has been uses as a folk medicine in China for a century in various clinical setting. Previous study documented that *D. Indica* has anti-inflammatory, anti-mutagenic , anti-biotic, cytotoxic and ant-oxidant activity (Umesh and Thoppil, 2014). It has been used as a main ingredient in traditionally use formula for the treatment of cancer in Japan and China (Zhu et al., 2015). *D. Indica* also called *Fargeria Indica* because of similarity with *Frageria* genus and various species *of frageria* like anasa, *frageria Vesca* which have proven antidepressant affect (Hazar and Fitri, 2015). *D. indica* is reported to be an excellent source of polyphenols, sterol, trephines and particularly Flavonoids such as quercitrin, myrecitin Ellagic Acid and keampferol which have proven CNS activities (Zhu et al., 2015).

Owing to its multiple traditional uses and strong chemical background, the current study was designed to investigate the anxiolytic and Anti-depressant like effects of *D. Indica* in different experimental models.

2. Material and Methods

2.1. Chemicals

n-hexane, Methanol 80 %, Water for injection, Diazepam, imipramine, sulfoxide (DMSO), carboxymethylcelloluse.

2.2. Experimental Animals

From veterinary and farm section of national institute of health (NIH) Islamabad male and female albino mice 20-35 gm, age 6-8 weak were obtained. Test mice were divided into different groups and each group having 5 test animals. The animals were acclimatized for 3 days with laboratory condition with standard feed, clean water for drinking and temperature that is 22 +3 C hours. The light and dark cycle was 12 h. The test animals were treated throughout the experiment in accordance to the rules and international guidelines.

2.3. Plant Collection

Plant *D. Indica* (6 kg) was collected from Darmai, Matta Swat, in the north of KPK Pakistan. Identification was carried out by Prof Pukhtoon Zada Department of Botany Government Afzal Khan Lala post graduate college of Matta Swat, voucher No GPGCM-67 Herbarium was kept as a reference in herbium of college.

2.4. Extraction

After collection of Plant *D. Indica*, the entire plant was washed and dried in shade and then grinded into 2 kg powdered form. Then dried powder was macerated with 80 % methanol and *n*-hexane for one weak with intermittent stirring and then the same process was repeated three times with fresh solvent. Solvent than evaporated with rotary evaporator at 42 C with reduces pressure and 80 gm of crude extract was isolated. The dried extract was stored in refrigerator and used for pharmacological investigation. There are 9 groups having 5 test experimental animals in each group for each model. Diazepam (1mg/kg) and Imiperamine (10mg/kg) were used as standard drugs while Normal saline (10ml/kg) were used as control. Aqueous methanolic and N-hexane extract of different specified doses were administered intra-peritoneal before 30 minutes of conducting activities. Behavioral changes were recorded by two recording cameras for six minutes.

2.5. Acute Toxicity Test

Acute toxicity test was conducted to determine toxic dose. Limit dose of 2000 mg/ kg methanolic extract and 25 mg n-hexane was selected following the standard procedures of organization for the economic cooperation and development guidelines (Chemicals, 2005) Three groups each containing 3 albino mice having

average weight of 25-30 gm were selected to conduct the experiment. The experimental mice were restricted from food and drinks before 4 hours conducting the experiments. Different doses of different strength like 500 mg 1000 mg and 2000 mg of methanolic extract and 15mg, 20 mg and 25 mg per kg given to each group respectively. After two hours of the dose food and drink were provided and start and start observation of each mouse individually for death and toxicity signs for early 30 min and then intermittently for 24 h.

2.6. Anxiolytic Activity

2.6.1. Light and Dark Models

The apparatus composed of wooden Box with two compartments (25 x 33 x 24) of size. Both are connected with one another by the help of opening 10 x 10 through which mice were move from one compartment to other. One compartment was lighted and open while other remained dark. The time spent by mice in open lighted compartment was recorded for 5 minutes as quickly as the mice mouse was stepped into dark box. The mice were treated with respective doses 30 minute before being placed in box (Campos et al., 2013).

2.6.2. Elevated Plus Maze Model Test

Elevated plus Maze model have four Arms which are 40 cm long and 20 cm wide, these arms are arranged that two arms of a same type were opposite to each other. The EPM contain two open arms and two close arms. The EPM is elevated 50 cm above the floor (Rabbani et al., 2003).

2.6.2. Antidepressant like activity tests

2.6.2.1. Force Swim Test

It consists of cylinder tanks having (30cm x 20cm diameter) parameters. The water level is 15 cm above from the bottom. The dimension of cylinder is selected that the mice will not be able to touch the bottom of tank either with their tail or feet. The mice will allow to swim in cylinder filled with water having temperature 25 ^{0}C. The floating behavior where the animals remain almost immobile and its head above the water is used as parameter to analyze.

2.6.2.2. Tail Suspension Test

Tail suspension test is employed on rodents to predict antidepressant potential by decreasing immobility period by several different classes of antidepressant drugs. Tail suspension test is behavior despair model of depression. It has been reported that tail suspension test is higher pharmacological sensitivity than force swim test (Kothari et al., 2010). In this test mice were suspended on the edge of table approximately 1 cm from the tip of the tail with help of adhesive tape. Immobility duration induced by tail suspension was recorded during 6 mint period. When the mice did not show any movement of the body except for those required for respiration and hang passively the animal were considered as immobile (Can et al., 2012).

3. Results

3.1. Effect in Acute Toxicity Test

Acute toxicity test was performed on the crude and *n*-hexane extract of *D. Indica*. Showed its non-toxic nature at different doses. The methanolic extract at the
doses of 500 mg 1000 mg and 2000 mg/kg and *n*-hexane extract at the doses of 15 mg 20 mg and 25 mg as shown in table 3.1. There is no sign of toxicity or mortality observed in mice to which high dose of crude and *n*-hexane drug extract were administered. As mentioned above which shows that LD50 of the extract of *D. Indica* could be above 2000 mg/kg of crude methanolic extract and 25 mg of *n*-hexane extract. There were no even behavioral change likes immobility, alertness, breathing problem, diarrhea, restleness and convulsion problems observed during 24 hours of examination period.

3.2. Anxiolytic Models

3.2.1. Effect in Dark and Light Model

Methanolic extract of *D. Indica* was administered intra-peritoneal to different experimental groups and observed in Light and Dark model table 3. Increase the dose in LDT, there is significantly increased in the time spends by mice in Light side of light and dark chamber. In Control group which is treated with normal saline, the time spend by mice was recorded 104 ± 6.6 seconds. While the mice treated with standard that is benzodiazepine was 160 ± 6.8 second. Treating different experimental groups of mice with methanolic extract with 50 mg and 100 mg and 200 mg/kg, the time spend by mice increased significantly ($P< 0.0001$) to 132 ± 7.1 and 159 ± 7.2 as compared with control group that is normal saline. The highest time spend by mice in light region was observed at 200 mg that was 164 ± 4.4 seconds. Similarly, the time spend by mice in light region was significantly ($^{***}P< 0.001$) increased by administration of *n*-hexane extract

with different dose that are 2.5 mg, 5 mg and 10 mg/kg. The highest time spend in light region was observed at 5 mg/kg that was 180 ± 9.1 sec, while at 2.5 mg the time spend in light region was observed 35 sec while at 10 mg it was 126 sec.

Table 1: Toxicity profile and percentage of mortality on animal model

Extract	Dose (extract)	Animal Died	Animal survived	% Mortality
Methanolic	500 mg	Nill	All	0
	1000 mg	Nill	All	0
	2000 mg	Nill	All	0
n-Hexane	15 mg	Nill	All	0
	20 mg	Nill	All	0
	25 mg	Nill	All	0

Table 2: Physical Behavior of mice after 24 h of administration of extracts in acute toxicity test

Extract	Dose	Convulsion	Sedation	Immobility	Breathing problems
Methanolic	500 mg	_	_	_	_
	1000 mg	_	_	_	_
	2000 mg	_	+	_	_
n-Hexane	15 mg	_	_	_	_
	20 mg	_	_	_	_
	25 mg	_	++	_	_

Different doses of *D. Indica* were administered to a different mice group and are observed at EPM test apparatus. The result shows that by increasing dose the time spend by mice in open area increases as compared with control group. There was highly significant (P<.0001) increased in time spend by in the open arm at 50 mg 100 mg and 200 mg to 36 ± 4.4, 105 ± 8.2 and 109 ± 9.1 as compare to control group that is 27.8 ± 6.1 seconds. The highest time spend by mice was observed with dose of 200 mg / kg. Also, the number of entries of mice increased significantly P< 0.01 as a compare to control group. Different doses of *n*-hexane extract administered to different test groups and observed in Elevated plus maze model. The time spends by mice in open arm was significantly (P<0.001) increased as compare to control group. At 2.5 mg the time spend by mice increased to 46 ± 6.1 seconds and at 100 mg it increased to 73 ± 13 seconds and the maximum time spend by mice in open arm was observed at 10 mg /kg that is 109 ± 8.8 seconds

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Table 3: Effect of *D. Indica* crude extract and hexane fraction on the behavior of mice in Light and Dark model

Group	Dose (mg/kg)	Light region		Percentage
		Time Spent(sec)	No of Entries	
Diazepam	1	160±6.8	9±1.6***	44
Methanolic Extract	50	132±7.1	6.4±2.5**	36
Methanolic Extract	100	159±7.2	9.2±1.6***	44
Methanolic extract	200	164±4.4	11±1.8***	45
n-hexane extract	2.5	35±5.7	4.6±2.6	9.1
n-hexane extract	5	180±9.1	11±2.8***	50
n-hexane extract	10	126±8.5	8.8±2.7**	35
Normal saline	10	104±6.6	5.8±1.1	

Data are represented as mean ±SEM (n=5), one way ANOVA used with Graph pad prism. N/S = normal saline, DZM= Diazepam, administered at 1mg/kg, methanolic extract 50 mg, 100 mg and 200 mg /kg, *n*-hexane extract 2 mg, 5 mg and 10 mg /kg used as test group. The level of statistical significance was *P< 0.05.

Table 4: Effect of *Duchesnae Indica* methanolic and n-hexane extract on the behavior of mice in Elevated plus maze model

Group	Dose (mg/kg)	Open Arm		% Effect
		Time Spent(sec)	No. of Entries	
Diazepam	1	110±8.1	8.7±0.5***	30.5
Methanolic Extract	50	36±6.1	4±1.2	10
Methanolic Extract	100	105±8.2	5.2±1.3	29
Methanolic extract	200*	109±9.1	8.7±2.01	30.1
n-hexane extract	2.5	46+6.1	4.5+1.7	12
n-hexane extract	5	73+13	7+1.15	20
n-hexane extract	10	109+7.8	7.5+1.9	30.1
Normal saline	10	27±8.1	6.2±2.2	7.5

Data are represented as mean ±SEM (n=5), one way ANOVA used with Graph pad prism. N/S = normal saline, DZM= Diazepam, administered at 1mg/kg, methanolic extract 50 mg, 100 mg and 200 mg /kg, *n*-hexane extract 2 mg, 5 mg and 10 mg /kg used as test group. The level of statistical significance was *P< 0.05.

3.3. Depression Models

3.3.1. Effects in Tail Suspension

There is significant ****(P< 0.0001) decrease in time of immobility when different doses of methanolic extract was administered and compared with control group and observed for six minutes in tail suspension model (figure 1). At 50 mg the time of immobility was recorded 140 ± 8.5 and at 100 mg the time of immobility was reduced to 129 ± 6.9 seconds and at 200 mg the time reduced to 109 ± 5.1 seconds as compared to control group which is recorded 155 ± 8.5 seconds. By administering different doses of *n*-hexane extract to different experimental group there is significant **** (P<0.0001) decrease in immobility time as compare to control group. At 2.5 mg the time of immobility was reduced to 126 ± 10.1 seconds as compared to control group of normal saline that was 162.4 seconds, it 5 mg the time of immobility was recorded 143 ± 5.7 sec and it 10 mg it was reduced to 114 ± 4.6 sec.

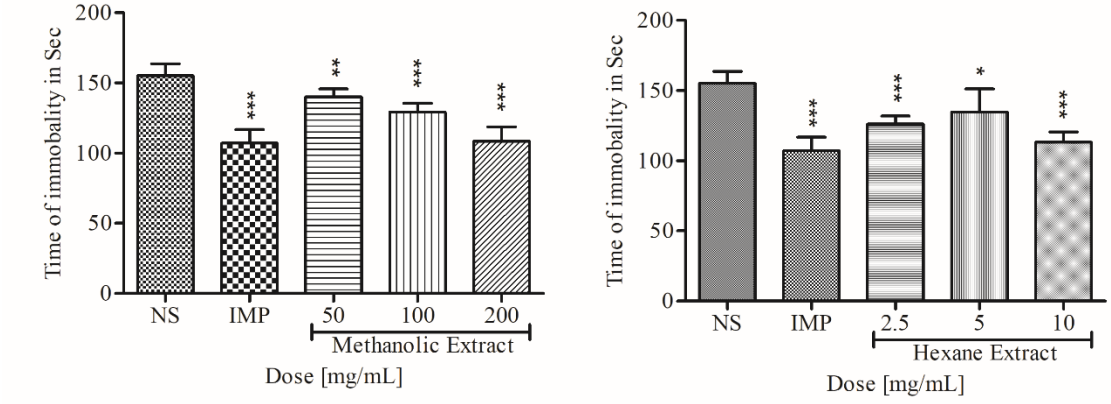

Figure 1. Effect of *Duchesnae Indica* Methanolic extract and *n*-hexane extract on the behavior of mice in Tail Suspension Model. Data are represented as mean ±SEM (n=5), one way ANOVA used with Graph pad prism. N/S = normal saline, IMP= Imiperamine (10mg/kg), methanolic extract 50 mg, 100 mg and 200 mg /kg, *n*-hexane extract 2 mg, 5 mg and 10 mg /kg used as test group. The level of statistical significance was *P< 0.05.

3.3.2. Effect in Forced Swim Test

Different doses of *D. Indica* were administered to the different group of mice and observed in FST (figure 2). The result showed that the immobility time decreases as we increase the dose. Different doses of Methanolic extract administered intra-peritoneal to different experimental groups and observed in force swim test. The result showed that there was significant ****(P< 0.0001) decrease in immobility time occur as compare to control groups. At 50 mg the time of immobility was reduced to 131 and at 100 mg was noted 146 and at 200 mg the time of immobility was reduced to 115 as compared to control group which is 178 sec. *n*-hexane extract was administered to different experimental groups and immobility time was observed for six minutes in Forced Swim model. As the dose increased the immobility time was significantly (P< 0.0001) decreased as compare to control group. The minimum time of immobility was observed at 10 mg which was 93 sec as compared to control group.

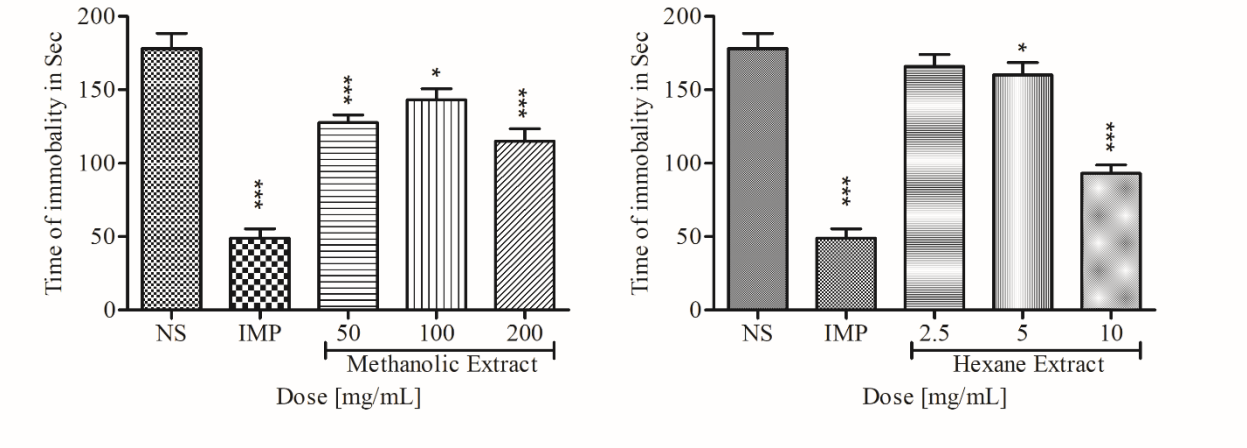

Figure 2. Effect of *Duchesnae Indica* Methanolic extract and *n*-hexane extract on the behavior of mice in Force swim model.

4. Discussion

The current study deals with the acute toxicity, anxiolytic and antidepressant like effects of crude and *n*-hexane extract of *D. Indica* in animal paradigms.

Acute toxicity test was performed on the crude and *n*-hexane extracts of *D. Indica* which showed its non-toxic nature at different doses including methanolic extract at the doses of 500 mg 1000 mg and 2000 mg/kg and *n*-hexane extract at the doses of 15 mg 20 mg and 25 mg. There was no sign of toxicity or mortality observed in mice to which high dose of crude and *n*-hexane drug extract were administered. Which show that LD_{50} of the extract of *D. Indica* could be above 2000 mg/kg of crude methanolic extract and 25 mg of *n*-hexane extract. Similarly, there was no behavioral change like alertness, breathing problem, diarrhea, restleness and convulsion problems observed during 24 h of examination period.

Light and Dark and elevated plus maze model are the most commonly used models for the assessment of anxiolytic like effects of test articles (Sasidharan et al., 2011; Walf and Frye, 2007). In light and dark model, the movement of mice toward dark and close area shows the state of anxiety (Bourin and Hascoët, 2003; Jawad et al., 2017). The Light and Dark test is based on an approach-avoidance conflict between exploration of novel environment and avoidance of brightly lit open space (Arrant et al., 2013). In Elevated Plus Maze model, the movement toward close spaces and fear from height shows the state of anxiety. The anxiolytic drug increases the number of entries and time spend in open arm (Komada et al., 2008). Herein, we employed the same to study the anxiolytic like effect of *D. Indica* extracts including both crude and hexane fraction. Furthermore, the result of the experiment shows that by administering different doses of *D. Indica* in mice was the potential of anxiolytic effect without customizing motor activity. Some major outcomes were noted when anxiety base models that is LD and EPM

were used for examining of anxiolytic potential of *D. Indica*. At 200 mg/kg of crude extract and 10 mg of *n*-hexane extract showed the mice spend maximum time in light part of LD chamber and open arm of EPM model. The effects were significant for both extracts at various test doses, while hexane was more potent therefore revealed that the nature of pharmacological active compounds is non-polar.

The elevated Plus maze model is one of the important and most popular models of all currently available animals' models for anxiety. This test has a strong predictive validity for screening. Anxiolytic drugs. The anxiogenic drug decreases and anxiolytic drug specially increases the number of entries and time spend in the open arm. The open arm and close arm considered to evoke the same exploratory drive, but the avoidance of open arm is considered to be the result of high level of anxiety. Therefore, the reduction in the aversion of open arm are considered to be caused by anxiolytic agent (Komada et al., 2008).

Forced Swim and tail suspension models were used to study antidepressant activity of *D. Indica* which are frequently used (Chang et al., 2016; Mohseni et al., 2017; Naidu et al., 2007). In tail suspension test the mice were suspended from the edge of table over the height of 50 cm. After suspension of mice by tail through sticking, the immobility time of mice is measured with stop watch for the total duration of 6 minutes. Mice were considered immobile when they hung passively and completely motionless. The result of the experiment showed that by administering different doses of plant extract to the Experimental groups significantly ($P< 0.001$) increases the time spend in open arm as compare to control group. By administering different doses of methanolic and *n*-hexane extract of *D. Indica* exhibited diminution of immobility of time of mice expose to force swim and tail suspension. The immobility time reflect depressive behavior. By administering different doses of methanolic and *n*-hexane extract of *D. Indica* reduce the immobility time significantly as compare to control group.

Forced swim test were used to evaluate antidepressant activity (Mohseni et al., 2017). In this test immobility time are measured by placing the mice in inescapable cylinder filled with water. In this assessment the climbing behavior, swimming behavior and immobility time was recorded. Immobility was considered when the mice made no further attempt to escape and makes only the movement to keep its head above the water. The immobility of mice shows the depress state. By administering different doses of methanolic and *n*-hexane extract of *D. Indica* reduce the immobility behavior significantly as compare to control group

As we compare the activity of both crude methanolic extract and *n*-hexane extract, *n*-hexane have a little higher anxiolytic activity than crude extract, at 5 mg /kg of *n*-hexane the mice spend more time in open arm of elevated Plus maize model as compare to methanolic extract but in Light and dark model both have almost same activity. Similarly, n-hexane extract has more antidepressant activity than crude methanolic extract because at 10 mg of *n*-hexane extract the time of immobility was recorded 93 sec while the minimum immobility time of methanolic extract was observed at 200 mg /kg that was 115 seconds in force swim model while in tail suspension both extracts have same value.

5. Conclusion

The result of our analysis showed that *D. Indica* has significant potential of reducing anxiety and depression. The maximum time spent in open arm in light and dark model at high dose and more type spend in open arm as compare to closed arm in elevated plus maze model at high dose as compare to control group. The plant also reduced the immobility time of mice in tail suspension and force swim models which show its antidepressant like activity at different doses. Keeping in view these effects, further in-depth and advance molecular studies are suggested to elucidate the exact mechanism and further possible clinical uses.

Author's contribution: Mohibullah and Abdul Saboor Pirzada carried out experimental work. Michael Aschner designed the study while Haroon Khan supervised the study.

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