

SHORT COMMUNICATION

Evaluation of antianxiety and antidepressant properties of *Carthamus tinctorius* L. (Safflower) petal extract

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Abstract: Nowadays anxiety and depression are most commonly encountered diseases. They are not only difficult to diagnose but even difficult to treat since both are sometimes seen together or one predisposes the other. Apart from this side effect profile of these drugs is also high; hence there is immense scope for the herbal drugs to treat these disorders. Present study was therefore performed to evaluate the antianxiety and antidepressant effect of *Carthamus tinctorius* petal extract. 28 white albino rats bred in the animal house of Department of Pharmacology, University of Karachi weighing 180-220gm were randomly divided into four groups (n=7/group) to assess behavioral effects. The anxiolytic and antidepressant effects of *Carthamus tinctorius* petal extract were evaluated using elevated plus maize and forced swim test respectively at 100 and 200mg/kg. These effects were compared with standard drugs Diazepam (anxiolytic) 2mg/kg and Nortriptyline (antidepressant) 12.5mg/kg. Results show that CT produced highly significant anxiolytic and antidepressant effects at both doses as compared to control, similar to standard anxiolytic and antidepressant drugs diazepam and nortriptyline. It increased the latency of first entry to closed arms and the time spent in open arms very significantly at both doses while entries to open arm were increased significantly at 100mg/kg and very significantly at 200mg/kg in EPM test and increased the immobility time very significantly in FST. Hence it can be concluded that CT may be used as an alternative therapeutic agent while treating patients with anxiety and depressive disorders.

Keywords: *Carthamus tinctorius* L., antidepressant, antianxiety, forced swim test, elevated plus maize.

INTRODUCTION

Depression and anxiety has gained attention as the incidence of mental illnesses is increasing significantly owing to multiple reasons (Norquist & Regier, 1996). These disorders represent a considerable amount of the global load of disease, and are expected to form the second common cause of morbidity by year 2020 (Murray and Lopez 1996).

Anxiety disorders are among the most common behavioral and emotional problems (Kessler *et al.*, 2005a, 2005b; Olatunji *et al.*, 2007), affecting one-eighth of the population worldwide, and have gained significant attention in research performed in the field of psychopharmacology (Eisenberg *et al.*, 1998; Dopheide & Park, 2002). Different types of psychological treatments have been used to relieve the symptom of stress and anxiety but still none of these have achieved a successful and satisfactory response as almost all of these agents produce side effect (Gold berg, 1984; Aisha *et al.*, 2005).

On the other hand 21% of the world's population is estimated to be affected by depression alone (Paykel

2006). Depression manifests itself as impairment of mood, certain symptoms of disorders of depression overlie with the anxiety symptoms together with phobias and panic agoraphobia and obsessive-compulsive disorder. Being a significant global public health issue depression is associated with substantial disability (Goodwin & Bunney, 1971; Gold *et al.*, 1988). Though a number of drugs are easily available in the market; but most of them are allied with some restrictions and drugs to combat both anxiety and depression are very few (Gupta *et al.*, 2011). Hence the search is again focused on herbal products as these are usually thought to be devoid of the side effects. *Carthamus tinctorius* L. (CT) is a thistle like plant grown in Pakistan, China, Iran, India and many others parts of the world. It is Latinized synonym of the Arabic world quartum or gurtum which refers to the color of the dye extracted from safflower flowers. In Pakistan and India it is known as kusumba or kusum, in China as hong hua while in Persian as gulrang (Li and Mundel, 1996). Petals are important source of traditional medicine (More *et al.*, 2005). According to traditional herbal literature it has wide spectrum of pharmacological activity effective against variety of disorders e.g. gynecological diseases, osteoporosis, hyperlipidemia and inflammation (Zhou *et al.*, 2014) while it has also been explored for neuroprotective properties against

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excitotoxic neuronal Death (Yang *et al.*, 2010) reducing the size of cerebral infarct and edema (Ye and Gao 2008). It can also regulate dopamine, 5HT and nor epinephrine transporter levels in the newborn rat brain. These neurotransmitters are essential for regulating cognition, emotion and affection and protect against neuronal injury (Zhao *et al.*, 2009). These monoamines are also held responsible in the pathogenesis of depression and anxiety, thus if their concentration is increased it can reverse the condition (Matrenza *et al.*, 2004; Richell *et al.*, 2005; Roiser *et al.*, 2005). Serotonin (5-HT) receptor family consists of seven main families (5-HT₁–5-HT₇), 5-HT₁ receptors are widely distributed in the central nervous system and other tissues, implicated in pathophysiological processes of several human diseases such as depression, anxiety, migraine, and cognitive dysfunction (Lanfume and Hamon, 2004). Due to the physiological importance of 5-HT₁ receptors, their agonists and antagonists have been explored and developed in order to treat human diseases associated with 5-HT₁ receptors. Interestingly, serotomide and safflomide have chemical structures similar to 5-HT₁ receptor ligands. Thus due to presence of serotomide and safflomide in petals of CT this study was performed to observe its anxiolytic and anti depressant effects as such studies can be a major breakthrough for managing patients of neuropsychiatric disorders after conducting clinical trials in future.

MATERIALS AND METHODS

The study was carried out in Research Institute of Pharmaceutical Sciences, Pharmacology Section after approval from Board of Advance Studies and Research, University of Karachi, Karachi, Pakistan.

Experimental design

28 white albino rats bred in the animal house of Department of Pharmacology, Faculty of Pharmacy weighing 180-220gm were randomly divided into four groups (n=7/group) to assess behavioral effects. All animals were housed in plastic cages at 24±2°C in a natural day and night cycle for one week. Animals were shifted to laboratory for testing at least one hour before start of the experiment. All the experimental procedures were performed during daytime. Animals were kept under standard laboratory conditions with food and water ad libitum. Anxiolytic effect was evaluated through elevated plus maze and anti-depressant activity by forced swim test.

Extraction of plant material

Dried safflowers (petals of *Carthamus tinctorius* L.) were obtained from herbal dealer at Peshawar, Pakistan and identified by Professor Ghazala H Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The dried flowers (100 gm) were soaked in ethanol for eight weeks, filtered and then evaporated in rotary evaporator at 40°C followed by freeze drying at

-30° at HEJ Research Institute of Chemistry. Extract yield was approximately 10gm from 100gm.

Drugs used in experiment

Diazepam and Nortriptyline tablets were purchased from local pharmacy and used as standard anxiolytic and antidepressant drugs respectively. Each tablet was powered in mortar and pestle separately, dissolved in distilled water and administered by orogastric tube. Nortriptyline was given at a dose of 12.5mg/kg (Telner and Singhal, 1981), and diazepam at a dose of 2mg/kg (Gupta *et al.*, 2011). All preparations were given after an overnight fast.

Behavioral tests

Elevated plus maze (EPM)

The elevated Plus maze is a widely used behavioral assay for rodents to assess the anti-anxiety effects of pharmacological agents and mechanisms underlying anxiety related behavior (Walf and Frye, 2007). Animals of each group i.e. control, standard and treated were given a single oral dose of normal saline, standard drugs and extracts of CT at 100 and 200mg/kg respectively one hour prior to testing. Rats were placed at the junction of the four arms of the maze, facing an open arm. Dose schedule was adjusted in a way that every animal had its 5 min session after an hour of administration of drug. Number of entries/duration in each arm was recorded by observer for 5 min. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior. Precautions were taken to avoid any provoked and abnormal response by maintaining noise free environment.

Forced swim test (FST)

The swim test involves the scoring of active (swimming and climbing) or passive (immobility) behavior when rodents are forced to swim in a cylinder from which there is no escape. It is the most commonly used test for assessing anti-depressant activity (Porsalt *et al.*, 1978, Drugan *et al.*, 2010), 28 rats were divided in four groups i.e. a, b, c and d with seven animals in each group. Group a served as control (saline 10ml/kg), b as standard (Nortriptyline 12.5mg/kg) and c and d as treated groups received extract in the doses of 100 and 200mg/kg. Individual animals were placed for swimming in a glass jar filled with water up to 15cm at 25±2°C. The height of jar was 40cm and diameter 24cm. The initial two minutes vigorous activity was followed by a characteristic stationary posture with minimum movements of limbs to hold the head just above water was considered immobile. In a total 6-minute test the immobility time was noted during the last four minutes. Each animal was used only once and subjected to direct immersion after giving drugs one hour before test session.

RESULTS

Table 1 and fig. 1 reveals the anxiolytic effect of *Carthamus tinctorius* petal extract (CT) when compared

to control group, which was similar to the effect of standard drug diazepam. At 100mg/kg CT showed a highly significant change in latency of first entry to closed arms i.e. 32.14 ± 1.67 and 200mg/kg it was 45.00 ± 0.8 seconds as compared to control group i.e. 15.43 ± 1.39 seconds. While there was a highly significant increase in this duration in standard group i.e. 25.00 ± 1.91 seconds as compared to control. Time spent in open arms was changed highly significant in group c and d as compared to control while standard drug diazepam also showed increase in time spent in open arms from 24.57 ± 1.27 seconds to 136.00 ± 2.64 seconds as compared to control, which was highly significant. Similarly number of entries to open arm were also increased significantly at 100mg/kg and highly significantly at 200mg/kg by CT i.e. 1.71 ± 0.48 and 2.14 ± 0.37 respectively as compared to control i.e. 0.71 ± 0.48 , while in Diazepam treated group it increased up to 1.78 ± 0.5 which was also significant.

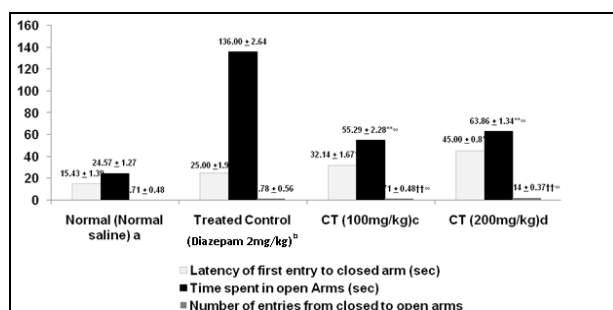


Fig. 1: Comparison of anxiolytic effect of *Carthamus tinctorius* (n=28)

Table 2 and fig. 2 shows antidepressant effect of *Carthamus tinctorius* petal extract, there was highly significant reduction in immobility time of animals in forced swim test at both doses i.e. 100 and 200mg/kg as compared to control, which was similar to the effect of standard drug nortriptyline. The immobility time was reduced to 93.5 ± 3.4 seconds and 74.0 ± 2.5 seconds at 100 and 200mg/kg respectively as compared to control i.e. 153.2 ± 2.0 seconds, whereas decrease in immobility time by nortriptyline was 64.14 ± 2.7 seconds as compared to control, which was also highly significant.

DISCUSSION

People are again diverting towards Complementary and alternative medicine (CAM) due to increased burden of poly pharmacy and adverse effect profile of the drugs. World Health Organization has reported that 80% of the world's population is now using herbal drugs for some reason (Pandey et al., 2011). The number of consumers using CAM therapies was raised significantly from 33.8% to 42.1% between 1990 and 1997 (Ernst, 2000).

Anxiety and depression are prevalent in all parts of the world and are commonly encountered ailments for a

general health care practitioner. Most of the time they appear as co morbid conditions along with chronic diseases like hypertension, diabetes mellitus, rheumatoid arthritis and many more, hence in present study behavioral effects of CT have been evaluated in rats using plus maze (table 1) and swimming induced depression (table 2). Results revealed significant anxiolytic as well as antidepressant effects.

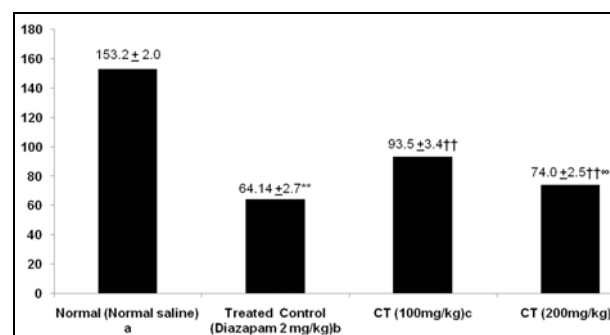


Fig. 2: Comparison of antidepressant effect of *Carthamus tinctorius* L. (n=28)

Since serotonin receptors are believed to be involved in depression and anxiety, these diseases are currently treated with tricyclic antidepressants, selective and non-selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and others. Although therapeutic agents for treating depression and anxiety undoubtedly help alleviate serious conditions derived from the diseases, however there are large number of studies pointing towards numerous adverse effects and gradual loss of efficacy of these drugs (Wernicke, 2004). Hence alternative treatments have been sought for years. In present study safflomid-type phenylpropanoid amides (serotomide and safflomid) have been investigated for their effects on serotonin receptors due to their chemical similarity to serotonin receptor agents used for treating depression, anxiety and cognitive dysfunction (Lanfume and Hamon, 2004; Leysen, 2004; Thompson and Lummis, 2006; Nelson, 2004). Although, several phytochemicals from plants have been investigated related to human diseases, but little is known about the effect of safflomid-type phenylpropanoid amides on serotonin receptors. Hence in this study, serotomide and safflomid were investigated and demonstrated to be as potent as pharmacologic compounds used as 5-HT₁ receptor ligands. Clinically, 5-HT₁ agonist have been utilized for these disorders, therefore, future research should be aimed on determining whether serotomide, safflomid and their analogues have improved efficacy and decrease adverse effects in treating human diseases associated to 5-HT₁ receptors. The preliminary study results of antidepressant activity in forced swimming test at 100 and 200 mg/kg doses revealed highly significant reduction in immobility time as compared to control.

Table 1: Comparison of anxiolytic effect of *Carthamus tinctorius*

Groups	Latency of 1 st entry to closed arm (sec)	Time spent in open arm (sec)	No. of entries from closed to open arm
Normal Control (Saline) ^a	15.43±1.39	24.57±1.27	0.71±0.48
Standard Control (diazepam 2 mg/kg) ^b	25.00±1.91	136.00±2.64	1.78±0.5
CT (100mg/kg) ^c	32.14±1.67* [∞]	55.29±2.28** [∞]	1.71±0.48†† [∞]
CT (200mg/kg) ^d	45.00±0.8* [∞]	63.86±1.34** [∞]	2.14±0.37†† [∞]

N=28 Results presented as Mean ± Standard Deviation *P<0.05; **P<0.01) compared to group a † p value<0.05 (significant), †† p value < 0.01 (highly significant) as compared to b Comparison among the group's c & d [∞]<0.05 (significant)

Table 2: Comparison of antidepressant effect of *Carthamus tinctorius* in rats

Groups	Immobility Time (seconds)
Normal Control (saline) ^a	153.2±2.0
Standard Control (Nortriptyline 12.5mg/kg) ^b	64.14±2.7**
CT (100mg/kg) ^c	93.5±3.4††
CT (200mg/kg) ^d	74.0±2.5†† [∞]

N=28 Results presented as Mean ± Standard Deviation *P<0.05; **P<0.01) compared to group a for CT† P value<0.05 (significant), †† P value<0.01 (highly significant) as compared to group a for standard drug

Calmness or immobility is either considered to be a sign of failure to carry on escape directed behavior after constant stress or the progress of passive behavior that disengages the animal from active forms of coping with stressful stimuli. Many antidepressants can reduce the immobility time after forced swimming (Gupta, 2011), thus *carthamus tinctorius* has been included to the list of herbal antidepressants due to its effect on regulating serotonergic transmission (Rajput, 2011), but clinical trials are still lacking.

Carthamus contains safflomidol and serotamidol, which are serotonin derived phenylpropanoid amides (Niwa *et al* 2000, Jenett-Siems *et al*, 2003 and Stark *et al* 2006) and are reported as potent as other pharmacological agents for the treatment of anxiety and depression (Lanfumay and Homon 2004, Leysen 2004, Nelson 2004, Park and Chen, 2007) by modulating 5HT receptor ligands. Thus anxiolytic and antidepressant effects of *carthamus tinctorius* seen in present study might be due to its monoamine transporter modulating activity like potent dopamine transporter (DAT) and Nor epinephrine transporter (NET) activators (Zhao *et al*; 2009).

CONCLUSION

Preliminary findings of this study in exploring behavioral effect can be manipulated in patients of anxiety and depression; however it needs further confirmation on large number of animals.

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