

Neuropharmacological effects of camel milk related to modulation of biogenic amines in experimental animals

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Abstract: Camel milk is reported as anti-diabetic, hepato-protective, anticancer, antioxidant, antiviral and neuroprotectant in numerous studies. Based on its neuroprotective profile, camel milk is investigated for its possible beneficial effect in treating anxiety and depression and its effect on brain biogenic amines in the present study. Head dip, cage crossing, stationary rod, elevated plus-maze, open field, light & dark box and forced swim tests were used to measure change in rodents' behavior after camel milk administration. Any possible change in brain biogenic amines level after camel milk treatment was evaluated using High Performance Liquid Chromatography (HPLC) technique. Camel milk administration resulted in significant increase ($p \leq 0.001$) in exploratory and locomotor activity and showing anxiolytic behavior in rodents. In depression-like model, rats showed significant increase ($p \leq 0.001$) in struggling time after 30-days administration of camel milk. HPLC detection of brain biogenic amines revealed significant increase ($p \leq 0.001$) in norepinephrine, insignificant increase in 5-hydroxytryptamine and significant decrease ($p \leq 0.001$) in dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid in camel milk treated group. Based on above findings, camel milk is suggested as anxiolytic and antidepressant in the administered doses. However, further experimental and clinical investigations are required to authenticate the same at different doses.

Keywords: Neuropharmacological, HPLC, biogenic amines, camel milk, anxiety, depression.

INTRODUCTION

According to World Health Organization, burden of mental disorders including stress, anxiety and depression is increasing globally and will be the second largest cause of disability and morbidity after cardiovascular disorders by 2020 (Kessler *et al.*, 2009). Widely available current psychotherapies are associated with sedation and dependence as major adverse effects (Kemp, 2014). Therefore, new drugs with fewer side effects are always in demand and treatments are now moving towards using alternative medicines such as herbal and other natural therapies (Ekor, 2014).

Camel milk has beneficial effects on human health and is reported as anti-diabetic, hepato-protective, anticancer, antioxidant and antiviral (Agrawal *et al.*, 2011; Darwish *et al.*, 2012; El-Agamy *et al.*, 1992; Homayouni-Tabrizi *et al.*, 2016; Jrad *et al.*, 2014; Salwa *et al.*, 2010). Recently in 2015 and 2016, Khatoon *et al.* evaluated anti-seizure and anti-Parkinson's effect of camel milk in experimental animals and reported potential benefits of camel milk administration as an adjuvant therapy in neurodegenerative diseases (Khatoon *et al.*, 2015; Khatoon *et al.*, 2016). As per our knowledge, there is no study so far reported neuropharmacological effects and modulation of brain biogenic amines after camel milk administration. Therefore our objective was to explore the

effect of camel milk on brain biogenic amines in experimental animals and to investigate if it could be administered as an adjuvant therapy in diseases like anxiety and depression.

MATERIALS AND METHODS

Camel's milk samples

Fresh camel milk samples were collected from local camel farm located about an hour distance from the place of study. Milk was collected in sterile screwed bottles and kept in ice-cool boxes until transported to the department for the study. To avoid early fermentation and to maintain the freshness of camel milk, samples were kept in frozen state until use. Frozen milk was thawed at countertops before administration. Omer and Eltinay (2009) reported no change in its physicochemical properties when stored at different temperatures.

Drugs and chemicals

All the chemicals used for the study were of analytical grade, obtained as follows: (-) noradrenaline (NA), 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), homovanillic acid (HVA) and ethylenediaminetetraacetic acid (EDTA) disodium salt (Sigma, USA). Dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrochloric acid (Sigma-Aldrich, Germany). 1-octane sulfonic acid, methanol and acetonitrile (Fischer chemicals, UK). Sodium dihydrogen citrate anhydrous (Fluka, Germany).

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Deionized water used in the study was obtained by using Millipore simplicity (185) deionization system. Dothiepin (25 mg tablets, Abbott Laboratories (Pakistan), Limited).

Animal selection and housing

Wistar albino mice and rats (either sex) weighing between 20-25 gram and 200-250 gram respectively were used for the study. Animals were obtained from animal house of department of Pharmacology, Faculty of Pharmacy, University of Karachi, housed randomly in stainless steel cages as groups of 3-4 animals per cage and kept under controlled environmental conditions at $25 \pm 1^{\circ}\text{C}$ and humidity (50-60%) with 12 hour light & dark cycle. Male and female animals were kept in separate cages to avoid mating during the study period. Food and water were available *ad libitum*.

Animals were handled as per guidelines provided by the National Institute of Health for the Care and Use of Animals (DHEW Publication, NIH, 80-23) and study is approved by institutional Board of Advanced Studies and Research vide Resol. No. 10(P) 01 dated: 03-03-2014.

Animals grouping and dosing

Head dip, cage crossing and stationary rod test

Twenty mice were randomly divided into two equal groups of 10 mice each for head dip, cage crossing and stationary rod activities, as follows:

- Control group animals received 0.1 ml distilled water p.o daily.
- Test group animals treated with 33ml/kg p.o daily of camel milk (Salwa *et al.*, 2010)
- All animals were started with 60-days treatment of camel milk as a test drug. Effects were monitored on 15th, 30th and 60th day of treatment. All observations were made during 9.00 to 14.00 hrs without any outside disturbances.

Elevated plus-maze, light & dark box and open field test

Thirty rats (200-250 gram) were equally divided into three groups of 10 rats each for elevated plus-maze, light & dark box and open field test, as follows:

- Animals of control group were administered 1 ml p.o of distilled water.
- Animals of test group were treated with 33 ml/kg p.o of camel milk.
- Standard group was treated with reference drug dothiepin (32 mg/kg p.o) (Bourin *et al.*, 1996). Dothiepin is selected as a reference drug after taking into consideration its better safety profile among various available tricyclic antidepressants and its dual effects as antidepressant and anxiolytic in same therapeutic doses (Bourin *et al.*, 1996).

All animals were subjected to the drugs treatment for 30 days. Effects were monitored on day 15th and 30th of treatment. On the day of experiment, 30 minutes after

drug administration rats were placed separately first in the Elevated Plus-Maze for 5 minutes then immediately in the Light and Dark box followed by Open Field paradigm for another 5 minutes for each test (Ishaq, 2014).

Forced swim test (FST)

Forced swim test was performed on separate group of 30 rats (n=10) divided into three groups similarly as described above.

Brain biogenic amine estimation

Twenty Wistar albino rats of either sex weighing 200-300 gram were equally divided into two groups of 10 animals each. Group one received 1 ml distilled water orally and served as control while group two was on 33 ml/kg camel milk daily via oral route (Salwa *et al.*, 2010) for a period of 30 days and served as treated.

Experimental protocol

Head dip test

The same method was adopted as described previously by Takeda and co-workers (1998). The apparatus was made up of 45cm x 45cm square wooden box with equally spaced 10 holes on all of its four sides. 30 minutes after distilled water or camel milk treatment on day 15th, 30th and 60th of dosing each subject was placed separately in the center of the apparatus and numbers of head poking were counted for 5 minutes (Sultana *et al.*, 2012).

Cage crossing test

For behavior and spontaneous locomotor activity, cage crossing test was performed. The apparatus consisted of a 26cm x 26cm x 26cm sized transparent home-cage (Sarfaraz *et al.*, 2014). Escaping was prevented by surrounded walls. On day 15th, 30th and 60th of dosing each mouse of both treatment groups was placed for 5 minutes in the cage 30 minutes after distilled water or camel milk treatment and number of crossings was counted (Sultana and Najam, 2012).

Stationary rod test

To assess learning ability and memory in animals, stationary rod test was performed. The apparatus was almost identical with that described by Sultana and Najam (2012) and consisted of a horizontal stainless steel rod with platform at both ends. Initially, animals were trained briefly to walk and maintain balance on the rod and then time required to reach the opposite platform was noted 30 minutes after distilled water or camel milk treatment on day 15th, 30th and 60th of dosing (Sultana and Najam, 2012).

Elevated plus-maze test

The apparatus was almost same as described by Ishaq (2014) and consisted of four equal sized (40x10 cm) arms (two opened and two closed) radiating from the central platform to form plus sign. The walls of closed arms were

17 cm high. The apparatus was placed on a 50 cm high wooden stand.

On the day of experiment, rats were presented in the neutral zone (central platform) of elevated-plus maze facing the open arm. Percent time spent in the open arm ($\text{Time spent in open arm} / \text{Total time} \times 100$), the percent open arm entry ($\text{Open-arm entries} / \text{Total entries} \times 100$) and total number of entries were calculated for each rat for the cut off time of 5 minutes (Braun *et al.*, 2011).

Light and dark box test

The apparatus comprised of two compartments. One small, square shaped dark compartment and another bigger rectangle shaped light compartment with an external size of 26 x 27 x 28 cm (Mahmood *et al.*, 2015). Light box was brightly illuminated by a lamp placed 35-40 cm above the floor of the apparatus (Ishaq, 2014). The two compartments were linked together by a midway door of size 7.5 x 7.5 cm. On the day of experiment, each rat was placed in the light box separately and the time spent in the light compartment and number of transitions between the boxes were monitored for 5 minutes (Kuleshkaya *et al.*, 2014; Mahmood *et al.*, 2015).

Open field test

Open field was used for assessment of behavior and spontaneous locomotor activity. The apparatus consisted of large square arena of 76 x 76 cm, made of white Plexiglas material with transparent floor. Floor was divided into 25 equal squares of 16 x 16 cm each. The walls were opaque and 40 cm in height (Ishaq, 2014). Control and treated group animals were introduced in the central square of the apparatus separately on 15th and 30th day of treatment and number of parameters such as central squares crossing, peripheral squares crossing and rearing were monitored for a cut off time of five minutes (Ishaq, 2014; Kuleshkaya *et al.*, 2014).

Forced swim test

Forced swim test was used for the assessment of antidepressant-like effect of camel milk in rats. The test procedure was first defined by Porsolt *et al* (1977 and 1978). The apparatus consisted of acrylic glass cylinder of 29 cm height with 6 cm of diameter. Apparatus was filled with water at $25 \pm 2^\circ\text{C}$ up to 21 cm height every time before introducing animal in it (Sultana and Najam, 2012).

For preparation of depression-like model same protocol was followed that was described by Ishaq and colleagues (2013). Since swimming behaviors of rats are not identical therefore in order to get them familiar with swimming and to get uniform animal models rats were forced to swim daily for 10 minutes for five days and then subjected to chronic mild stress (CMS). First CMS was applied on the 6th day by titling the cage not less than 30°

for two days. Second CMS was applied on eighth day by pouring enough water (approximately 150-250 ml) on sawdust to keep the bedding of the cage wet for 24 hours. Third CMS was applied on 9th day by keeping animals away from food for 24 hours. On 10th day of pretreatment period, rats were forced to swim again for 5 minutes and struggling time was recorded. This was considered as day 0 (pretreatment) reading. On the same day, drug treatment was started as per above described protocol. On 15th and 30th day of dosing, each rat of all treatment groups was presented in the apparatus and effect of drug treatment on struggling time was observed.

Brain biogenic amine estimation

Sample collection and preparation of homogenate

On 30th day of dosing, rats from control and milk treated groups were sacrificed by decapitation 30 minutes after the last dose. Brains from individual animals were removed carefully from cranial cavity, placed on ice pack and sent immediately to the Hussain Ebrahim Jamal (H.E.J) Research Institute of Chemistry, University of Karachi for HPLC analysis where samples were stored at -80°C until the day of analysis. On the study day, brain samples were weighed and homogenized separately in 1M perchloric acid at the ratio of 100 mg/ml. Homogenate was centrifuged at 14000 rpm for 20 minutes at 4°C . Supernatant was collected and filtered through syringe driven membrane filters of 0.22 μm size (Millex-GV, Millipore) (Abbas *et al.*, 2012). 20 μl of filtered supernatant was immediately transferred into Eppendorf tube and subjected to HPLC for estimation of brain bioamines and their metabolites.

HPLC analysis

The levels of monoamines and their metabolites in rat's whole brain were measured by reversed phase high performance liquid chromatography coupled with electrochemical detector (HPLC-ECD, Shimadzu). The system was almost identical as described by Abbas *et al.* (2012) and consisted of LC-20A pump, SIL-20A auto-sampler coupled with an electrochemical detector (L-ECD-6A). The hardware was connected to Shimadzu LC Solution software through communication bus module (LC-CBM-20A). Reversed phase C18 nucleosil column preceded by C18 nucleosil guard column were used as stationary phase and citrate buffer (pH 3.4) containing 10% acetonitrile as mobile phase (Abbas *et al.*, 2012).

On the day of analysis, stock solutions of noradrenaline, 5-HT and dopamine and their metabolites such as HVA, 5-HIAA and DOPAC were prepared in 0.01M hydrochloric acid at the ratio of 1mg/ml and then mixed to form standard solution using citrate buffer (pH 3.4) at the ratio of 50 ng/ml. An aliquot (10 μl) was injected in HPLC grade Eppendorf cuvette (Sigma-Aldrich) using syringe driven membrane filter unit (0.22 μm) and subjected to HPLC at the flow rate of 0.5 ml/min for analysis (Abbas *et al.*, 2012).

Before the analysis, the C18 nucleosil column (used as stationary phase) was pre-equilibrated with the mobile phase for 30 min at the flow rate of 0.5 ml/min. 10 μ l of standard mixture was injected twice, i.e. before and after the completion of sample analysis at the flow rate of 0.5 ml/min for 30 min for system calibration (Abbas *et al.*, 2012).

In order to minimize exposure of test and standard sample to high temperature and light the entire analytical procedure was conducted in dark and using refrigerated centrifugation (Hashemi *et al.*, 2014).

STATISTICAL ANALYSIS

Results were expressed as mean $\pm$ S.D and analyzed by two-way analysis of variance (ANOVA) followed by Scheffe test for post-hoc analysis. All statistical calculations were performed using IBM SPSS software for Windows. Results were statistically significant when $p \leq 0.05$, moderately significant at $p \leq 0.01$ and highly significant at $p \leq 0.001$. For HPLC analysis significance was calculated by independent sample t-test (student t-test) using SPSS version 20.0.

RESULTS

Effect of camel milk treatment on head dip and cage crossing activity

Two-way analysis of variance was significant for head dip ($F=19.18$; $df= 2, 54$; $p \leq 0.001$) and cage crossing activity ($F=17.50$; $df= 2, 54$; $p \leq 0.001$). Table 1 shows significant increase ($p \leq 0.001$) in head dip and cage crossing activity on day 15, 30 and day 60 of camel milk treatment when compared with control group.

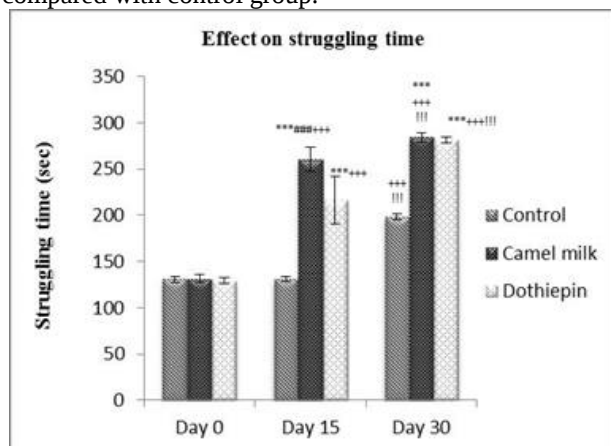


Fig. 1: Mean values $\pm$ S.D (n=10). Two-way ANOVA ($df=4, 81$) was used to calculate significance and post-hoc analysis by Scheffe test. ***, ### $p \leq 0.001$ in comparison to control and dothiepin respectively.; +, +, +, !!! $p \leq 0.001$ in comparison to baseline (Day 0) and day 15 values within same treatment group respectively.

Fig. 1: Effect of camel milk on struggling time in forced swim test

Effect of camel milk treatment on learning using stationary road test

Two-way analysis of variance was significant ($F=237.7$; $df= 2, 54$; $p \leq 0.001$). table 1 represents that animals of camel milk treated group required significantly ($p \leq 0.001$) less time to reach opposite platform when compared with animals of control group on 15th, 30th and 60th day of treatment. Significant reduction ($p \leq 0.001$) in the measured parameter was observed within camel milk treated group on day 30 and 60 when compared with day 15.

Effect of camel milk treatment on elevated plus-maze test

Two-way analysis of variance was significant for time spent in open arm ($F=1125.05$; $df= 4, 81$; $p \leq 0.001$), transitions in open arm ($F=8.80$; $df= 4, 81$; $p \leq 0.001$) and total number of entries ($F=9.22$; $df= 4, 81$; $p \leq 0.001$). In table 2 (a and b) results showed that camel milk treatment followed the same pattern of behavior as dothiepin (standard) treatment as shown by significant decrease ($p \leq 0.001$) in all measured parameters on day 15 and significant increase in all measured parameters ($p \leq 0.001$) after 30 days of dosing when compared with control group.

Effect of camel milk treatment on open field test

Two-way analysis of variance was significant for number of central squares ($F=6.49$; $df= 4, 81$; $p \leq 0.001$), number of peripheral squares ($F=1266.6$; $df= 4, 81$; $p \leq 0.001$), number of total squares ($F=1231.89$; $df= 4, 81$; $p \leq 0.001$) and number of rearing ($F=25.14$; $df= 4, 81$; $p \leq 0.001$). table 3 (a and b) reveal that camel milk treatment followed the similar pattern of behavior as standard (dothiepin) treatment as shown by initial decrease in number of peripheral square, total square crossings and number of rearing on day 15 and then significant increase ($p \leq 0.001$) on day 30 as compared to control group. However, number of crossings in central squares of open field was decreased in these two treatment groups after 15 and 30 days of dosing in comparison to control group.

Effect of camel milk treatment on light & dark box test

Two-way analysis of variance was significant for time spent in light area ($F=1674.29$; $df= 4, 81$; $p \leq 0.001$) and number of transitions between boxes ($F=10.05$; $df= 4, 81$; $p \leq 0.001$). In table 4 results reveal significant increase ($p \leq 0.001$) in time spent in light arena in camel milk treatment group after 15 days of dosing while after chronic dosing (30 days) significant decrease ($p \leq 0.001$) in the time spent in light compartment and number of transitions were found when compared with control and standard groups.

Effect of camel milk treatment on struggling time in forced swim test

Two-way analysis of variance was significant ($F=119.26$; $df= 4, 81$; $p \leq 0.001$). Results presented in fig. 1 reveal that

Table 1: Effect of camel milk treatment on head dip, cage crossing and stationary rod test in mice

Group	No. of head dips			No. of cage crossings			Time to reach opposite platform (sec) in Stationary rod test		
	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60
Control	23.5±3.0	22.5±3.0	31.7±4.7	23.3±3.3	26.0±2.5	29.5±3.0	63.0±3.2	58.6±2.9	48.5±3.0
Camel milk	32.5±3.0***	33.3±3.3***	55.0±5.8***,+++,!!!	26.5±3.0*	38.5±3.0***,+++	43.0±3.1***,+++,!!	55.5±3.0***	13.0±2.5***,+++	9.6±2.8***,+++,!

Values are mean ± S.D (n=10). Two-way ANOVA (df= 2, 54) was used to calculate significance and post-hoc analysis by Scheffe test. *p≤0.05, ***p≤0.001 in comparison to control. !p≤0.05, ++p≤0.01, +++p≤0.001 in comparison to 15 days and 30 days values respectively within same treatment group.

Table 2: Effect of camel milk administration on rats' behavior in elevated plus-maze test

Table 2(a)						
Treatment group	Percentage Time spent in open arms (%)			Percentage transitions in open arms (%)		
	Day 0	Day 15	Day30	Day 0	Day 15	Day30
Control	43.3±1.2	40.3±1.2	30.3±0.77 +++, !!!	72.1±5.5	62.5±10.6	46.5±8.2+++,!!
Camel milk	42.6±1.3	27.0±1.2 ***, ##,+++	34.8±0.95***, ###,+++,!!!	70.1±8.7	58.5±22.8 +	77.8±9.1***!!!
Standard (Dothiepin)	43.1±1.0	25.5±1.0 ***,+++	60.5±1.0***, +++, !!!	73.8±6.7	57.3±16.6++	76.9±8.1***,!!!
Table 2(b)						
Treatment group	Percentage transitions in closed arms (%)			No. of total arm-entries		
	Day 0	Day 15	Day30	Day 0	Day 15	Day30
Control	27.8±5.5	37.4± 10.6	53.4± 8.2+++,!!	18.0±4.5	16.3±3.8	16.1±5.6
Camel milk	29.8±8.7	41.4± 22.8 +	22.1± 9.1***,!!!	15.2±4.4	9.1± 2.8***, +++	20.6± 4.4*, ++ ,!!!
Standard (Dothiepin)	26.1±6.7	42.6± 16.6++	23.0± 8.1***,!!!	17.3±3.8	9.3± 3.0***,+++	24.1± 3.6***,+++, !!!

Mean values ± S.D (n=10). Two-way ANOVA (df=4, 81) was used to calculate significance and post-hoc analysis by Scheffe test. ***p≤0.001, *p≤0.05 in comparison to control. ###p≤0.001, ##p≤0.01 in comparison to dothiepin. +++p≤0.001, ++p≤0.01, +p≤0.05 in comparison to baseline values (Day 0). !!!p≤0.001, !!p≤0.01 in comparison to day 15 values within same treatment group.

camel milk treatment followed the similar pattern of behavior as dothiepin treatment as shown by significant increase (p≤0.001) in struggling time in these two treatment groups in comparison to control group when treated for 15 and 30 days.

Effects of camel milk treatment on brain biogenic amines

An independent-samples t-test was conducted to compare the effect of camel milk treatment on noradrenaline, dopamine, serotonin (5-HT), DOPAC, HVA and 5-HIAA level in rat brain as compared to control animals. Results presented in table 5 reveal significant increase in noradrenaline (t=31.60; df= 18; p≤0.001) insignificant increase in 5-HT level (t=0.25; df= 9.13; p>0.05) and significant decrease (p≤0.001) in Dopamine (t=-452.34; df= 18), DOPAC (t=-13.99; df= 9.76), HVA (t=-29.88; df= 14.39) and 5-HIAA (t=-13.54; df= 18) in camel milk treated group in comparison to control group.

DISCUSSION

Animal models are commonly used to predict therapeutic response to pharmacological agents. In previous studies increase in exploratory activity in mice e.g. total locomotor activity and number of head-dipping were assessed with respect to changes in their emotional state (Takeda *et al.*, 1998). Takeda and colleagues (1998) reported increase in number of head dips after anxiolytic treatment as compared to decreased head-dipping behavior in stress-induced animals. In the current study, camel milk treatment showed increase in exploratory and locomotor activity in mice, suggesting anxiolytic behavior.

Results of the present study also suggest that camel milk could be involved in enhancing learning ability and memory as observed in stationary rod test. The time required to reach the opposite platform is decreased after

long term treatment when compared with control animals. Stationary rod is also used to assess muscle coordination and locomotor activity. It has been reported that the striatum and cerebellum regions of brain are related to the muscular coordination and locomotor activity (Viggiano *et al.*, 2003). In these regions dopamine is highly expressed and is involved in muscle strength and coordination. In another study it has been found that not only dopamine, 5HT is also involved in muscle coordination and locomotor control (Sławińska *et al.*, 2014). The results of our present study are also in accordance with previous findings and showed increased level of norepinephrine and 5-HT (although insignificant) by camel milk (table 5).

The elevated plus-maze (EPM) test has been widely used in pharmacological studies to assess behavior related to anxiety in rodents. According to Braun and co-workers (2011), when animals are exposed to novel environment and allowed to make choice between two novel areas in EPM, they prefer to enter in closed arm and avoid open-arm. This animal behavior is widely accepted as index of anxiety in animals. The increased time spent and entries in the open arm showed anxiolytic effect (Braun *et al.*, 2011). Our results showed that long term administration of camel milk for 30 days caused significant increase in percentage of time spent in the open-arm and increased open-arm entries as compared to 15 days administration, suggesting anxiolytic effect.

In the light-dark box test anxiety can be assessed according to the number of transitions between light and dark compartments and the time spent in the light area where an increase in these parameters reflect anxiolytic behavior (Kuleskaya *et al.*, 2014). The results of current study revealed that the camel milk as compared to control group showed highly significant increase in time spent in the light compartment after initial dosing and then resulted in decrease in time spent in the lit area after 30 days of treatment. Additionally, a decrease in light and dark box transition in comparison to control group is also observed which might be due to the fact that camel milk would have made animals more familiar with the surroundings after few days of its administration. After continuous administration of camel milk animals would have become comfortable with the novel environment as shown by decreased exploratory and locomotor activity indicating calming behavior which is suggestive of anxiolytic profile.

The use of open field has been validated as an important behavioral model of anxiety in previous studies (Seibenhener *et al.*, 2015). The open field paradigm triggers anxiety behavior in rodents by inducing fear of novel and large arena and social isolation of animal from its group. The apparatus provides measurement of exploration and locomotion in terms of frequency of total squares crossing (Ajibade *et al.*, 2011). In previous

studies, it has been observed that anxiolytic treatment increases exploratory and locomotor activities (Ishaq, 2014). The results of our study also support above findings as observed by increase in total squares crossings showing increased locomotion and thus suggesting anxiolytic behavior in animals treated with camel milk.

Rearing is also reported as an important measure of exploration and animals show decrease in this behavior when they are introduced in a novel environment and reversal of this behavior is observed by anxiolytic treatment (Ishaq, 2014). It can be clearly seen from our results that rearing is increased in camel milk treated group, thus suggesting its anxiolytic potential.

In the open field test, animal did not show its preference to central squares after camel milk treatment and conversely showed increase in frequency of peripheral square crossings. Therefore, it has been suggested that it possesses anxiolytic activity without having sedative property.

The expression of anxiety involves a coordinated activity of numerous brain pathways involving different neurotransmitters including gamma amino-butyric acid (GABA), serotonin, noradrenaline and dopamine (Durant *et al.*, 2010). It has been demonstrated by Durant and co-workers (2010) that anxiety is actually linked with disturbance in balance between GABAergic and monoaminergic neurotransmission and thus serve as an important tool for the development of novel anxiolytics. In monoaminergic neurotransmission, serotonin has been involved in regulating emotional states. 5-HT is expressed in amygdala. This brain region plays its vital role in fear and anxiety responses and may be involved in pathogenesis of several neurological disorders such as acute state of anxiety and social phobias (Moya *et al.*, 2011). Serotonin hypothesis of anxiety suggest that increased level of 5-HT in synaptic cleft leads to anxiogenic behavior and thus reduced level can produce anxiolytic response (Johnston *et al.*, 1986). Results of our study support the previous findings and showed that camel milk treatment maintains stable internal environment and showed no significant increase in 5HT level after 30 days whereas decreased its metabolite 5HIAA. Decreased level of 5HIAA could be due to compensatory inhibition of 5HT metabolism probably as a result of 5HT homeostasis. Review of clinical data showed that SSRIs and TCAs are equally effective in treating patients with anxiety disorders such as aggression, obsessive compulsive disorders (OCDs) and generalized anxiety disorders (Rocca *et al.*, 1997). Results of our recent study also support previous findings and show that camel milk treatment requires at least two weeks to produce its anxiolytic effect. The results are comparable with dothiepin, the reference drug which showed its known dual activity as anxiolytic and antidepressant both (Lancaster *et al.*, 1989). However,

Table 4: Effect of camel milk administration on rats' behavior in light & dark box test

Treatment group	Time spent in light area (Sec.)			No. of Transitions between boxes		
	Day 0	Day 15	Day30	Day 0	Day 15	Day30
Control	58.0±2.5	35.0±4.0 ⁺⁺⁺	39.5±3.0 ⁺⁺⁺ , !!	12.5±3.6	12.1±2.8	9.0±2.5 ⁺ , !
Camel milk	60.0±3.6	50.5±3.0 ^{***, ###, +++}	26.0±3.6 ^{***, ###, +++} , !!!	11.4±2.0	6.5±3.0 ^{***, ###, +++}	5.6±2.2 ^{*, ###, +++}
Standard (dothiepin)	59.5±3.0	144.9±3.4 ^{***, +++}	179.0±3.6 ^{***, +++} , !!!	13.5±3.0	19.0±3.6 ^{***, +++}	17.1±3.0 ^{***, +++}

Mean values ± S.D (n=10). Two-way ANOVA (df=4, 81) was used to calculate significance and post-hoc analysis by Scheffe test. ^{***, ###}p≤0.001 in comparison to control and dothiepin respectively. ⁺⁺⁺p≤0.001, ⁺p≤0.05 in comparison to baseline values (Day 0). ^{!!!}p≤0.001, [!]p≤0.05 in comparison to day 15 values within same treatment group.

Table 5: Effect of camel milk on brain biogenic amines level

Biogenic Amines	Concentration (ng/g)	
	Control	Camel Milk
Noradrenaline	488.70±2.71	526.33±2.60 ^{***}
Dopamine	1076.90±1.14	821.37±1.37 ^{***}
5-HT	677.40±29.51	679.80±2.52
DOPAC	186.41±14.01	123.10±2.88 ^{***}
HVA	128.85±3.70	88.39±2.14 ^{***}
5-HIAA	423.80±7.19	389.40±3.57 ^{***}

Values are mean ± S.D (n=10). Independent sample t-test (student t-test) was used to calculate significance. ^{***}p≤0.001 in comparison to control group.

further research is required to confirm whether camel milk is actually involved in 5HT homeostasis and inhibition of its metabolism to 5HIAA.

Forced swim test (FST) is the behavioral model most commonly used to test drug having antidepressant potential. FST is specific to screen all major classes of antidepressants, including SSRIs, MAOIs, SNRIs and TCAs (Ishaq *et al.*, 2013). In the current study, animals on camel milk treatment spent more time in struggling, indicating antidepressant activity and results are comparable with reference drug dothiepin (Bourin *et al.*, 1996). It has been observed in earlier studies that when animals are treated with drug which increases serotonin, norepinephrine and dopamine levels in the nerve terminals they show antidepressant profile. This hypothesis appears to be supported by the mechanism of action of our reference drug dothiepin, which is classified as tricyclic antidepressant and structurally related to amitriptyline. The antidepressant activity of dothiepin is mediated through facilitation of noradrenergic neurotransmission by uptake inhibition and possibly also by increased 5HT level in synaptic cleft leading to enhanced serotonergic neurotransmission (Lancaster *et al.*, 1989).

Based on neuropharmacological profile of camel milk, levels of monoamines (norepinephrine, dopamine and 5HT) and their metabolites (DOPAC, HVA and 5HIAA) were quantified by HPLC-ECD in whole brain of rats treated with camel milk for 30 days and compared with

control animals. Previous studies reported high levels of all three amines in brain striatum of mice responsible for antidepressant behavior (Citó *et al.*, 2015). In accordance with previous reports that show changes in brain monoamine levels, present study showed significant increase in norepinephrine and insignificant increase in 5HT levels after chronic administration of camel milk. These results supported our hypothesis that the anxiolytic and antidepressant-like effect shown by camel milk is actually associated with the involvement of neurotransmitters and suggesting that the mechanism underlying its effect is possibly related to TCAs and depends on an increase in norepinephrine and possibly 5HT levels in the synaptic cleft. Moreover, decrease in dopamine, DOPAC and HVA level in the current study suggests that neuropharmacological profile of camel milk may further be investigated specifically with respect to its effect on dopamine. Since at present we examined rats' whole brain and it is quite possible that 5HT and dopamine level may be increased in other more specific brain regions, therefore, their levels in specific brain areas such as substantia nigra, striatum, cerebellum and hippocampus should be measured to justify the role of camel milk. Better muscle strength and coordination in FST and stationary rod of our study needs further correlation with respect to neurotransmitters.

CONCLUSION

Based on results of our study camel milk is suggested as anxiolytic and antidepressant in the administered doses

and the effect is linked to modulation of brain biogenic amines level. With these findings, it can be concluded that if camel milk is administered as a nutritional supplement in patients with diagnosis of anxiety and depression, they could get benefit from its dual action and may not require additional benzodiazepine therapy to control their mood swings and sleep cycle. However, its long-term safety profile at different quantities of its consumption needs to be further investigated experimentally as well as clinically.

REFERENCES

- Abbas G, Naqvi S and Dar A (2012). Comparison of monoamine reuptake inhibitors for the immobility time and serotonin levels in the hippocampus and plasma of sub-chronically forced swim stressed rats. *Pak. J. Pharm. Sci.*, **25**: 441-445.
- Agrawal R, Sharma P, Gafoorunissa SJ, Ibrahim SA, Shah B and Shukla DK *et al.* (2011). Effect of camel milk on glucose metabolism in adults with normal glucose tolerance and type 2 diabetes in Raica community: A crossover study. *Acta. Biomed.*, **82**(3): 181-186.
- Ajibade A, Adenowo T, Akintunde O, Fakunle P, Oyewo O, Ashamu E, *et al.* (2011). Suppression of exploration and locomotion in adult Wistar rats following quinine administration. *J. Neurosci. Behav. Health*. **3**: 32-37.
- Bourin M, Redrobe JP, Hascoet M, Baker GB and Colombel MC (1996). A schematic representation of the psychopharmacological profile of antidepressants. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **20**(8): 1389-1402.
- Braun AA, Skelton MR, Vorhees CV and Williams MT (2011). Comparison of the elevated plus and elevated zero mazes in treated and untreated male Sprague-Dawley rats: Effects of anxiolytic and anxiogenic agents. *Pharmacology Biochemistry and Behavior*, **97**(3): 406-415.
- Citó M, Silva M, Santos L, Fernandes M, Melo F, Aguiar J, *et al.* (2015). Antidepressant-like effect of Hoodia gordonii in a forced swimming test in mice: evidence for involvement of the monoaminergic system. *Braz. J. Med. Biol. Res.*, **48**(1): 57-64.
- Darwish HA, Abd Raboh NR and Mahdy A (2012). Camel's milk alleviates alcohol-induced liver injury in rats. *Food Chem. Toxicol.*, **50**(5): 1377-1383.
- Durant C, Christmas D and Nutt D (2010). The pharmacology of anxiety. In: Stein MB, Steckler T (eds). *Behavioral Neurobiology of Anxiety and Its Treatment*, edn. New York Springer-Verlag, Berlin Heidelberg, Germany, pp.303-330.
- El-Agamy EI, Ruppanner R, Ismail A, Champagne CP, Assaf R (1992). Antibacterial and antiviral activity of camel milk protective proteins. *J. Dairy Res.*, **59**(2): 169-175.
- Ekor M (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.*, **4**: 177.
- Hashemi F, Laufer R, Szegi P, Csomor V, Kalasz H and Tekes K (2014). HPLC determination of brain biogenic amines following treatment with bispyridinium aldoxime K203. *Acta. Physiologica Hungarica.*, **101**(1): 40-46.
- Homayouni-Tabrizi M, Shabestarin H, Asoodeh A and Soltani M (2016). Identification of two novel antioxidant peptides from camel milk using digestive proteases: Impact on Expression Gene of Superoxide Dismutase (SOD) in hepatocellular carcinoma cell line. *Int. J. Pept. Res. Ther.*, **22**(2): 187-195.
- Ishaq H (2014). Anxiolytic Effect of Herbal medicine, Khamira Gaozaban Ambri Jadwar Ood Salib Wala (KGJ) in experimental rat models. *Pak. J. Pharm. Sci.*, **27**(2): 289-294.
- Ishaq H, Mahmood R, Javed I, Tariq T and Mahmood I (2013). Antidepressant activity of the herbal extract, Khamira Gaozaban Ambri Jadwar Ood Salib Wala. *Int. J. Pharm.*, **3**(3): 450-456.
- Johnston AL and File SE (1986). 5-HT and anxiety: Promises and pitfalls. *Pharmacol. Biochem. Behav.*, **24**(5): 1467-1470.
- Jrad Z, Girardet J-M, Adt I, Oulahal N, Degraeve P and Khorchani T and El-Hatmi H (2014). Antioxidant activity of camel milk casein before and after *in vitro* simulated enzymatic digestion. *Mljekarstvo.*, **64**(4): 287-294.
- Kemp D (2014). Managing the side effects associated with commonly used treatments for bipolar depression. *J. Affect. Disord.*, **169**: S34-S44.
- Kessler RC, Aguilar-Gaxiola S, Alonso J, Chatterji S, Lee S, Ormel J, Üstün TB and Wang PS (2009). The global burden of mental disorders: An update from the WHO World Mental Health (WMH) Surveys. *Epidemiologia e Psichiatria Sociale*, **18**(1): 23-33.
- Khatoun H, Najam R, Mirza T and Sikandar B (2016). Beneficial anti-Parkinson effects of camel milk in Chlorpromazine-induced animal model: Behavioural and histopathological study. *Pak. J. Pharm. Sci.*, **29**(5): 1525-1529.
- Khatoun H, Najam R, Mirza T, Sikandar B, Ishaq H and Anser H (2015). Evaluation of anticonvulsant and neuroprotective effects of camel milk in strychnine-induced seizure model. *Asian Pac. J. Trop. Dis.*, **5**(10): 817-820.
- Kuleskaya N and Voikar V (2014). Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: Role of equipment and procedure. *Physiol. Behav.*, **133**: 30-38.
- Lancaster SG and Gonzalez JP (1989). Dothiepin. *Drugs*. **38**(1): 123-147.
- Mahmood R, Najam R, Rizwani GH and Khatoun H (2015). Evaluation of neuropharmacological activity of

- Cardiospermum halicacabum* (Linn) leaf extract. *World J. Pharm. Pharm. Sci.*, 5(3): 896-906.
- Moya PR, Fox MA, Jensen CL, Laporte JL, French HT, Wendland JR and Murphy DL (2011). Altered 5-HT_{2C} receptor agonist-induced responses and 5-HT_{2C} receptor RNA editing in the amygdala of serotonin transporter knockout mice. *BMC Pharmacol.*, 11(1): 3.
- Omer RH and Eltinay AH (2009). Changes in chemical composition of camel's raw milk during storage. *Pak. J. Nutr.*, 8(5): 607-610.
- Porsolt R, Bertin A and Jalfre M (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Archives internationales de pharmacodynamie et de thérapie*, 229(2): 327-336.
- Porsolt RD, Bertin A and Jalfre M (1978). Behavioural despair in rats and mice: Strain differences and the effects of imipramine. *Eur. J. Pharmacol.*, 51(3): 291-294.
- Rocca P, Fonzo V, Scotta M, Zanalda E and Ravizza L (1997). Paroxetine efficacy in the treatment of generalized anxiety disorder. *Acta Psychiatr. Scand.*, 95(5): 444-450.
- Salwa MQ and Lina AK (2010). Antigenotoxic and anticytotoxic effect of camel milk in mice treated with cisplatin. *Saudi J. Biol. Sci.*, 17(2): 159-166.
- Sarfaraz S, Najam R, Azhar I, Riaz B and Anser H (2014). Anxiolytic and CNS depressant effects of ethanolic extract of *Cleome brachycarpa* revealed after neuropharmacological screening. *World J. Pharm. Sci.*, 2(7): 605-610.
- Seibenhener ML and Wooten MC (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *JoVE*, 96: e52434-e52434.
- Sławińska U, Miazga K and Jordan LM (2014). The role of serotonin in the control of locomotor movements and strategies for restoring locomotion after spinal cord injury. *Acta Neurobiol. Exp.*, 74: 172-187.
- Sultana N and Najam R (2012). Anxiolytic activity of *Aloe vera* (L.) Burm. F tested in rodents. *Pak. J. Pharmacol.*, 29(1): 7-15.
- Takeda H, Tsuji M and Matsumiya T (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Eur. J. Pharmacol.*, 350(1): 21-29.
- Viggiano D, Ruocco LA and Sadile AG (2003). Dopamine phenotype and behaviour in animal models: In relation to attention deficit hyperactivity disorder. *Neurosci. Biobehav. Rev.*, 27(7): 623-637.