Beneficial anti-Parkinson effects of camel milk in Chlorpromazine-induced animal model: Behavioural and histopathological study

Humera Khatoon[1]*, Rahela Najam[2], Talat Mirza[3,4] and Bushra Sikandar[3]
[1]Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan
[2]Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan
[3]Department of Pathology, DOW University of Health Sciences, Karachi, Pakistan
[4]DOW Research Institute of Biotechnology and Biomedical Sciences, DOW University of Health Sciences, Karachi, Pakistan

Abstract: Potential roles of natural products have been identified for preventing or treating various diseases. Our aim was to investigate the effectiveness of camel milk in an animal model of Parkinson's disease and compare it with standard treatment (levodopa + carbidopa combination). 40 Wistar albino rats weighing 200-250 gram were divided into four groups of 10 animals each. Group I was kept on water and served as normal control, group II served as negative control, treated with chlorpromazine (5mg/kg i.p.), group III was given camel milk (33ml/kg p.o) and group IV the standard combination of levodopa + carbidopa (100+10mg/kg) respectively, 30 minutes after chlorpromazine treatment. All animals were subjected to the drugs treatment for 30 days. Catalepsy was assessed by Bar test on day 21 and day 30 at 30, 60, 90 and 120 minutes interval. On 30th day animals were sacrificed and whole brains were examined for histo-pathological changes. The results revealed highly significant ($p \leq 0.001$) anti-cataleptic effect of camel milk on day 21 and 30 in comparison to chlorpromazine. When compared with standard therapy, the results showed that anti-Parkinson's activity of camel milk was significant ($p \leq 0.01$) on day 21. However, the difference in activity was non-significant on day 30. Histopathology of the brain showed that administration of camel milk reveals intact architecture with mild degenerative changes than chlorpromazine and levodopa + carbidopa treated animals. In conclusion, camel milk possesses anti-Parkinson's activity. However, its long term efficacy and safety needs to be evaluated clinically.

Keywords: Camel milk, Parkinson's disease, chlorpromazine-induced catalepsy.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease in elderly. It is characterized by tremor, rigidity, bradykinesia, and postural instability. Damage to dopaminergic neurons in substantia nigra and other brainstem nuclei suggests pathogenesis of PD (Ionov and Severtsev, 2012). Currently available treatments (e.g. levodopa, carbidopa, bromocriptine etc.) for Parkinson's disease not only offer temporary improvement in the brain's dopamine levels and reverse the symptoms of Parkinson's disease but also show various side effects on long term therapy (Stocchi *et al.*, 2008). Since potential roles of natural products have been identified for the prevention or treatment of various diseases, our aim was to investigate the effectiveness of camel milk (CM) in an animal model of PD and compare it with standard treatment (combination of levodopa and carbidopa).

Camel milk has traditionally been used as a great source of human nutrition in most of the countries (which have camels as cattle). Its use is now being extended in the field of medicine because of its unique composition and characteristics due to which it also showed beneficial effects in diabetes (Agrawal *et al.*, 2011). Camel milk also possesses hepatoprotective (Darwish *et al.*, 2012), antibacterial (Cardoso *et al.*, 2013) and anticancer (Korashy *et al.*, 2012) activity. However, medical practice is still awaiting its scientific scrutiny. It has also been used in the treatment of autism, a neurodegenerative disorder, because of its antioxidant potential (Al-Ayadhi and Elamin, 2013).

Chemical composition of camel milk is almost similar to goat milk but when compared with cow milk, its 1 kg meets 100% daily human requirement for sodium, calcium and potassium. It is rich in copper, zinc and magnesium and contains about 40% of iron. Its vitamin E levels are similar (0.56 vs 0.60mg/L), but possess three times greater level of vitamin C (37.4 vs 11.0mg/L) when compared with cow milk (Akbar, 2011). Because of its unique composition and high content of vitamin C, E, zinc, magnesium and copper, camel milk possesses strong antioxidant characteristic and has potential to protect the brain from neurodegeneration. Al-Ayadhi and Elamin (2013) verified a substantial increase in plasma level of superoxide dismutase, myeloperoxidase and glutathione following camel milk consumption.

Various animal models of catalepsy are used for better understanding of the disease and to develop appropriate therapy. In order to evaluate anti-cataleptic effect of camel milk, the present study was conducted in a chlorpromazine (CPZ)-induced animal model (Wistar rat) of Parkinson Disease (PD). Since chlorpromazine toxicity

**Corresponding author:* e-mail: humerak_phr@hotmail.com

is involved in producing oxidative stress and decreasing the level of reduced glutathione in the brain, it results in producing motor defects (Sandhu and Rana, 2013). Therefore, the use of camel milk, due to its antioxidant properties, could be beneficial.

MATERIALS AND METHODS

Experimental animals

40 adult albino rats (either sex) of Wistar strain weighing 200-250gram, bred locally in the animal house of the Department of Pharmacology, University of Karachi were used in the present study. The animals were housed in iron cages, fed on regular rat pellet diet and water ad libitum. All the animals were maintained under constant environment with temperature (21±1°C) and humidity (50-60%). Animals were handled as per specifications provided in Helsinki Resolution 1964 and the study was approved by the institutional Board of Advanced Studies and Research vide Resol. No. 10(P) 01 dated: 03-03-2014.

Camel's milk samples

Milk samples were collected from local market early in the morning and kept in cool boxes until transported to the department for the study. To avoid early fermentation, samples were kept in frozen state until use.

Drugs and chemicals

Chlorpromazine (CPZ) was taken from Sigma, Chemicals Company, USA. Levodopa and Carbidopa (100+10mg tablets, Merck Sharp & Dohme, Pakistan) was procured from alocal pharmacy. All other chemicals used for the experiment were of analytical grade.

Experimental protocol

All animals were divided into four groups of 10 animals each. Group I was kept on water and served as control, group II was treated with chlorpromazine (5mg/kg i.p.) (Kulkarni *et al.*, 2009) and served as negative control (NC) Group III and IV were administered chlorpromazine (5mg/kg i.p.). After 30 minutes, animals of group III were administered camel milk (33ml/kg p.o) (Salwa and Lina, 2010) and animals of group IV were administered combination of levodopa and carbidopa (100+10 mg/ kg, p.o) as a standard drug (Goel *et al.*, 2005). All animals were subjected to the drug treatment for 30 days. All observations were made at room temperature without any outside disturbances during 9.00 to 14.00 hrs.

Evaluation of catalepsy

Catalepsy was induced by chlorpromazine and assessed by bar test method as described in previous studies (Costall and Naylor, 1974). In this test, both front paws of rats were placed in a half rearing position on a horizontal metal bar with 5mm diameter and 9cm above and parallel from the base of the test apparatus. The intensity of catalepsy was determined as length of time the test subject maintained this posture and measured with the stopwatch. When the animals removed at least one paw from the bar the stopwatch was stopped and time was noted. Score of 0 second was assigned if the animal fails to hold the bar after three attempts. The maximum cutoff time for bar test was 180 seconds. On day 21 and 30 animals were tested frequently at 0, 30, 60, 90, 120 minutes intervals after drug administration to determine the onset and intensity of catalepsy throughout the duration of the drug effect.

Histopathological evaluation

Histopathological evaluation was conducted at the Dow University of Health Sciences, under supervision of Prof. Dr. Talat Mirza and Dr. Bushra Sikandar.

On day 30 of the treatment, all 40 animals were sacrificed by cervical dislocation. Dissection of complete brain tissues from each of the normal, control, camel milk and drug treated animals were performed. The tissues were preserved in 10% of buffered formalin overnight. In order to examine morphological changes in brain tissue, gross examination was performed for size estimation; subsequently sections from mid brain were submitted in representative cassette. Tissues were processed for 12 hours in an automated "Medite TPC 15" tissue processor. Subsequently paraffin blocking was performed on "TES 99 Medite", automated paraffin embedding station (Presnell, 1997). Representative sections from paraffin embedded block, each measuring 3 to 4µm in thickness were sectioned using a microtome "SLEE 4062". Dissected sections were transferred into a water bath (46-48°C). Sections were then mounted on a glass slide, marked with a representative number. The slides were kept in an oven at 60°C for 30 minutes. Sections were de-waxed in two concentrations of xylene for 5 minutes each. Sections were rehydrated in different strength of alcohols and subsequently slides were plunged in running tap water for 1 minute. Sections were stained for 5 minutes with Harri's Haematoxylin and Eosin (Humason, 1962). Finally mounting was performed.

STATISTICAL ANALYSIS

The statistical analyses were carried out using SPSS version 20.0 for Windows. Data was analyzed using three-way analysis of variance (ANOVA) followed by Scheffe test for comparisons between various treated groups. The results were presented as means ± SD, n=10. Values with $p<0.05$ were considered to be statistically significant.

RESULT

Catalepsy

Tables 1 & 2 shows the catalepsy score of the present study assessed by the bar method. Data analyzed by three-way ANOVA showed a significant difference (F=11.56,

p≤0.001) between treatment groups on day 21 and 30 at 30, 60, 90 and 120 minutes interval. Post-hoc analysis by Scheffe test shows that chlorpromazine produced significant catatonia in CPZ treated group (II) as indicated by the highly significant (p≤0.001) increase in time spent on the bar in the bar test when compared with control group (I) (tables 1 & 2).

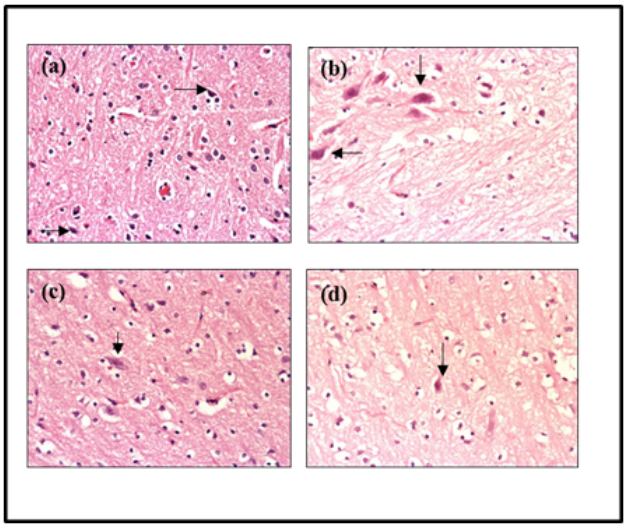

Fig. 1: Microphotographs of H&E at ×400, (a) Water treated control, arrow pointed out the reactive astrocytes. (b) Chloropromazine treated animal showing degenerative neuron with hyperchromatic nuclei (pointed by arrow) and surrounding tissue showing edema and proliferative blood vessels. (c) camel milk treated animal showing neuron with mild hyperchromasia (pointed by arrow) along with proliferative blood vessels.(d). Levodopa/ carbidopa treated animal showing neurons with mild neurodegenerative changes along with proliferative blood vessels.

Chlorpromazine-induced catatonia was considerably prevented in animals treated with camel milk (Group III) and on standard therapy (Group IV) as evident by significant (p≤0.001) at all intervals, p≤0.05 at 60 and 90 minutes respectively) decrease in catalepsy score on 21st day of treatment (table 1). However, decrease in catatonia in group IV was non-significant at 30 and 120 minutes interval on same day in comparison to group II (table 1). Administration of camel milk and standard therapy in chlorpromazine-treated animals (group III and IV respectively) showed the maximal decrease (p<0.001) in catalepsy on day 30 at all intervals in comparison to animals treated with chlorpromazine alone (table 2).

Antiparkinson's activity of camel milk was more significant (p≤0.01) in comparison to standard therapy on 21st day of treatment at all intervals except at 120 minutes where difference was significant (p≤0.05). On 30th day of treatment there was no significant difference in anti-cataleptic effect in group III when compared with group IV at 30 and 60 minutes interval. However, catalepsy score was significantly increased (p≤0.05) at120 minutes interval in group III in comparison to group IV on same day (table 2).

Histopathology

Histopathology of our samples showed that chlorpromazine caused degeneration in the mid brain region of the rats as neurons were under oxidative stress when compared with normal rats as can be seen from fig. 1a & 1b. Presence of hyperchromatic nuclei with eosinophilic vacuolated cytoplasm in edematous background having proliferative blood vessels indicates hypoxic insult to neurons in chlorpromazine treated animals (fig.1b). However sections examined from administration of camel milk (fig. 1c) exhibits intact architecture with occasional hyperchromatic nuclei and mild vacuolization suggestive of mild degenerative changes than chlorpromazine (fig. 1b) and levodopa + carbidopa treated animals (fig. 1d).

DISCUSSION

Haloperidol/chlorpromazine-induced animal models are commonly accepted among various available experimental models of PD. Previous studies (Parikh *et al.*, 2003) revealed that use of typical antipsychotics is associated with increased free radical-mediated oxidative cellular injury in rats in comparison to atypical ones e.g. risperidone, clozapine or olanzapine. One of the biochemical mechanisms in the pathogenesis of neuroleptic associated extra pyramidal side effects is increased production of reactive oxygen species (ROS) (Sagara, 1998). If these ROS are not removed by natural enzymatic antioxidant defense it can cause cellular damage. Glutathione peroxidase, catalase and superoxide dismutase are involved in normal antioxidant defense and protect cells against oxidative injury. In 2013, Sandhu and Rana have suggested that neuronal damage and development of catatonia as a result of chronic administration of chlorpromazine is associated with oxidative stress due to increased lipid per oxidation as evident by elevated level of thiobarbituric acid reactive substances (TBARs) and nitrite, decreased levels of super -oxide dismutase (SOD) and reduced glutathione (GSH). Extent of oxidative stress was also determined by measuring the level of superoxide dismutase (SOD) and reduced glutathione (GSH) in another study (Sharma and Nehru, 2013) and their decreased levels were suggestive of damage to motor control system.

In the present study, our results suggest that anti-Parkinson's activity of camel milk was more significant when compared with standard therapy on day 21. On day 30, results indicate that after chronic administration, anti-Parkinson's effect of camel milk was similar to that of standard drug initially at 30, 60 and 90 minutes interval.

Table 1: Effect of camel milk on catalepsy score (sec) in comparison with chlorpromazine and levodopa+ carbidopa treatment on Day 21

Treatment Group#/Drug (Dose)	Time spent on the Bar (sec) at various time intervals			
	30 min	60 min	90 min	120 min
I/Control (water treated)	0.91±0.20	1.00±0.00	1.00±0.00	1.16±0.40
II/CPZ (5 mg/kg)	54.66±18.56***	61.33±17.96***	64.83±16.63***	67.83±15.77***
III/CPZ+CM (5mg/kg+33ml/kg)	6.50±3.01$^{\$\$\$,\#\#}$	7.16±2.71$^{\$\$\$,\#\#}$	8.33±2.58$^{\$\$\$,\#\#}$	18.16±6.85$^{\$\$\$,\#}$
IV/CPZ+LD+CBD (100+10mg/kg)	36.50±29.53	37.00±26.38$^{\$}$	41.83±35.84$^{\$}$	46.50±36.87

Table 2: Effect of camel milk on catalepsy score (sec) in comparison with chlorpromazine and levodopa+ carbidopa treatment on Day 30

Treatment Group#/Drug (Dose)	Time spent on the Bar (sec) at various time intervals			
	30 min	60 min	90 min	120 min
I/Control (water treated)	1.00±0.54	1.16±0.40	1.00±0.00	0.83±0.25
II/CPZ (5 mg/kg)	61.17±26.00***	67.5±30.45***	77.00±31.56***	91.66±51.63***
III/CPZ+CM (5mg/kg+33ml/kg)	9.00±3.57$^{\$\$\$}$	26.33±4.36$^{\$\$\$}$	32.33±10.76$^{\$\$\$}$	57.33±27.86$^{\$\$,\#}$
IV/CPZ+LD+CBD (100+10mg/kg)	19.33±11.89$^{\$\$\$}$	27.00±12.0$^{\$\$\$}$	28.50±11.09$^{\$\$\$}$	28.83±12.49$^{\$\$\$}$

Values are mean ±SD, n=10. Significant difference by Scheffe test, ***p≤0.001 when compared with control, $^{\$}$p≤0.05, $^{\$\$}$p≤0.01, $^{\$\$\$}$p≤0.001 when compared with CPZ and $^{\#}$p≤0.05, $^{\#\#}$p≤0.01 when compared with LD+CBD treated animals, following three-way ANOVA. CPZ; Chlorpromazine, CM; camel milk, LD+CBD; levodopa +carbidopa.

Its anti PD effect persisted for longer duration (till 120 minutes) when compared with chlorpromazine treatment. The generation of free radicals due to excessive lipid per oxidation is most important cause of oxidative stress and neuronal cell death, therefore neuroprotective effect of camel milk could be attributed to its antioxidant property. Our findings are in accordance with previous studies, which revealed a significant increase in plasma level of super oxide dismutase, myeloperoxidase and glutathione following camel milk consumption (Al-Ayadhi and Elamin, 2013). These enzymes are the basis of neuroprotection.

Previous studies (Fahn, 1992) showed that combination of vitamin C and high dose of vitamin E when administered in patients with early Parkinson's disease resulted in slow disease progression and delay in the need for drug treatment. Neuroprotective effects of camel milk could be due to its high content of these two vitamins (Akbar, 2011). Histopathological findings of our study are also in agreement with earlier studies which reported a neuroprotective role of vitamin E in animal model of catalepsy which is contributing to this study (Sharma and Nehru, 2013). As shown by fig. 1. camel milk administration produced more neuroprotective effect when compared with chlorpromazine and almost similar degree of neuroprotection in comparison to levodopa + carbidopa treated animals.

Results of our present study concluded that due to its rapid onset and longer duration of action camel milk might possess advantages in the long-term management of Parkinson's disease over other conventional therapies. However, clinical study for its long-term efficacy and safety assessment is suggested.

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