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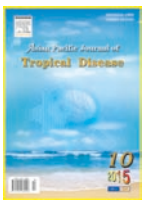

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Evaluation of anticonvulsant and neuroprotective effects of camel milk in strychnine-induced seizure model

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ABSTRACT

Objective: To discover the use of camel milk as an alternate medicine for the treatment and prevention of convulsions using strychnine-induced seizure model.

Methods: Thirty animals were divided into three equal groups. Group I was on distilled water, Group II was on camel milk for 15 days prior to experiment and Group III was on reference drug diazepam. On the day of experiment, strychnine was administered in all treatment groups after distilled water, camel milk and diazepam treatments respectively. Animals were observed for 30 min for latency of seizure onset, frequency of convulsions and duration of jerks. The mortality rate was also evaluated for each group.

Results: Camel milk treatment showed significant seizure protection as observed by delayed seizure onset ($P \leqslant 0.001$), decreased total duration of convulsions ($P \leqslant 0.001$) and mortality rate ($P \leqslant 0.001$) when compared with Group I.

Conclusions: Anticonvulsant activity of camel milk could be due to potentiation of glycinergic and GABAergic activities both. Antioxidant activity can also amplify its antiepileptic activity. Further studies are required to confirm the exact mechanism of action.

1. Introduction

Epilepsy is one of the common neurological disorders that are characterized by unpredictable, recurrent, rhythmic electrical firing of brain neurons called seizures. Epileptic seizures are broadly classified into two categories partial (focal) and generalized, depending on the portion of the brain affected[1]. Epilepsy is associated with chemical imbalance and may be due to hypoxia, hypoglycemia, low serum levels of sodium and calcium *etc*. Commonly hippocampal lesions or any injury to brain can induce seizures. Other causes of epileptic seizures include high fever, birth trauma, infections or head injuries[2]. There are 8 traditional antiepileptics available for use since 1990, namely, bromide, primidone, ethosuximide, phenytoin, carbamazepine, valproate, phenobarbital and certain benzodiazepines[3]. Based on antiepileptic drug utilization studies, it has been found that among various available anticonvulsant therapies, most commonly prescribed were phenytoin and sodium valproate along with carbamazepine[4]. It has been observed that the conventional antiepileptic therapy with drugs like phenytoin, carbamazepine and phenobarbital is unable to control seizures effectively in some patients and their continued use may precipitate or aggravate certain type of seizures[5]. Moreover, in some patients in order to achieve desired antiepileptic effects, these drugs are required in doses which are associated with several serious adverse effects including neurotoxicity[6]. However, despite of developing many new anti-epileptic drugs (like gabapentin, vigabatrin, lamotrigine, *etc*) which are used as adjunct to the conventional agents, large population of epileptic patients still have uncontrolled seizures and are also suffering from the side effects of these drugs[3]. The objective of anti-epileptic therapy is not only to eradicate the incidence of seizures but also enable patients to lead a self-sustained life[7]. Therefore, there is a need to scrutinize for highly effective as well as safe antiepileptic therapy in order to minimize drug related toxicity.

With the discovery of natural products as an alternative medicine for the treatment and prevention of many diseases, use of camel

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milk has been extended and studied in the field of medicine. Because of its unique composition, camel milk has therapeutic potential in diabetes[8]. Camel milk possesses powerful immune system boosting components and may become a potential treatment option in autoimmune disorders like Crohn's disease, multiple sclerosis and autism[9]. Previous studies showed that it also possessed anticancer[10] and hepatoprotective activity[11]. Despite its conventional use, scientific evidences are insufficient to prove the effectiveness of camel milk in the treatment of various other diseases and medical practice still requires some scientific investigations for its long term efficacy and safety assessment.

This study is designed to evaluate the anticonvulsant activity of camel milk using strychnine-induced seizure model.

2. Materials and methods

2.1. Experimental animals

A total of 30 albino mice (either sex) of Wistar strain weighing 20-25 g were used in the present study. The animals were bred locally in animal house of Department of Pharmacology, University of Karachi, housed in iron cages, fed on regular pellet diet and water *ad libitum*. All the animals were maintained under constant environment with temperature (21 ± 1) ℃ and humidity (50%-60%). Animals were handled as per specifications provided in Helsinki Resolution 1964. All the experimental procedures and protocols were reviewed and approved by institutional Board of Advanced Studies and Research vide Resol. No. 10(P) 01 dated: 03-03-2014.

2.2. 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.

2.3. Drugs and chemicals

We purchased diazepam 10 mg/2 mL injection (Roche Pharmaceuticals) from local market. All other chemicals used were of analytical grade.

2.4. Experimental protocol

The animals were divided into three groups of 10 animals each:

Group I: Control group (distilled water treated).

Group II: Test group, on camel milk (33 mL/kg *p.o.*)[12].

Group III: Standard group, on reference drug (Diazepam, 5 mg/kg *i.p.*)[13].

All the drugs were administered 30 min prior to the administration of strychnine (1 mg/kg, *i.p.*)[14]. Animals of Group II were on 15 days prior treatment of camel milk. On Day 16, strychnine was administered 30 min after camel milk treatment in the same group. Any mouse that did not convulse within 30 min was considered protected.

2.5. Evaluation of strychnine-induced seizure

Strychnine, as a chemo-convulsant, 1 mg/kg was administered intraperitoneally. The protective effect of camel milk against strychnine-induced seizures was evaluated and compared with diazepam, as a standard drug. Camel milk was administered at the dose of 33 mL/kg orally for 15 days. On Day 16 (*i.e.* on day of experiment), it was administered 30 min prior to strychnine. Distilled water (1 mL, *p.o.*) or diazepam (5 mg/kg, *i.p.*) were administered 30 min and 15 min respectively before injecting strychnine to evaluate the anti-convulsive activity of camel milk and compared with diazepam. The animals were placed in individual cage (20 cm × 15 cm) and observed for 30 min for latency of seizure onset, frequency of convulsions and duration of jerks. The mortality rate was also evaluated for each group. The number of animals, which survived after 30 min, served as criterion of protection[15]. The animals which remained alive after 30 min were continued with camel milk administration for the next 24 h. The animals which survived after that time period were considered to be protected from lethal effects of strychnine.

2.6. 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 the day of experiment, animals were sacrificed by cervical dislocation and complete brain tissues from each of the normal, control and drug treated animals were immediately dissected out, washed with ice-cold 0.9% NaCl and were preserved in 10% buffered formaline overnight. In order to assess morphological changes, gross examination was performed. Subsequently, sections from hippocampal region were submitted in representative cassette and processed in an automated "Medite TPC 15" tissue processor for 12 h. Afterwards, tissues were embedded in paraffin wax using automated paraffin embedding station, "TES 99 Medite"[16]. Tissue sections of 3 to 4 μm in thickness were cut from paraffin embedded block using a microtome "SLEE 4062". Dissected sections were transferred into water bath maintained at 46-48 ℃ and mounted on positively charged glass slides. Finally, sections were subjected to haematoxylin and eosin staining as described by Kumar and Kiernan[17].

2.7. Statistical evaluation

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

3. Results

3.1. Effect of camel milk on strychnine-induced seizures

Table 1 shows the effect of camel milk on strychnine-induced seizures in mice.

Table 1

Effect of camel milk on strychnine-induced seizure model.

Group/Treatment	Dose (Route)	Seizure onset in s (Latency)	No. of seizures (Frequency)	Total duration of jerks in s
I/Distilled water	10 mL/kg(*p.o.*)	116.50 ± 69.59	2.00 ± 0.94	35.30 ± 3.33
II/Camel milk	33 mL/kg (*p.o.*)	716.00 ± 194.23$^{***,\#\#\#}$	4.30 ± 0.94$^{***,\#\#\#}$	23.60 ± 7.02***
III/Diazepam	10 mg/kg (*i.p.*)	443.20 ± 71.86***	6.00 ± 0.66***	24.50 ± 7.87**

Values are mean ± SD (n = 10). Significant difference by Scheffe test; ***: $P \leqslant 0.001$, **: $P \leqslant 0.01$ when compared with distilled water, and $^{\#\#\#}$: $P \leqslant 0.001$ when compared with diazepam treated animals, following One-way ANOVA.

3.1.1. Latency of seizure onset

One way ANOVA showed significant difference between groups, (F = 56.61, df = 2, 27, $P \leqslant 0.001$) on latency of seizures onset. *Post hoc* analysis by Scheffe test revealed that camel milk (Group II) significantly ($P \leqslant 0.001$) delayed the onset of seizures when compared with control (Group I) and standard (Group III) mice (Table 1).

3.1.2. Frequency of convulsions

One way ANOVA showed significant difference between groups, (F = 54.13, df = 2, 27, $P \leqslant 0.001$) on frequency of convulsions. *Post hoc* analysis by Scheffe test revealed that frequency of convulsions was significantly ($P \leqslant 0.001$) increased when Group II was compared with Group I, but significantly ($P \leqslant 0.001$) decreased in comparison to Group III (Table 1).

Reference drug, diazepam (Group III) also revealed similar effects as the camel milk on seizure onset and frequency when compared with control (Group I).

3.1.3. Total duration of jerks

Results showed highly significant difference between groups, (F = 10.37, df = 2, 27, $P \leqslant 0.001$) on total duration of jerks. *Post hoc* analysis by Scheffe test revealed that total duration of jerks was significantly ($P \leqslant 0.001$) decreased when Group II was compared with Group I. However, decrease was non-significant when compared with Group III (Table1). Standard group revealed moderately significant ($P \leqslant 0.01$) decrease in total duration of convulsions when compared with control.

3.2. Effect of camel milk on mortality rate

Table 2 shows the effect of camel milk on mortality rate after 30 min and after 24 h.

Table 2

Effect of camel milk on mortality rate in strychnine-induced seizure model after 30 min and 24 h.

Group/Treatment	Animal status (n = 10)				Mortality rate (%)		Protection (%)	
	30 min		24 h		30 min	24 h	30 min	24 h
	Dead	Alive	Dead	Alive				
I/Distilled water	8	2	8	2	80	80	20	20
II/Camel milk	2	8	4	6	$20^{***,\#\#\#}$	$40^{***,\#\#\#}$	80	60
III/Diazepam	0	10	0	10	0^{***}	0^{***}	100	100

Values are expressed as percentage. ***: $P \leqslant 0.001$ when compared with distilled water; $^{\#\#\#}$: $P \leqslant 0.001$ when compared with diazepam treated animals.

Data was analyzed by Pearson's *Chi* square test of correlation. It showed a significant effect of treatment on prevention of mortality, [χ^2 = 15.6 (df = 2), $P \leqslant 0.001$] after 30 min of administration of strychnine *i.e.*, 80%, as well as after 24 h, χ^2 = 13.33 (df = 2), $P \leqslant 0.001$ *i.e.*, 60% by camel milk. Diazepam showed its known effect of 100% prevention in both instances.

3.3. Histopathology

Histopathology of our samples showed that strychnine treatment produced neuronal damages as observed with hippocampal injury showing neuron with eosinophilic necrosis (Figure 1a) and surrounding tissue showing edema and proliferative blood vessels. Shrunken basophilic neurons were also observed with very few healthy neurons (Figure 1a) when compared with normal mice (Figure 1b). Treatment with diazepam showed preserved architecture in representative section of the brain with mild reactive changes as observed in Figure 1c. However, camel milk treatment showed significant preservation of hippocampal neurons as observed with relatively normal healthy neuron (Figure 1d) and few pyknotic nucleus suggestive of mild neuronal damage than animals treated with strychnine alone (Figure 1a).

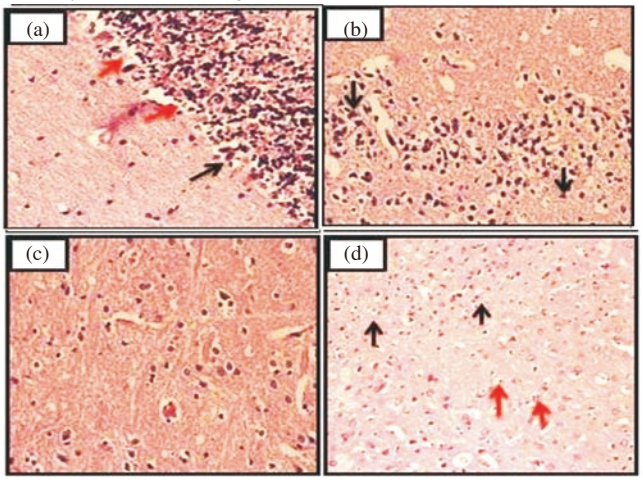

Figure 1. Histopathological changes in mice hippocampus (microphotographs of hematoxylin and eosin at ×400).
(a): Representative section of strychnine treated mice hippocampus showing neuron with eosinophilic necrosis (pointed by black arrow) and surrounding tissue showing edema and proliferative blood vessels. Note the shrunken basophilic neurons with very few normal neurons (pointed by red arrows); (b): Representative section of distilled water treated normal control, arrow pointed out the reactive astrocytes; (c): Representative section of diazepam treated brain showing preserved architecture; (d): Representative section of camel milk treated animal showing relatively normal neuron (pointed by black arrows) along with proliferative blood vessels and few pyknotic nucleus (pointed by red arrows).

4. Discussion

The results of present study demonstrate that camel milk possesses anticonvulsant effect triggered by strychnine, a glycine-receptor antagonist. Strychnine is a poisonous alkaloid, obtained from *Strychnos nux-vomica* seeds. It is a neurotoxin and acts as spinal cord stimulant. Strychnine exerts its effect by blocking the binding of glycine to the glycine-sensitive chloride channel within the spinal cord. Normally, when glycine binds to this channel, it causes increased inward flow of chloride resulting in hyperpolarization and blocking cells to propagate nerve signals. These inhibitory effects of glycine are blocked by strychnine intoxication, which consequently result in increased nerve signal transmission. Strychnine induced excitation leads to unregulated muscle spasm, extensor muscle contractions, convulsions followed by death due to respiratory paralysis[18].

Pretreatment with camel milk showed its protective effects against convulsions. Of the measured parameters, the latency to seizure onset and frequency of seizures seemed to be most sensitive to anticonvulsant effect of camel milk when compared with reference drug diazepam. However, decrease in total duration of jerks was most significant with camel milk treatment when compared with control. The effect was almost similar to that of reference drug, diazepam. Previous studies revealed depressed seizure activity in animal model of epilepsy after exogenous glycine administration[19]. Furthermore, previous work also suggested synergistic anticonvulsant effects when glycine was administered with γ-aminobutyric acid-A receptor

agonist[20]. Anticonvulsant effect of camel milk may be attributed to the activation of strychnine-sensitive glycine receptors alone or by concomitant potentiation of glycinergic and GABAergic activities which in turn may amplify its antiepileptic activity.

Mice induced seizures after strychnine 1 mg/kg *i.p.* were also protected from death when pretreated with 33 mL/kg *p.o.* of camel milk for 15 days and showed only 20% mortality rate in comparison to control group, which showed 80% mortality rate. Diazepam treated animals were completely protected (0% mortality) from death at the dose of 5 mg/kg *i.p.*

Neuroprotective effects of vitamin E have already been identified previously[21]. Due to its antioxidant characteristic, vitamin E could exert antiepileptic effect via inhibiting free radical formation[21]. Results of another study also showed that vitamin E administration reduces seizure frequency and intensity by decreasing oxidative stress in brain cells of experimental animals[22]. Chemical composition of camel milk showed high content of vitamin C, E, zinc, magnesium and copper[23]. Hence, it also possesses strong antioxidant characteristic and has neuroprotective potential. Results of our study are also in accordance with previous studies and concluded that delayed seizure onset and decreased total duration of convulsions in camel milk pretreated group could also be due to its high content of vitamin E (0.56 mg/L) and vitamin C (37.4 mg/L) which increase total antioxidant potential of brain cells[23,24].

Anticonvulsive effect of camel milk could also be attributed to its protective effect against cellular injury induced by strychnine in hippocampal neurons. Exact mechanism of this resistance to hippocampal injury is currently unclear. However, previous studies indicate that neurons involved in oxidative injury create a toxic environment within the hippocampus as a result of induction of reactive oxygen species activation and progression to apoptosis which ultimately destroy hippocampal neurons[25]. Therefore, it is suggested that neuroprotective potential of camel milk is due to its strong antioxidant characteristics. It should be confirmed by estimating superoxide dismutase and glutathione levels.

The results of our study indicate that camel milk possesses anticonvulsant activity, which may justify its use as an alternative medicine in order to prevent drug related toxicities and drug resistance. The proposed anticonvulsive mechanism may involve potentiation of both GABAergic and glycinergic stimulatory mechanisms. Antioxidant activity can also amplify its antiepileptic activity. The exact mechanism of action of camel milk should be assessed and requires detailed study.

Conflict of interest statement

We declare that we have no conflicts of interest.

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