Investigation of anti-inflammatory and analgesic activities of camel milk in animal models

Humera Khatoon[1]*, Rahila Ikram[2], Humaira Anser[3], Sadaf Naeem[3], Saira Saeed Khan[2] Sakina Fatima[4], Nuzhat Sultana[2] and Sana Sarfaraz[2]

[1]Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan;
[2]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan
[3]Faculty of Pharmaceutical Sciences, Jinnah Sind Medical University, Karachi, Pakistan
[4]Department of Pharmaceutics, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

Abstract: Opioids and non-opioids have long been used as analgesic, anti-inflammatory and antipyretic. Long-term use of these drugs may lead to severe toxicities. Therefore natural remedies are now being explored to avoid risk of adverse effects associated with the use of these conventional medicines. Bioactive components from milk of different species have been identified as nutraceuticals, but no experimental or clinical study is conducted so far to explore the analgesic and anti-inflammatory potential of camel milk. In this study we evaluated camel milk for its possible analgesic and anti-inflammatory activity. The anti-inflammatory effects of camel milk was studied in rats using paw edema method (induced by acetic acid) while tail-flick method was used to evaluate its analgesic effect in mice. Significantly increased tail-flick latency was shown after camel milk (33ml/kg) treatment when compared with acetylsalicylic acid at all time intervals. Anti-inflammatory activity of camel milk was significant ($p \leq 0.001$) at 4th hour of treatment as shown by maximum percentage inhibition in edema volume (46.84%) in comparison to control. Results of our present study suggested possible use of camel milk as adjuvant therapy in treating various chronic pain and inflammatory ailments. Camel milk could further be investigated in future for recognition of biochemical constituents responsible for its anti-inflammatory and pain relieving activities.

Keywords: Camel milk, inflammation, pain, paw edema, tail-flick.

INTRODUCTION

Pain and inflammation are nonspecific symptoms of many chronic diseases such as inflammatory bowel disease, rheumatoid arthritis, psoriasis, many types of infections as well as tumor and malignancies (del Rincón *et al.*, 2003; Fujino *et al.*, 2003; Macarthur *et al.*, 2004).Our body responds naturally to injurious stimuli and provides first line defense via initiating inflammatory response. After tissue injury excessive production and accumulation of arachidonic acid metabolites such as leukotrienes, thromboxanes and prostaglandins via lipooxygenase (LOX) and cyclooxygenase (COX) pathways play a vital role in eliciting pain and inflammatory responses (Higuchi *et al.*, 1985; Vane *et al.*, 1987).

Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids have widely been used to relieve pain, inflammation and fever for many years (Yaksh *et al.*, 2011). Even in current clinical practice COX inhibitors are mainstay in analgesia among available medicines(Kehlet *et al.*, 1999; Raffa, 2001). Newly developed formulations are well absorbed, easy to administer and economical, but unfortunately chronic use of these classical medicines carry a high risk of severe adverse effects including gastrointestinal complications, renal damage, and possible cardiovascular toxicity (Sostres *et al.*, 2010; Whelton, 2000). New generation selective COX-2 (cyclooxygenase-2) inhibitors although have an improved safety profile (Coruzzi *et al.*, 2007) but their use still require constant vigilance. Therefore, new safer replacements for current analgesic and anti-inflammatory drugs without those reported side effects are being explored globally.

Milk from different animal species including bovine, goat and camel has been served as good source of nutrition for many years. Bioactive active substances in fresh and fermented camel milk have now been identified as functional food and many research studies in recent years reported health benefits with the use of these milk-derived constituents(Khatoon and Najam, 2017; Meisel, 1997). Review of literature recommend that camel milk has remedial potential and might be utilized in the treatment of diabetes, viral and bacterial infections, hypertension and serve as immune booster and anti-carcinogenic (Yadav *et al.*, 2015). Neuroprotective potential of camel milk was also identified recently (Khatoon *et al.*, 2016; Khatoon *et al.*, 2015).Yamaguchi and colleagues (2009) reported anti-inflammatory and anti-nociceptive effects of milk from cow and other species as its novel function, but no in vivo or in vitro studies have been conducted so far on camel milk to identify its anti-inflammatory and pain-relieving potential. Therefore, the present investigation

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

was intended to determine the efficacy of camel milk as an alternate source to alleviate pain and inflammation if consumed orally.

MATERIALS AND METHODS

Experimental animals

Swiss albino mice (20-30g) and albino rats (200-250g) of either sex were used for analgesic and anti-inflammatory activities respectively. Animals were handled according to the guidelines for the care and use of laboratory animals provided by National Institute of Health (NIH publication #80-23). BASR (Board of Advanced Studies and Research), University of Karachi, Pakistan approved the study (Resol.#10(P)01) dated: 03-03-2014.

Camel milk

Milk of one-humped camel was purchased from local farm house and kept at -6 to -8^{o}C till its use.

Drugs and chemicals

Ibuprofen (Abbott Laboratories, Pakistan, Ltd.), acetic acid and acetylsalicylic acid from Sigma Chemicals, USA were purchased for our study.

Analgesic activity

Tail-flick test

Mice (Swiss albino) were selected randomly, distributed into 3 groups (gp), each comprises of ten animals. First gp (control) received distilled water (0.5ml), second gp (experimental) administered 33ml/kg of camel milk (Salwa *et al.*, 2010) and 3rd gp (standard) received 70 mg /kg of acetylsalicylic acid (Olaleye *et al.*, 2000). Each of the drugs was given by oral means 30 minutes before the investigation.

The reaction time was measured as tail-flick latency as per the technique given by Olaleye *et al*. (2000) utilizing preheated water bath maintained at 50.0 ± 1.0^{0}C temperature. According to the procedure, one-third of the tail was immersed in preheated water bath and response was recorded in seconds as tail-flick latency. The response was measured when animal flicks its tail from hot water bath. Twenty seconds were imposed as a cut-off time to protect tissue from thermal damage. Pre-treatment reaction time of all treatment groups was measured at 0 minute as baseline. Reaction time was measured every 30 min interim after respective treatment to each group till 210 minutes (Olaleye *et al.*, 2000).

Anti-inflammatory activity

Acetic acid-induced paw edema

The study was conducted on rats (Wistar albino). Thirty animals were distributed into three groups as follows:

Control group assigned number 1, was on distilled water treatment (0.5 ml p.o), test group assigned number 2, kept on camel milk treatment (33ml/kg, p.o) (Salwa *et al.*, 2010) and reference group assigned number 3, on ibuprofen 100 mg /kg p.o (Choi *et al.*, 2005).

All groups were subjected to their respective treatments on the day of experiment. Later on, 30 min after treatment 1% w/v acetic acid was injected (0.1ml) in sub-plantar region of left hind paw to induce edema. Baseline paw size of each animal in all treatment groups was measured via Plethysmometer (Ugobasile, Italy) before treatment (at 0 hour). Subsequently, paw size was measured every one-hour interval after acetic acid injection till four hours (Anser *et al.*, 2015).

Determination of paw size using Plethysmometer

Anser and Najam (2015) used the same apparatus in their study, consisted of two interconnected measuring tubes made up of transparent plastic, each having a platinum electrode. Water was used to conduct electricity and filled in each measuring chamber. The paw was immersed in the first measuring tube. Water displacement as a result of paw-immersion brought about change in conductance between the two electrodes. The change in conductance was detected by the control unit of Plethysmometer and transmitted to the digital display, designated as the volume displacement in ml (Anser *et al.*, 2015).

The anti-inflammatory activity was measured by Rachehh and colleagues (2011) as % inhibition calculated by the formula :

% inhibition= [1-(ΔVt/ΔVc)]x100

Where,

ΔVt is mean increase in paw size for camel milk/ibuprofen-treated animals.

ΔVc is mean increase in paw size for control animals.

STATISTICAL ANALYSIS

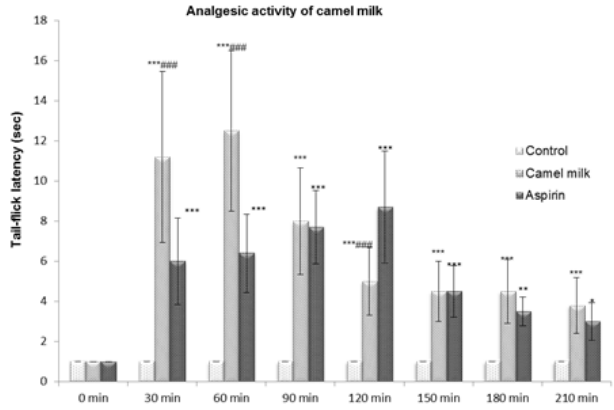
Two-way ANOVA (analysis of variance) using SPSS (Statistical Package for the Social Sciences version 20 for windows) was used to statistically analyze the data, presented as mean ± standard deviation (SD). Significance between groups was analyzed by Scheffe post-hoc. The difference between groups was considered significant at P value less than 0.05.

RESULTS

Analgesic activity of camel milk

The outcomes of anti-nociceptive activity of camel milk are shown in fig. 1. Mice in control group did not exhibit any substantial change in response latency throughout the whole experiment. Camel milk revealed significant increase in tail-flick latency when compared to control group at all intervals, which was reached to its maximum (12.50±4.00) at 60 minutes. On the other hand, the maximum activity of acetyl salicylic acid was 8.70 seconds (±2.79) appeared at 120 minutes. The results

showed that increase in tail-flick latencies for camel milk remains significant ($p \leq 0.001$) till 210 minutes when compared with control. The fig. 1 also illustrated that till 90 minutes camel milk was more effective than acetyl salicylic acid with reaction time of 8.00 seconds compared to 7.7 seconds respectively. However, at 150 minutes both groups showed same activity i.e. 4.5 seconds.



Mean values ± S.D (n=10). Two-way ANOVA was used to calculate significance and Scheffe test for post-hoc analysis. $^{***}p \leq 0.001$, $^{**}p \leq 0.01$ and $^{*}p \leq 0.05$ when compared with control .$^{###}p \leq 0.001$ in comparison to standard (Acetyl salicylic acid).

Fig. 1: Analgesic activity of camel milk

Anti-inflammatory activity of camel milk

The results of anti-inflammatory activity showed that strong edema was produced in the rats' paw of all treated groups following sub-plantar injection of acetic acid (table 1). The edema volume reached to its peak after 3 hours and started to decrease at fourth hour in group 1 and 2 (control and camel milk respectively). In comparison to control group, camel milk showed significant reduction ($P < 0.001$) in paw edema volume (0.59±0.18) at fourth hour of treatment with percentage inhibition of 46.84. The reference group (group 3) revealed its previously recognized anti-inflammatory effect as shown by significant inhibition ($P < 0.001$) in edema volume and time-dependent increase in percentage inhibition at all intervals throughout the experiment. Within camel milk treated group, the edema size at fourth and first hour of treatment was nearly same (0.59±0.18 and 0.59±0.30 ml respectively), suggested its delayed onset anti-inflammatory activity.

DISCUSSION

NSAIDS are commonly prescribed for symptomatic relief of pain, fever and inflammation but these agents have some side effects including gastric ulcer, cardiac abnormalities, renal insufficiency and bronchospasm. Therefore, researchers are now focusing on plants and other natural dietary products such as turmeric, fish derived omega 3 and 6 fatty acids, camel milk, olive oil and other polyphenols, flavonoids, steroids, and terpenes containing products as they have different pharmacological activities including analgesic, antipyretic and anti-inflammatory effects (Khalid *et al.*, 2018). Analgesics act either centrally or peripherally and producing pain-relieving effect without fluctuating consciousness (Hijazi *et al.*, 2017). Centrally acting analgesics act by modifying the physiological response to pain via increasing pain threshold. However, via peripheral pathway pain is relieved by aspirin and other drugs by blocking detection of pain impulses by nociceptors in the periphery (Shreedhara *et al.*, 2009). In the current study camel milk has been investigated for its possible analgesic and anti-inflammatory activity by using tail flick and acetic acid-induced rat paw edema method. The results of the present study showed that camel milk elicited acute and prolonged analgesic activity and exhibited substantial increase in reaction time at 30 minutes, reached to its peak at 60 minutes and the effect was maintained throughout the experiment when compared with control and aspirin treated groups. AL-Awadi and Srikumar, (2001) reported that other than all dietary benefits camel's milk also possesses higher zinc content than milk of any other specie including human milk. Zinc functions as a modulator of the immune response by improving adaptive immunity and inhibition of prostaglandins, interleukin and other inflammatory cytokines production (Bonaventura *et al.*, 2015; Prasad *et al.*, 2011). Other than zinc the protein in camel's milk (casein and whey proteins) is present in nearly the same proportion as that in cow's milk, whereas protein content is lowest in human milk when compared milk from other mammals (Yadav *et al.*, 2015; Jenness, 1974). Camel milk contains about 3% protein content, of which caseins represent 80% of total proteins (Khatoon and Najam, 2017). Caseins are precursors of a significant number of opioid peptides described as β-casomorphins (Carrillo *et al.*, 2018). These opioid peptides have been evaluated in different animal and human models, demonstrating their efficacy against pain (Grecksch *et al.*, 1981; Matthies *et al.*, 1984).

It is manifested that acetic acid-induced edema is biphasic and used as a standard model to assess the anti-inflammatory activity of drugs and chemicals. Initially, after first to second hour of acetic acid administration inflammation is mediated by the synthesis of prostaglandins and release of kinin, serotonin and histamine which is persisted in late phase due to the release of bradykinins, prostaglandin and other mediators (Das *et al.*, 2011). In the present study camel milk treated group revealed delayed but significant reduction in paw edema after 3 to 4 hour as compared to control that represents that its slow-onset anti-inflammatory activity could be due the inhibition of synthesis and release of second-phase inflammatory mediators. Our results are in

Table 1: Anti-inflammatory activity of camel milk

Treatment groups	Before acetic acid treatment (displacement in ml) (mean±SD)	1 hr after acetic acid treatment (displacement in ml) (mean±SD)	After acetic acid treatment Difference in paw size of rat (volume displacement by paw edema in ml) (Percent inhibition of edema)			
	Initial paw size	Paw size after acetic acid	1hr	2hr	3hr	4hr
Control	2.99±0.16	3.63±0.11	0.64±0.20	0.93±0.22	1.32±0.15	1.11±0.14
Camel milk	3.60±0.19	4.19±0.27	0.59±0.30 (7.81%)	0.79±0.24$^{###}$ (15.05%)	0.99±0.22$^{***, ###}$ (25.00%)	0.59±0.18$^{***,###}$ (46.84%)
Ibuprofen	3.08±0.05	3.63±0.08	0.55±0.06 (14.06%)	0.41±0.12*** (55.91%)	0.38±0.06*** (71.21%)	0.23±0.17*** (79.27%)

Results are shown as percentage inhibition and difference in paw size. Values are presented as Mean ± S.D (n=10). Two-way ANOVA was used to calculate significance and Scheffe test for post-hoc analysis. $^{***, ###}$ $p \leq 0.001$ as compared to control and ibuprofen treated animals respectively.

accordance with Rasheed et al. (2016) who reported that anti-inflammatory effect of camel milk in human osteoarthritis chondrocytes is produced by inhibiting COX-2 expression and PG-E2 production (Rasheed *et al.*, 2016). Another study demonstrated that camel milk has unique property of containing immunoglobulins that regulate innate immunity and promote a powerful anti-inflammatory effect (Habib *et al.*, 2013).

The results of our investigation support the conventional use of camel milk as nutrient in alleviating pain and inflammation and recommend its use in combination with NSAIDs in arthritis and other chronic pain conditions. Since high doses of NSAIDs are generally required for management of such painful conditions, therefore combined use of camel milk in such conditions could reduce the daily doses of NSAIDs and hence help in avoiding unwanted effects accompanying their use. The current study also suggests further investigation and elucidation of camel milk for the presence of biologically active components responsible for its pain-relieving and anti-inflammatory activities.

CONCLUSION

Our study suggest that camel milk can successfully attenuates pain perception either by its central (by stimulating opioid receptors) or peripheral (by inhibiting COX pathway) action. However further studies are required to interpret the precise molecular mechanisms that underpin their role in pain management and the ability to stimulate the immune system.

REFERENCES

AL-Awadi FM and Srikumar T (2001). Trace elements and their distribution in protein fractions of camel milk in comparison to other commonly consumed milks. *J. Dairy Res.,* **68**(3): 463-469.

Anser H and Najam R (2015). Evaluation of anti inflammatory activity of Argentum nitricum, Staphysagria and Ignatia amara in experimental animal model. *Int. J. Adv. Pharm., Biol. Chem.,* **4**(1): 76-83.

Bonaventura P, Benedetti G, Albarede F, Miossec P (2015). Zinc and its role in immunity and inflammation. *Autoimmun Rev.,***14**(4): 277-285.

Carrillo W, Monteiro KM, Spindola H, Ramos M and de Carvalho JE (2018). Antiulcerative and antinociceptive activities of casein and whey proteins. *J. Med. Food*, **21**(6): 605-611.

Choi J, Lee KT, Choi MY, Nam JH, Jung HJ, Park SK and Park HJ (2005). Antinociceptive anti-inflammatory effect of monotropein isolated from the root of Morinda officinalis. *Biol. Pharm. Bull.,* **28**(10): 1915-1918.

Coruzzi G, Venturi N and Spaggiari S (2007). Gastrointestinal safety of novel nonsteroidal antiinflammatory drugs: Selective COX-2 inhibitors and beyond. *Acta Biomed.*, **78**(2): 96-110.

Das S, Haldar PK, Pramanik G, Panda SP, Bera S (2011). Evaluation of analgesic and anti-inflammatory activity of *Diospyros cordifolia* extract. *Afr. J. Tradit. Complement. Altern. Med.,* **8**(1): 11-14.

del Rincon I, Williams K, Stern MP, Freeman GL, O'leary DH and Escalante A (2003). Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. *Arthritis Rheum.*, **48**(7): 1833-1840.

Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T and Fujiyama Y (2003). Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*., **52**(1): 65-70.

Grecksch G, Schweigert C and Matthies H (1981). Evidence for analgesic activity of β-casomorphin in rats. *Neurosci. Lett.,* **27**(3): 325-328.

Habib HM, Ibrahim WH, Schneider-Stock R and Hassan HM (2013). Camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities. *Food Chem.,* **141**(1): 148-152.

Higuchi S, Osada Y, Shioiri Y, Tanaka N, Otomo S and Aihara H (1985). The modes of anti-inflammatory and analgesic actions of aspirin and salicylic acid. *Nihon Yakurigaku Zasshi.*, **85**(1): 49-57.

Hijazi MA, El-Mallah A, Aboul-Ela M and Ellakany A (2017). Evaluation of analgesic activity of papaver libanoticum extract in mice: Involvement of opioids receptors. *J. Evid. Based Complementary Altern. Med.*, 2017: 1-13.

Jenness R (1974). Biosynthesis and composition of milk. *J. Invest. Dermatol.,* **63**(1): 109-118.

Kehlet H, Werner M and Perkins F (1999). Balanced analgesia. *Drugs*, **58**(5): 793-797.

Khalid S, Ullah MZ, Khan AU, Afridi R, Rasheed H, Khan A, Ali H, Kim YS and Khan S (2018). Antihyperalgesic properties of honokiol in inflammatory pain models by targeting of NF-κB and Nrf2 signaling. *Front Pharmacol.,* **9**: 140.

Khatoon H and Najam R (2017). Bioactive components in camel milk: Their Nutritive Value and Therapeutic Application. In Nutrients in Dairy and their Implications on Health and Disease, Academic Press, University of Arizona, USA, pp. 377-387.

Khatoon 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.

Khatoon 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.

Macarthur M, Hold GL and El-Omar EM (2004). Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **286**(4): G515-G520.

Matthies H, Stark H, Hartrodt B, Ruethrich HL, Spieler HT, Barth A and Neubert K (1984). Derivatives of β-casomorphins with high analgesic potency. *Peptides*. **5**(3): 463-470.

Meisel H (1997). Biochemical properties of bioactive peptides derived from milk proteins: potential nutraceuticals for food and pharmaceutical applications. *Livest Prod. Sci.* **50**(1-2): 125-138.

Olaleye S, Farombi E, Adewoye E, Owoyele B, Onasanwo S and Elegbe R (2000). Analgesic and anti-inflammatory effects of kolaviron (a *Garcinia kola* seed extract). *Afr. J. Biomed Res*., **3**: 171-174.

Prasad AS, Bao B, Beck FW and Sarkar FH (2011). Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB. *Nutrition*., **27**(7-8): 816-823.

Rachehh M, Yadav P, Cokani R and Jain S (2011). Antiinflammatory activity of *Benincasa hispid* fruit. *Int J. Phar. Biosci*., **2**(3): 98-106.

Raffa R (2001). Pharmacology of oral combination analgesics: Rational therapy for pain. *J. Clin. Pharm. Ther.*, **26**(4): 257-264.

Rasheed N, Alghasham A and Rasheed Z (2016). Lactoferrin from camelus dromedarius inhibits nuclear transcription factor-kappa B activation, cyclooxygenase-2 expression and prostaglandin E2 production in stimulated human chondrocytes. *Pharmacognosy Res.*, **8**(2): 135.

Salwa MQ, Lina AK (2010). Antigenotoxic and anticytotoxic effect of camel milk in mice treated with cisplatin. *Saudi J. Biol. Sci.,* **17**(2): 159-166.

Shreedhara C, Vaidya V, Vagdevi H, Latha K, Muralikrishna K and Krupanidhi A (2009). Screening of *Bauhinia purpurea* Linn. for analgesic and anti-inflammatory activities. *Indian J. Pharmacol.*, **41**(2): 75.

Sostres C, Gargallo CJ, Arroyo MT and Lanas A (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Pract. Res. Clin. Gastroenterol.*, **24**(2): 121-132.

Vane J and Botting R (1987). Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J.,* **1**(2): 89-96.

Whelton A (2000). Renal and related cardiovascular effects of conventional and COX-2-specific NSAIDs and non-NSAID analgesics. *Am. J. Ther.*, **7**(2): 63-74.

Yadav AK, Kumar R, Priyadarshini L, Singh J (2015). Composition and medicinal properties of camel milk: A Review. *Asian J. Dairy and Food Res.*, **34**(2): 83-91.

Yaksh TL and Wallace MS (2011). Opioids, analgesia, and pain management. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Edited by Brunton L, Chabner B, Knollman B: McGraw-Hill Medical, New York, USA, pp.481-526.

Yamaguchi M, Yoshida K and Uchida M (2009). Novel functions of bovine milk-derived α-lactalbumin: anti-nociceptive and anti-inflammatory activity caused by inhibiting cyclooxygenase-2 and phospholipase A2. *Biol Pharm Bull.*, **32**(3): 366-371.