The egyption journal of hospital medicine (2009) vol., 35 : 335-346

Whey Protein, α-Lactalbumin and β-Lactoglobulin in Sprague Dawley Rat

Somaia A. Nada

Pharmacology Department, National Research Centre, Dokki, Cairo, Egypt

Abstract

Background: Whey is a by-product of cheese production; it is one of the components which separate from milk after curdling, when rennet or an edible acidic substance is added. Whey protein (WP) is typically a mixture of beta-lactoglobulin (β-lg) (~65%), alpha-lactalbumin (α-la) (~25%), and serum albumin (~8%), which are soluble in their native culture forms and it has the highest biological value of any known protein.

Materials and Methods: Comparative studies were performed to assess the efficacy of WP, α-la and β-lg (100, 200 and 300 mg/Kg, Os) in tow animal models: hot plate-induced thermal pain and carrageenan-induced paw inflammation and antioxidant activities in rats.

Results: Results revealed that the higher doses of WP, α-la and β-lg caused significant analgesic effect versus paracetamol (50 mg/Kg) especially after 3 hr-post treatment (potency: 3.01, 3.21 and 3.45, respectively). Whereas after 4hr., WP and α-la (300 mg/Kg) treatments had similar analgesic effect. While, β-lg (200 and 300 mg/Kg) was the most potent in its analgesic effect when compared with the paracetamol and the other treated groups.

In acute anti-inflammatory activity, it was shown that the two doses of β-lg (100 and 200 mg /kg) significantly reduced paw oedema after 30 min (potency versus indomethacine was: 1.11 and 1.13). While after 4 hr, the higher dose of α-la (300 mg/Kg) had similar effect to that induced by the two doses of β-lg (200 and 300 mg/Kg) treatment. The potency of the two doses (100 and 200 mg/Kg) of WP nearly had similar anti-inflammatory effect (time dependent effect).

All treatments caused significant antioxidant activity when compared with the control group. The increase in SOD value was dose dependent manner. In which, 300 mg/Kg showed remarkable increase in SOD level with the following rank, α-la > β-lg > WP > indomethacine (5 mg/Kg) treated groups. These results indicated that β-lg produced powerful analgesic and anti-inflammatory activities than α-la and WP. As well as, α-la possess strong antioxidant activity than β-lg and WP treatments.

Conclusion: It suggested that β-lg, α-la and WP could be used safely as natural analgesic and anti-inflammatory drug instead of NSAIDs, which have side effects when used for chronic disorders.

Key words: Whey Protein, α-Lactalbumin, β-Lactoglobulin, analgesic, anti-inflammatory, SOD, rat.

Introduction

In recent years, milk constituents have become recognized as functional foods, suggesting that their use has a direct and measurable effect on health outcomes. Milk contains two primary sources of protein, the caseins and whey. After processing, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. Whey has been touted as a functional food with a number of health benefits **(Marshall *et al.*, 2004)**. The protein fraction in whey comprises four major protein fractions and six minor protein fractions. The major protein fractions in whey are α -lactalbumin (α -la), β-lactoglobulin (β lg), bovine serum albumin, and immuneoglobulins.The biological components of whey demo-nstrate a range of immune-enhancing properties **(Low *et al.*, 2003)**. In addition, whey has the ability to act as an antioxidant **(Brown *et al.*, 2004)** antihypertensive **(Saito (2008),** antitumor **(Bounous *et al.*, 1991)**, hypolipidemic **(Marshall, 2004),** antiviral **(Neurath *et al.*, 1996),** antibacterial **(Shah, 2000)** and chelating agent **(Hurrell *et al.*, 1989).** It is well-known that lactoferrin, the minor component of whey proteins, inhibits production of the inflammatory cytokines tumor necrosis factor (TNF)-α , interleukin (IL)- 1b , and IL-6 in monocytes. **Yamaguchi *et al.*, (2001)**

confirmed that lactoferrin inhibits TNF-α production caused by sensitization of hepatic monocytes (kupffer cells) by lipopolysaccaride **(Yamaguchi *et al.*, 2001).** It has been reported that lactoferrin produces analgesia in the thermal, visceral and formalin-evoked nociceptions in rats **(Hayashida *et al.*, 2004).** Prostaglandins (PGs) formed by the phospholipase A2 (PLA2) and cyclooxygenase (COX) enzymes are important mediators of nociception and inflammation **(Smith, 2006).** On the other hand, emerging information has pointed to the role of another arachidonic acid metabolic pathway (the 5-lipoxygenase pathway) in producing and maintaining inflammation **(Yamakawa *et al.*, 2009)**. There is evidence that COX-2 and 5-lipoxygenase are co-expressed and up-regulated in a number of inflammatory diseases and that COX-2 as well as 5-lipoxygenase inhibitors have beneficial effects in inflammatory diseases **(Claria and Romano, 2005)**.

The aim of the present study was to investigate: (1) if the oral administration of WP, α-la and β-lg could induce analgesic and anti-inflammatory effects; (2) which the therapeutic doses can exert the powerful effect and their potencies versus the corresponding market drugs (paracetamol and indomethacine). (3) Comparison between the three tested whey proteins as antioxidant which play a
critical role to exert their anti-inflammatory activities.

Materials and Methods

1. Drugs and chemicals: Indomethacine, paracetamol and carrageenan, (Behringwake Ag, Marburg, Germany).

2. Whey proteins α- la and β-lg were isolated and kindly provided by Prof. Dr. Abedl-Khalek Elnemr, Diary Science Department, National Research Centre, Dokki, Cairo, Egypt.

3. Animals: Sprague Dawley rats of both sexes weighing 100 - 120 gm were used throughout the experiments. The animals were divided into 11 equal groups (six rats each), housed under standard environmental conditions (23 ± 1◦C, 55 ± 5 % humidity and a 12-h light: 12-h dark cycle) and maintained on a standard laboratory diet and water *ad libitum*. "The experimental protocols were approved by The National Research Centre, Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues (Zimmermann, 1983).

Experimental Design

Dose Response of WP, α- la and β-lg . Three dose levels were choosen100, 200 and 300 mg/Kg) to determine the most effective dose at exerting physiological activity. In preliminary study, the tested doses 50 mg and 75 mg /Kg had insignificant changes from control group. All treatment solutions were given orally and 1 hr before assessment of anti-inflammatory activity.

1. Analgesic effect: The hot plate method (Roszkowski *et al.*,1971) was used. The mean reaction time was calculated 30 min post- drug administration during 4 hrs to the following groups : control (group 1), given distilled water (10 ml / Kg b.wt.), (Groups 2, 3 and 4) were administered WP, α- lact alb and β-lactglu (100 mg/Kg b.wt., orally); (Groups 5 ,6 and 7) given 200 mg / kg b.wt., and (Groups 8, 9 and 10) treated with 300 mg/ Kg. b. wt. , while group 11 administered the reference drug paracetamol (50 mg/ Kg b.wt.).
2. Assessment of antiinflammatory activity: The carrageenan- induced rat paw oedema was employed according to the method of **Winter *et al.*, (1962)** using a plethsmometer system**.** Plethysmometer is a volume meter and the standard instrument for measurement of rodent paw volume. This is a test to screen potential anti-inflammatory or anti-edema agents. The paw measured is

inserted into water in a clear acrylic cell, up to the wrist joint. The volume of water displaced is measured by a transducer **(Sharma *et al.*, 2004)**. Eleven groups of rats (six rats each) were treated as previously mentioned as in analgesic experimental design: control (group 1), given distilled water (10 ml / Kg b.wt.), (Groups 2, 3 and 4) were administered WP, α-la and β-lg (100 mg/Kg b.wt., orally); (Groups 5 ,6 and 7) given 200 mg / kg b.wt., and (Groups 8, 9 and 10) treated with 300 mg/ Kg. b. wt. , while group 11 administered indomethacine (anti-inflammatory reference drug) (5 mg / Kg

b.wt.), using a plethsmometer system (Ugo Basile Instruments, Italy) after 30 min of drugs administration till the end of experimental duration 4 hrs . The results were expressed as the difference of oedema inhibition. After 24 hr of treatments, all groups subjected for SOD analysis.

3. Antioxidant activity: After 24 hr, blood samples were collected from rtro-orbital venus plexus from all animals (in antiinflammatory assay) in plain test tubes. Serum was separated for determination of superoxide dismutase (SOD) according to Suttle (1986).

4. Statistical analysis: The obtained results were analyzed by ANOVA two ways using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package.

Results

Analgesic activity

Data in table (1) showed the analgesic effect of WP, α-la & β-lg using hot plate –induced pain in rats with the three dose levels for whey protein and its two components.

WP analgesic effect was observed after 1hr, 2hr and 2.5 hr post-administration at doses 300 mg, 200, and 100 mg /Kg., respectively. WP exerts its maximum analgesic effect after 3 hr at dose level (300 mg/Kg). Then, its effect declined after 3.5 hr.

α-Lactalbumin and β-lg -administration caused significant analgesic effect starting from 1/2 hr in dependent dose manner and their effects became more potent by time when compared with paracetamol treatment. Moreover, β- lg had a powerful analgesic effect than α-lactalbumin treatment when compared with the time or with the dose level.

Paracetamol treatment showed significant analgesic effect after 1hr –post treatment and its effect was comparable to that of α-la and β-lg in doses of 200 mg and 300 mg/Kg., while, they had resulted significant increase in threshold time more than paracetamol effect during the experimental period.

β- Lactoglobulin treated group had prolonged analgesic effect more than the other treated groups. The potency of the tested WP and protein fractions reached its higher level after 3 hr- post-treatment in groups orally given 300 mg/Kg with the following ranking in potency: β-lg > α-la > WP *vs.* paracetamol (3.45, 3.21 and 3.01) , respectively (table 1). After 4 hr., analgesic effect was prominent in groups treated with the higher doses (200 and 300 mg/Kg) of WP, α-la and β- lg when compared with paracetamol-treated group. As well as, the administration of WP with the lower dose (100mg /Kg) had analgesic effect similar to that of paracetamol effect starting from 1.5 hr – 4 hr.

Anti-inflammatory effect

Acute anti-inflammatory effect of the studied WP and its two major fractions α-la and β-lg in comparable with indomethacine was demonstrated in table (2). After 30 min - post treatment, groups treated with WP and α-la at doses of 100 mg and 200 mg /Kg showed significant reduction in paw oedema when compared with the control group, beside that, they insignificantly different between each other. Moreover, β-lg administration either with (100 mg or 200 mg/Kg) caused significant reduction in paw oedema when compared with WP and α-la treated animals, whereas, these two doses non-significantly different from each other. Potency was: 1.11 and 1.13 in groups treated with β-lg (100 mg and 200 mg / Kg) after 1/ 2 hr-post treatment *vs.* indomethacine–administration (Table 2). The higher dose of β-lg (300 mg /Kg) had similar effect to that of indomethacine after 30 min - 1hr -post treatment. Insignificant differences were demonstrated after 1hr- 3hr of treatment between groups treated with WP and α-la (300 mg/Kg) in their anti-inflammatory effect when compared with each other, while they had moderate anti-inflammatory effect (potency : 0.66, 0.68 and 0.78 *vs.* indomethacine, respectively). While, the higher dose of α-la (300 mg/Kg) produced potent anti-inflammatory effect as induced by the two doses of β-lg (200 mg and 300 mg /Kg) after 4hr (Table 2).

Interestingly, after 2 hr, both doses of β-lg (200 mg and 300 mg /Kg) had anti-inflammatory activity. β-lg (200 mg/Kg) had similar antiinflammatory effect to that of indomethacine, while the effect of β-lg (300 mg/Kg)was more potent than indomethacine treatment. After 2.5 hr,, both doses of β-lg (200 and 300 mg/Kg non-significantly different from indomethacine - administration. Furthermore, the two doses of β-lg (200 mg and 300 mg /Kg) showed persistent significant

reduction in paw oedema when compared with the standard drug indomethacine at the last time of experimental period (3hr-4hr).

Antioxidant activity

Antioxidant activity of the studied WP and its two fractions was demonstrated in Table 3. Data revealed that SOD level increased significantly in all treated groups when compared with the control group. The increase in SOD value was dose dependent manner. Treatment with α-la (300 mg/Kg) resulted the maximum increase in SOD level comparing with the same dose of WP and β-lg treatment. Indomethacine also increased SOD significantly when compared with the control group; while, it is non-significantly different from WP (100 mg, 200 mg/Kg) and β-lg (100 mg /Kg) treated groups.

Discussion

Bovine milk contains approximately 0.9 g/L of α- la and 0.3 g/L of β-lg, while human milk contains 1.6 g/L of α- la but no endogenous β-lg **(Hambrus , 1998).**

The antinociceptive activity of the three tested doses of whey protein and its two major fractions α-la and β-lg was clearly demonstrated at the higher dose (300 mg/Kg) in all treatment. Whereas the maximum recorded potency was 3.45 *vs.* paracetamol (after 3hr of treatment) in group treated with β-lg (300 mg/Kg). At the same time, both WP and α- la (300 mg/Kg) are nearly equal in their analgesic effect during 1.5 hr - 4 hr . This analgesic effect due to whey proteins contain opioid –like sequences in their primary structure, namely α- la f(50-53) and β-lg f(102- 105). These peptides have been termed α- and β- lactorphins **(Chiba and Yoshikawa, 1986).** Proteolysis of α- la with pepsin produced α- lactorphin, while digestion of β-lg with pepsin and then with trypsin, or with trypsin and chymotrypsin, yielded β-lactorphin (**Pihlanto-Leppala, 2001)**. α- lactorphin exerts weak but consistent opioid activity in the guinea pig ileum and in connection with receptor-binding; whereas β-lactorphin – despite its similar receptor-binding affinity – exerts an apparent non-opioid stimulatory effect on guinea pig ileum. These peptides show very low affinity for opioid receptors and μ-type receptor ligands. Both α- and β-lactorphin were found to displace ^{3}H-naloxone from its binding sites at micromolar concentrations **(Paakkari *et al.*, 1994)**. Furthermore, it was shown that digestion of β-lg with chymotrypsin produced β-lactotensin and β-lg f(146 – 149). The pharmacological activity of β-lactotensin was similar to that of β-lactorphin **(Pihlanto-Leppala, *et al.*, 1997)**.

In an animal model of acute inflammation (injection of carrageenan into the hind paw), edema was produced that was associated with marked accumulation of cyclooxygenases (COX) mRNA and thromboxane **(Seibert *et al.*, 1994 and Tantisira *et al.*, 2009).** Carrageenan injection induced a marked edema of the hind paw with coincident local production of PGE2 associated with upregulation of COX mRNA and protein in the affected paws **(Anderson *et al.*, 1996)**. Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the COX enzyme **(Schmelzer *et al.*, 2006).** On its own, COX enzyme synthesizes prostaglandins, creating inflammation. On the whole, the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain. COX-2 selective inhibitor is a form of NSAID that directly targets COX-2, an enzyme responsible for inflammation and pain. Selectivity for COX-2 reduces the risk of peptic ulceration. It has been reported that COX-2-selectivity does not affect other adverse effects of NSAIDs (most notably an increased risk of renal failure and gastric ulcer) **(Stichtenoth , 2004).** Recent clinical trials provide further evidence that COX-2 inhibitors may increase risk of cardiovascular events **(Bombardier *et al.*, 2000 and Wong *et al.*, 2005)** and delayed the wound healing process **(Gilory *et al.*, 1999 and Futagami *et al.*, 2002)**. A novel finding in this study is that WP, α-la and β-lg had anti-inflammatory effect when used in higher dose (300 mg/Kg) and their effect was persists after 4 hr –post treatment as well as, more potent than that of indomethacine. **Yamaguchi *et al*.(2009)** reported that α-la inhibited COX. Moreover, α-la showed selectivity on COX-2 as compared with COX-1. These results suggest that the tested WPs reduce the gastrointestinal side-effects. It has been reported that α-la fortifies the mucus gel layer by stimulating mucin production and secretion in gastric mucus-producing cells, and that this enhancing

effect is independent of endogenous PGE2 **(Ushida *et al.*, 2007)**. Whey proteins , α-la and β-lg stimulate mucin synthesis and secretion in mucus producing cells and induces increased thickness of the mucus gel layer in the gastric mucosa, suggesting that stimulation of mucus metabolism by α-la contributes to its gastroprotective actions **(Ghosh and Playford, 2003 and Stern *et al.*, 1984)**.

Previous studies have suggested that inhibition of cyclooxygenases can result in a shift of the arachidonic acid (AA) metabolism to produce leukotrienes (LTs) via the lipoxygenase pathway **(Brune, 2004).** As a consequence of shutting down the cyclooxygenase pathway, the accumulation of AA and the products from lipoxygenase can induce up-regulation of pro-inflammatory cytokines at transcriptional and post-transcriptional levels through the NF-kB pathway **(Bonizzi *et al.*, 1999).** The changes in gene expression related to lipoxygenase family members (ALOXE3, ALOX12B and ALOX15B) which reflect compensatory reactions from the interruption of the cyclooxygenase pathway by inhibition of COX-2, which affecting other inflammatory mediators**.**

In carrageenan-evoked inflammatory pain, the pro-inflammatory cytokines-including TNF-α , IL-1b , and IL-6-play an early and crucial role in the subsequent inflammatory responses **(Chou *et al.*, 2003)**. In this study, we demonstrated that WPs has a preventive and therapeutic analgesic effect in inflammatory pain. It was found that α-la inhibits the formation of IL-6, which may contribute to its analgesic and anti-inflammatory effects **(Yamaguchi *et al*.,2009)**. This finding supported our results that α-la, β-lg and WP had analgesic and anti-inflammatory effect without the side effects produced by COX-2 selective NSAID.

Carrageenan induced paw edema is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-hydroxytryptamine in the early stage followed by kinin release and then PG in the later phase **(Arunachalam *et al.* , 2002)**. It has been reported that the second phase (3 h) of edema is sensitive to most clinically effective anti-inflammatory agents. Anti-inflammatory effects of whey proteins (WPs) in 3 h of edema suggest involvement of inhibition of PG in the action of WPs.Aspirin and paracetamol are widely used as oral analgesic that act as an inhibitor of COX. Various proinflammatory cytokines injected into the central nervous system produce pain behavior. It has been reported that aspirin significantly and dose-dependently attenuates the pain behavior induced by TNF-α , IL-6, or IFN-γ administered intrathecally **(Kwon *et al.*, 2005)**.

Yamaguchi and Uchida (2007) found that α-la has a marked suppressive effect on pro-inflammatory cytokine release in various animal models, and it inhibited IL-6 production in carrageenan-injected paw. Our results suggest that WP, α-la, and β-lg may attenuate pain behavior induced by pro-inflammatory cytokines. In addition to the anti-nociceptive and anti-inflammatory effects presented here, it is known that whey proteins had many peripheral functions, including immuno-modulation and gut maturation **(Burd *et al.*, 2009)**. Casein clots in the stomach, whereas whey proteins are a soluble protein, which accelerates its gastric emptying. These unique characteristic of whey proteins are useful in maintaining physiological activities in the intestinal tract **(Lonnerdal and Lien, 2003)**. However, some of the biological activity of milk protein components is latent, and is released only upon proteolytic action **(Pillanto-Leppala, 2001).** Moreover, the physiological effects of bioactive peptides depend on their ability to reach their target sites intact, which may involve absorption through the intestinal epithelium prior to travel to the peripheral organs (**El-Zahar *et al.*, 2005)**. The cleavage of latent bioactive peptides from milk proteins normally occurs during digestion by pepsin and pancreatic enzymes (trypsin, chymotrypsin, carboxy and aminopeptidases), thus suggesting that WP and its fractions (α-la and β-lg)- derived peptides may possess remarkable anti-nociceptive and anti-inflammatory activities.

Our findings suggest that WP, α-la and β-lg could exert their antinociceptive effect may due to their antioxidant activity through increased the level of SOD after 4 hr of administration in dose dependent manner. It was found that expression of SOD2 (encoding superoxide dismutase 2) was significantly up-regulated by the treatment of rofecoxib and ibuprofen following tissue injury in this clinical model (**Wang *et al.*, 2007)**. SOD2 is the most prominent and widely distributed form of the SOD family and plays a critical role in modulating the production of

inflammatory mediators via its antioxidant defensive properties (**White *et al.*, 1991**). High level of SOD2 inhibits the over-expression of PLA2 and downstream PGE2 production via the nuclear factor –kappa B(NF-$_k$B)-dependent pathway **(Fakhrzadeh *et al.*,2004),** and thereby abrogates the development of inflammation. The up-regulation of SOD2 following inhibition of COX-2 by the rofecoxib or ibuprofen treatment during acute inflammation in this study may also contribute to the anti-inflammatory and analgesic effects via affecting the activation of PLA2 in the AA pathway (**Wang *et al.*, 2007)**.

It was reported that the LD50 of WPs were no less than 2000 mg/kg body weight **(Hayasawa *et al.*, 2004)** indicating that the toxicity of α-la and β-lg were extremely very low. Thus, WP, α-la and β-lg were found to be safe in the anti-nociceptive and anti-inflammatory dose range.

In conclusion, we have reported a novel function of WP, α-la and β-lg as anti-nociceptive, anti-inflammatory and antioxidant activities. These results suggest that WP, α-la and β-lg can be a safe and useful natural drug for patients with severe pain or that requires treatment from chronic inflammatory diseases.

Table (1) : Analgesic activity of Whey proteins, α- lactalbumin and β-lactoglobulin as compared to control and paracetamol (50 mg/ Kg)- treated groups on hot plate- induced pain in rats. (Means ± SE, n = 6 rats / group).

Group	Control	100 mg/kg			200 mg/kg			300 mg/kg			50 mg /kg Paracetamol
		WP	α-la	β-lg	WP	α-la	β-lg	WP	α-la	β-lg	
1/2 hr Potency	A a 5.73±0.32	A a 6.42±0.28 0.73	B a 9.20±1.06 1.05	C ac 12.65±0.50 1.44	A a 6.35±0.31 0.72	BD a 10.53±0.37 1.20	C a 12.87±0.45 1.46	A a 6.33±0.39 0.72	CD a 12.32±0.50 1.40	E a 14.77±0.65 1.68	CD a 11.38±0.42 1.00
1hr Potency	A a 5.35±0.30	A a 6.17±0.41 0.62	B a 9.73±0.31 0.98	C a 12.22±0.68 1.23	A a 6.73±0.23 0.68	B a 9.97±0.34 1.00	C a 12.50±0.58 1.26	B b 8.73±1.37 0.88	D b 15.55±0.80 1.56	E b 18.30±1.27 1.84	B a 10.05±0.49 1.00
1.5 hr Potency	A a 5.53±0.24	AE a 6.40±0.23 0.48	B b 12.78±0.86 0.96	C b 9.67±0.53 0.73	AE a 6.88±0.13 0.52	B be 12.73±0.65 0.96	C b 10.08±0.25 0.76	D c 19.55±1.12 1.47	D c 19.12±1.01 1.43	D bc 19.18±0.66 1.44	E b 7.50±0.36 1.00
2 hr Potency	A a 5.20±0.18	AG a 6.87±0.23 0.51	B ac 10.57±0.20 0.79	C b 10.18±1.41 0.76	D b 12.53±0.61 0.93	D bde 14.40±0.84 1.07	E c 16.75±0.43 1.25	F cd 20.22±0.66 1.50	F d 21.52±0.70 1.60	F ce 20.97±0.97 1.56	G b 7.43±0.24 1.00
2.5 hr Potency	A a 5.15±0.28	B a 7.38±0.31 0.56	C b 13.15±0.66 0.99	CD cd 14.20±0.52 1.07	D c 14.68±0.57 1.11	E c 17.03±0.89 1.28	E cd 18.18±0.63 1.37	F d 21.47±0.58 1.62	FG de 22.60±1.26 1.70	G d 24.15±0.69 1.82	B b 7.53±0.17 1.00
3hr Potency	A a 5.48±0.20	B a 7.60±0.18 1.04	C b 13.50±0.46 1.85	C d 14.98±0.55 2.05	C c 15.03±0.52 2.05	D c 17.22±0.80 2.35	E d 19.58±0.43 2.68	F d 22.00±0.61 3.01	FG e 23.52±1.07 3.21	G d 25.22±0.55 3.45	B b 7.32±0.10 1.00
3.5 hr Potency	A a 5.43±0.16	B a 7.50±0.17 1.03	C bc 11.87±0.55 1.63	C ac 13.03±0.46 1.79	D c 15.70±0.50 2.15	D cd 16.07±0.87 2.20	D c 17.08±0.61 2.34	E d 21.47±0.58 2.94	EF de 22.60±1.26 3.10	F d 24.15±0.69 3.31	B b 7.30±0.16 1.00
4 hr Potency	A a 4.95±0.25	B a 6.85±0.26 0.97	C bc 11.80±0.66 1.68	CDE ac 12.93±0.30 1.84	CD b 12.63±0.47 1.80	DE e 13.62±0.51 1.94	E a 14.57±0.50 2.07	F e 16.67±0.42 2.37	F b 17.08±1.87 2.43	G e 21.13±0.69 3.00	B b 7.03±0.12 1.00

Two –way ANOVA , at $P < 0.05$. , LSD = 1.87

The capital letters are significantly different between groups, while the small letters are significantly different in time.

Table (2) : Anti-inflammatory effect of Whey proteins, α- lactalbumin and β-lactoglobulin. on rat paw oedema as compared to control and indomethacine - treated groups (Means ± SE, n = 6 rats / group).

Group	Control	100 mg /kg			200 mg /kg			300 mg /kg			5 mg /kg Indomethacine
		WP	α-la	β-lg	WP	α-la	β-lg	WP	α-la	β-lg	
1/2 hr Potency	A a 0.392± 0.013	B a 0.337± 0.011 0.57	B ac 0.328± 0.009 0.58	C a 0.260± 0.012 0.74	B ad 0.330± 0.015 0.58	B acd 0.340± 0.012 0.56	C ac 0.240± 0.012 0.80	D a 0.292± 0.009 0.66	D ad 0.300± 0.009 0.64	E a 0.172± 0.005 1.12	E a 0.192±0.006 1.00
1hr Potency	A ab 0.378± 0.010 0.00	B a 0.332± 0.009 0.52	C a 0.305± 0.006 0.57	D ab 0.242± 0.011 0.72	BC ab 0.318± 0.010 0.54	BC bc 0.302± 0.015 0.57	D a 0.223± 0.009 0.78	D b 0.262± 0.014 0.66	D bd 0.257± 0.006 0.68	E ab 0.148± 0.009 1.17	E a 0.173±0.008 1.00
1.5 hr Potency	A b 0.367± 0.005	B a 0.338± 0.009 0.53	C a 0.307± 0.008 0.59	D bc 0.228± 0.010 0.79	C b 0.305± 0.012 0.59	C b 0.293± 0.008 0.61	E b 0.198± 0.009 0.91	D bc 0.248± 0.006 0.73	D bc 0.251± 0.005 0.72	F b 0.144± 0.004 1.25	G a 0.180±0.004 1.00
2 hr Potency	A ab 0.370± 0.004	B b 0.275± 0.008 0.62	C b 0.245± 0.008 0.70	C bc 0.222± 0.009 0.77	B c 0.278± 0.009 0.62	B b 0.278± 0.005 0.62	D b 0.190± 0.007 0.90	C bc 0.238± 0.010 0.72	C b 0.237± 0.005 0.73	E b 0.142± 0.009 1.21	D a 0.172±0.006 1.00
2.5 hr Potency	A ab 0.370± 0.009	B a 0.340± 0.007 0.54	B c 0.335± 0.007 0.55	B cd 0.325± 0.009 0.57	B ad 0.333± 0.005 0.56	C b 0.288± 0.007 0.64	D b 0.185± 0.004 1.00	E c 0.232± 0.009 0.80	E bc 0.242± 0.009 0.77	D c 0.205± 0.008 0.90	D a 0.185±0.006 1.00
3 hr Potency	A ab 0.373± 0.013	A c 0.367± 0.011 0.75	AB cd 0.350± 0.015 0.79	BC de 0.343± 0.010 0.81	BC de 0.345± 0.008 0.80	C cd 0.323± 0.008 0.86	D b 0.207± 0.005 1.34	E a 0.287± 0.005 0.97	E cd 0.265± 0.006 1.04	D c 0.228± 0.010 1.21	E b 0.277±0.011 1.00
3.5 hr Potency	A ab 0.372± 0.009	A c 0.372± 0.009 0.80	A d 0.363± 0.011 0.82	AB e 0.355± 0.013 0.84	ABD de 0.352± 0.007 0.84	BD cd 0.335± 0.008 0.89	CE c 0.258± 0.006 1.15	D d 0.327± 0.008 0.91	CF de 0.280± 0.006 1.06	E d 0.255± 0.008 1.16	F bc 0.297±0.008 1.00
4 hr Potency	A b 0.365± 0.008	A c 0.368± 0.009 0.84	AC d 0.360± 0.013 0.86	A e 0.362± 0.011 0.86	AC e 0.358± 0.005 0.87	AC d 0.343± 0.008 0.90	BD d 0.287± 0.008 1.08	C d 0.337± 0.007 0.92	BD ae 0.295± 0.004 1.05	B d 0.272± 0.005 1.14	D c 0.310±0.005 1.00

Two –way ANOVA, at P< 0.05. LSD = 0.025.

The capital letters are significantly different between groups, while the small letters are significantly different in time.

Table (3) : Effect of Whey proteins, α- lactalbumin and β-lactoglobulin treatments on serum SOD level (IU/L) in rat- induced paw oedema comparing to the control and indomethacine (5 mg/Kg) - treated groups after 4hr. of treatment. (Means ± SE, n = 6 rats / group).

Groups	Control	100 mg/kg			200 mg/kg			300 mg /kg			
		WP	α-la	β-lg	WP	α-la	β-lg	WP	α-la	β-lg	Indomethacine
SOD (IU/L)	A 31.38±0.64	B 34.22 ± 0.86	CE 38.42±0.71	CH 36.82±0.52	CH 37.03±0.86	DF 44.10±1.03	E 40.45±0.44	EF 41.83±0.68	G 54.55±1.29	D 45.80±1.31	BH 34.78±0.70

One-way ANOVA , at P< 0.05 , LSD = 2.450
The capital letters are significantly different between groups.

References

1. **Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, and Gregory SA (1996).** Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis., J. Clin. Invest., 97: 2672 - 2679
2. **Arunachalam G, Chattopadhyay D, Chatterjee S, Mandal AB, Sur TK, and Mandal SC (2002).** Evaluation of anti-inflammatory activity of Alstonia macrophylla Wall. ex A.DC. leaf extract, Phytomedicine, 9, 632- 635.
3. **Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, and Schnitzer TJ (2000)**. Comparison of Upper Gastrointestinal Toxicity of Rofecoxib and Naproxen in Patients with Rheumatoid Arthritis. N. Engl. J. Med., 343:1520 - 1528.
4. **Bonizzi G, Piette J, Schoonbroodt S, Greimers R, Havard L, Merville MP, and Bours V(1999).** Reactive oxygen intermediate-dependent NF-kappaB activation by interleukin-1beta requires 5-lipoxygenase or NADPH oxidase activity. Mol. Cell Biol., 19:1950 - 60.
5. **Bounous G, Batist G, and Gold P (1991).** Whey proteins in cancer prevention Cancer Lett., 57: 91- 94.
6. **Bounous G (2000).** Whey protein concentrate (WPC) and glutathione modulation in cancer treatment, Anticancer Res. 20 : 4785 – 4792.
7. **Brown EC, DiSilvestro RA, Babaknia A , and Devor ST (2004).** Soy versus whey protein bars: Effects on exercise training impact on lean body mass and antioxidant status . Nutr J., 3: 22- 26 .
8. **Brune K (2004).** Safety of anti-inflammatory treatment--new ways of thinking. Rheumatology Oxford; 43 (Suppl) :i 16–20.
9. **Burd AN, Tang JE, Moore DR, and Phillips SM (2009)**.Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences J Appl Physiol 106: 1692-1701.
10. **Chou TC, Chang LP, Li CY, Wong CS, and Yang SP (2003)**.The Antiinflammatory and Analgesic Effects of Baicalin in Carrageenan-Evoked Thermal Hyperalgesia, Anesth. Analg., 97: 1724 – 1729.
11. **Clària J., Romano M. (2005).** Pharmacological intervention of cyclooxygenase-2 and 5-1ipoxygenase pathways. Impact on inflammation. Curr. Pharm. Des., 11: 3431- 3447.
12. **El-Zahar Kh., Sitohy M , Choiset Y, Metro FQ, Haertle T, and Chobert JM (2005).** Peptic hydrolysis of ovine b-lactoglobulin and a-lactalbumin Exceptional susceptibility of native ovine b-lactoglobulin to pepsinolysis. International Dairy Journal 15 : 17-27.
13. **Fakhrzadeh L, Laskin JD, Gardner CR, and Laskin DL(2004).** Superoxide dismutase-overexpressing mice are resistant to ozone-induced tissue injury and increases in nitric oxide and tumor necrosis factor-alpha. Am. J. Respir. Cell Mol Biol. 30:280 -7.
14. **Futagami A, Ishizaki M, Fukuda Y, Kawana S, and Yamanaka N (2002).** Wound healing involves induction of cyclooxygenase-2 expression in rat skin. Lab. Invest. 82:1503–13.
15. **Ghosh S and Playford RJ (2003).** Bioactive natural compounds for the treatment of gastrointestinal disorders. Clinical Science,104: 547–556
16. **Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, and Willoughby DA (1999).** Inducible cyclooxygenase may have anti-inflammatory properties. Nat. Med., 5:698–701.
17. **Hambrus L (1998).** Dietary assessments: how to validate primary data before conclusions can be drawn Leif Hambaeus. Scandinavian Journal of NutritinlNaringrforskning, 142:66-68,1998.
18. **Hayasawa H, Toida T, Shimokawa Y, and Matsumoto H (2004).** Antiulcer agent. U.S. Patent, 6815419.
19. **Hayashida K, Kaneko T, Takeuchi T,**

Shimizu H, Ando K., and Harada E(2004). Oral administration of lactoferrin inhibits inflammation and nociception in rat adjuvant-induced arthritis. J. Vet. Med. Sci. 66: 149–154.

20. **Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, and Cook JD (1989).** Iron absorption in humans as influenced by bovine milk proteins Am. J. Clin. Nutr., 49: 546 - 552
21. **Kwon MS, Shim EJ, Seo YJ, Choi SS, Lee JY, Lee HK, and Suh HW(2005).** Effect of aspirin and acetaminophen on proinflammatory cytokine-induced. Pharmacology, 74:152-156
22. **Lönnerdal B and Lien EL (2003).** Nutritional and physiologic significance of alpha lactalbumin in infants. Nutr Rev., 61: 295–305.
23. **Low PP, Rutherfurd KJ, Gill HS, and Cross ML (2003).** Effect of dietary whey protein concentrate on primary and secondary antibody responses in immunized BALB/c mice. Int. Immunopharmacol., 3: 393- 401
24. **Yamaguchi M, Yoshida K, and Uchida M (2009).** Novel Functions of Bovine Milk-Derived a-Lactalbumin: Anti-nociceptive and Anti-inflammatory Activity Caused by Inhibiting Cyclooxygenase-2 and Phospholipase A2. Biol. Pharm. Bull. 32: 3, 366- 371.
25. **Marshall K (2004).** Therapeutic applications of whey protein. Altern. Med. Rev., 9: 136 - 156
26. **Neurath AR, Li YY, Strick N, and Jiang S (1996)**. A herpesvirus inhibitor from bovine whey.Lancet, 347:1703 - 1704
27. **Paakkari I, Jarvinen A, Anttila MJ, and Pihlanto- Leppala A(1994).** Opioid effects of the milk wehy-protein derived peptides α- and β- lactorphin in β-casomorphins and related peptides. Recent development (Brantl, V., Teschemacher, H.eds.), pp 33 -37, VCH, Weinheim.
28. **Pihlanto-Leppala A, Paakkari I, Rinta-Koski M, and Antila P(1997).** Bioactive peptide derived from in vitro proteolysis of bovine β-lactoglobulin and its effect on smooth muscle. J. Diary Res., 64 : 149 – 155.
29. **Pillanto-Leppala A (2001):** Review: Bioactive peptides derived from whey proteins: Opioid and ace inhibitory peptides. Trends in Food Science and Technology. 11: 347 -356
30. **Roszkowski A, Rooks WH, Tonolonis AJ, and Miller LM (1971).** Anti-inflammatoryand analgesic properties of Naproxien. J.Pharmacol. Exp.Thera., 179, 1.
31. **Saito T (2008)**, Antihypertensive peptides derived from bovine casein and whey proteins. Adv. Exp. Med. Biol., 606: 295 - 317.
32. **Schmelzer KR, Inceoglu B, Kubala L, Kim IH, Jinks SL, Eiserich JP, and Hammock BD, (2006).** Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. Proc. Natl. Acad. Sci. U.S.A., 103:13646 - 13651
33. **Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W,Lee L, and Isakson P (1994).** Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc. Natl. Acad. Sci. U.S.A., 91: 12013 – 12017.
34. **Shah NP(2000)**. Effects of milk-derived bioactives: an overview Br. J. Nutr., 84: (Suppl. 1), S3 -S10 .
35. **Sharma J N, Samud AM and Asmawi MZ (2004).** Comparison between plethysmometer and micrometer methods to measure acute paw oedema for screening anti-inflammatory activity in mice. Inflammopharmacology, 12: 1, 89 – 94 .
36. Smith HS (2006). Arachidonic acid pathways in nociception. J. Support. Oncol., 4: 277 - 287
37. **Stern M, Pang KY, and Walker WA (1984**). Food proteins and gut mucosal barrier. II. Differential Interaction of Cow's Milk Proteins with the Mucous Coat and the Surface Membrane of Adult and Immature Rat Jejunum. Pediatric Research. 18:1252-1257.
38. **Stichtenoth DO (2004).** Mini Rev. The second generation of COX-2 inhibitors: clinical pharmacological point of view. Med. Chem., 4: 617- 624
39. **Suttle NF (1986).** Copper deficiency in ruminants, recent developments. The Veterinary Record , 119: 519 -522.
40. **Tantisira KG, John L, Jody S, Klanderman B , and Weiss ST (2009).** 5-Lipoxygenase pharmacogenetics in asthma: overlap with Cys-leukotriene receptor antagonist loci. Pharmacogenetics and Genomics, 19 : 244 - 247
41. **Ushida Y, Shimokawa Y, Toida T, Matsui H, and Takase M.(2007)**. Bovine α-Lactalbumin Stimulates Mucus Metabolism in Gastric Mucosa, J. Dairy Sci., 90: 541 - 546
42. **Wang XM, Wu TX, Hamza M, Ramsay ES, Wahl SM and Dionne RA (2007).** Rofecoxib modulates multiple gene expression pathways in a clinical model of acute inflammatory pain. Pain. 128: 136–147.
43. **White CW, Avraham KB, Shanley PF, and Groner Y (1991).** Transgenic mice with expression of elevated levels of copper-zinc superoxide dismutase in the lungs are resistant to pulmonary oxygen toxicity. J Clin Invest., 87:2162–68.
44. **Winter GA, Rislfy EA and Nuss GW (1962)**. Cargeenin induced oedema in hind paw of rats as assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med., 111:121 .
45. **Wong D, Wang M, Cheng Y, and FitzGerald GA (2005).** Cardiovascular hazard and non-steroidal anti-inflammatory drugs. Curr Opin Pharmacol., 5: 204 –10.
46. **Yamaguchi M, Matsuura M, Kobayashi K, Sasaki H, Yajima T, Kuwata T (2001).** Lactoferrin Protects against Development of Hepatitis Caused by Sensitization of Kupffer Cells by Lipopolysaccharide. Clin. Diagn. Lab. Immunol., 8: 1234 - 1239

47. **Yamaguchi M and Uchida M (2007).** α-Lactalbumin suppresses interleukin-6 release after intestinal ischemia/reperfusion via nitric oxide in rats Inflammopharmacology, 15: 43 - 47

48. **Yamakawa K, Matsunaga M, Isowa T, Kimura K, Kasugai K, Yoneda M, Kaneko H, and Ohira H (2009)**. Transient responses of inflammatory cytokines in acute stress. Biol Psychol. May 14. [PMID: 19446599 [PubMed – as supplied by publisher]

49. **Zimmermann M (1983)**. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16:109 –110.

مضادات جديدة للآلم و مسكنات للإلتهاب و مضادات للأكسدة جديدة وذات فاعلية قوية باستخدام بروتين شرش اللبن و الالفالاكت ألبومين والبيتالاكتجلوبيولين فى الجرذان البيضاء

سمية أحمد ندا

قسم الفارماكولوجى – المركز القومى للبحوث- الدقى – الجيزة – مصر

لقد تم حقن ثلاثة مجموعات من فئران التجارب البيضاء بثلاث جرعات لكل من بروتنات شرش اللبن و شرش اللبن الكلى و كانت هذة الجرعات : 100 و 200 و 300 مجم / كجم من وزن الجسم عن طريق الفم ، كما تم احداث ألم بواسطة السطح الساخن (55 درجة مؤية) بعد الحقن على فترات بداية من 30 دقيقة وحتى 4 ساعات بعد الحقن،. كان هناك مجموعة ضابطة أخذت محلول ليس به دواء و أخرى أخذت 50مجم / كجم عن طريق الفم من عقار الباراسيتامول .

كانت نتائج التركيز العالى فى كل من البيتالاكتوجلوبيولين أفضل ثم يليه الفالاكتالبيومين ثم بروتينات الشرش الكلية بالمقارنه بالمجموعة التى أخذت الباراسيتامول ، كما لوحظ ان التأثير المضاد للألم يزداد بزيادة الجرعة و الوقت .

كما تم تقييم التأثيربروتينات الشرش الكلية, الفالاكتالبيومين و بيتالاكتو جلوبيولين كمضاد للآلتهاب تجريبيا على فئران التجارب .

لقد تم حقن ثلاثة مجموعات من فئران التجارب البيضاء بثلاث جرعات لكل من بروتينات شرش اللبن و شرش اللبن الكلى و كانت هذة الجرعات : 100 و 200 و 300 مجم / كجم من وزن الجسم عن طريق الفم ، المجموعة ضابطة أخذت عقار الاندوميثاسين ، تم احداث التهاب فى اليد اليمنى للفئران بواسطة مادة الكراجينان. وبعد مرور نصف ساعة من احداث الالتهاب تم قياسه حتى 4 ساعات من الجرعات المذكورة. كانت أفضل النتائج للبيتالاكتو جلوبيولين فى الجرعة العالية وكانت أفضل من الاندوميثاسين خلال وقت القياس ، كما كان هناك تأثير مماثل لكل من بروتينات الشرش الكلية و الفالاكتالبيومين فى الجرعتين 200 و 300 مجم / كجم و فى كل الحالات كان لهم تأثير مضاد للألم مماثل فى الجرعة الصغيرة (100 مجم /كجم).

أحدث العلاج ببروتينات الشرش الكلية , الفا لاكتالبيومين وبيتالاكتوجلوبيولين إلى زيادة ملحوظة فى مستوى SOD مقارنة بجرذان المقارنة وان هذا التأثير يزداد طرديا فى الجرعة العالية. كانت نسبة SOD فى الفئران التى اخذت علاج بالاندوميثاسين أقل من النسب المتحصل عليها من بروتينات شرش اللبن بعد 4 ساعات من الحقن.

نستنتنج من هذة الدراسة : ان هذة البروتينات لهم تأثير مضاد للآلم ومخفض للالتهابات وكان هذا التأثير أقوى من الادوية المقارنة (الباراسيتامول و الاندوميثاسين) وكانت النتائج على النحو التالى:

بيتالاكتوجلوبيولين ثم يليه بروتينات الشرش الكلية ثم الفالاكتالبيومين.