

**STUDY ON THE EFFECT OF PROCESSED MILK PROTEIN
ON ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN
RATS**

BY

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DECLARATION BY THE CANDIDATE

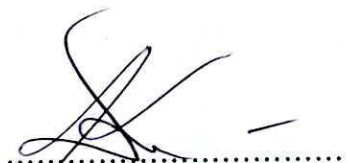
The work described in this thesis was carried out by me under the supervision of Prof. Hemantha Peiris (Head of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Dr. S.D. Jayaratne (Head of the Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any University or any other Institution for another Degree/Diploma.

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ABBREVIATION

ACE	Angiotensin Converting Enzyme
ANG	Angiotensin
APC	Aerobic Plate Count
AT	Angiotensin receptor
BGB	Brilliant green blue
BW	Body weight
°C	Degrees centigrade
CN	Casein
Con	Concentrated
CPPs	Caseinophosphopeptides
DBP	Diastolic Blood Pressure
<i>E.Coli</i>	<i>Escherichia Coli</i>
FAO	Food and Agriculture Organization
FAPGG	Furylacryloyl-phenylalanyl-glycyl-glycine
GALT	Gut Associated Lymphoid Tissue
GFR	Glomerular Filtration Rate
GIT	Gastrointestinal tract
HDL	High Density Lipoproteins
HHL	Hippuryl -L-Histidyl-Leucine
HT	Hypertension
IL	Interleukines
INF	Interferon

ABBREVIATION

Ig	Immunoglobulin
<i>L</i>	<i>Lactobacillus</i>
LA	Laboratory Accident
LAB	Lactic Acid Bacteria
LDL	Low Density Lipoprotein
MA	Milk Agar
MI	Myocardial Infarction
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRI	Medical Research Institute
mw	Molecular Weight
PAGE	Poly Acrylamide Gel Electrophoresis
PDA	Potato Dextrose Agar
RAS	Renin Angiotensin System
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SHR	Spontaneously Hypertensive Rats
Subsp	Sub species
TC	Total Cholesterol
TG	Triacylglycerol/Triglycerides
TNF	Tumour Necrosis Factor
WHO	World Health Organization

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ABSTRACT

Milk peptides are known to have antihypertensive effects by inhibiting the Angiotensin Converting Enzyme (ACE) and thereby inhibiting the formation of angiotensin II (ANG II). Due to lack of available literature pertaining to SAARC countries including Sri Lanka on antihypertensive effects of milk proteins, this study was carried out to investigate the effects of milk proteins and curd on ACE activity and their effects on lipid profile using *Wistar* rats.

Milk and curd of a commercially available local brand was used in all the experiments. Protein fractions of milk and curd were isolated and separated using SDS PAGE electrophoresis. Results revealed that the ACE inhibiting activity due to in-vitro digested products, curd, milk, whole casein, whole whey, α -casein, β -casein and κ -casein were 78.98%, 48.09%, 83.6%, 53.5%, 43.2%, 82.1% and 80.1% respectively. Prior to enzymatic digestion, ACE inhibiting activity of milk and curd were 3.99% and 49.41% respectively. The enzymatic digest of total casein which had the highest ACE inhibition (83.6%) in the *in-vitro* assay was further subjected to an animal study. Three different animal experiments were carried out to determine the effects of casein, whey and curd on serum ACE activity and long term intake on the lipid profile of *Wistar* rats. In experiment I, the test groups were fed with World Health Organization (WHO) standard feed incorporated with casein or curd, while the control group received the standard feed. In experiment II, test groups were

orally fed with either 2 ml of hydrolyzed casein or curd whilst experiment III test group received 2 ml of whey while control groups received 2 ml of water in addition to the standard feed. The mean differences obtained for ACE activity for individual animals were analyzed after eight weeks. In Experiment I, casein fed group had a higher serum ACE reduction when compared with the control group. The mean difference of ACE (U/L) in both casein (-37.2 ± 33.5) and curd (-12.3 ± 16.2) treated groups were significantly lower ($p < 0.05$) when compared to the control group (54.7 ± 66.2). In experiment I, curd treated group also had a lesser increase in serum cholesterol (1.65 ± 12.8 g/dL) when compared to the control group (16.5 ± 4.8 g/dL) whilst the whey treated group in experiment III had a mean reduction of -2.8 ± 4.0 g/dL for serum total cholesterol. The mean difference in both curd and whey fed groups showed a significantly lower value for serum cholesterol ($p < 0.05$). However, high density lipoprotein cholesterol, triglycerides, feed intake and body weight did not show a significant difference between the animals in the treatment group and the control groups. These results suggest that both casein and curd have an inhibitory effect on serum ACE activity. In addition to the ACE inhibitory effect, curd also has a serum cholesterol lowering effect on *Wistar* rats.