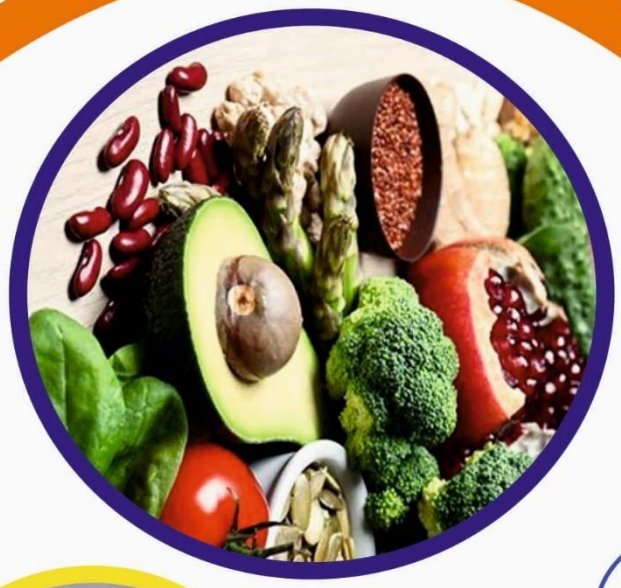
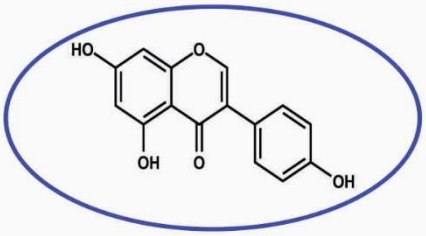
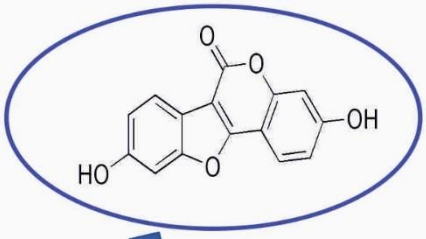
PHYTOESTROGENS: AN INVESTIGATION ON THEIR SYNERGISTIC EFFECTS







Dr. Nitu Debnath

Kripa Drishti Publications, Pune.

PHYTOESTROGENS:

AN INVESTIGATION ON

THEIR SYNERGISTIC EFFECTS

Dr. Nitu Debnath
Assistant Professor,
Department of Zoology, Cachar College,
Silchar, Assam.

Kripa-Drishti Publications, Pune.

Book Title: **Phytoestrogens: An Investigation on Their Synergistic Effects**

Author By: **Dr. Nitu Debnath**

1st Edition

ISBN: **978-93-94570-80-1**

Published: **Jan 2022**

Publisher:

Kripa-Drishti Publications

A/ 503, Poorva Height, SNO 148/1A/1/1A,
Sus Road, Pashan- 411021, Pune, Maharashtra, India.
Mob: +91-8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

PREFACE

With growing concern about environmental health worldwide, the safety of foods or diet has also received great attention over the years. Consequently, the analysis of chemical composition of various types of vegetables, fruits and other natural plant derived foods combined with determination of the possible role of various such natural compounds on human and animal health became a major scientific research focus over the last two decades. Phytoestrogens are one class of those natural compounds found in many plant derived foods which exhibit unique biological activities by mimicking the actions of estrogen, a natural hormone available in humans and other mammals.

The Estrogens play significant role in reproductive physiology of organisms possessing them. Therefore, the interference of normal endocrine functions by external agencies or environmental factors has led to the development of the new area of research known as “Environmental Endocrine Disruption”.

Tremendous progress of scientific research in this field around the world have produced sufficient evidences in support of both the beneficial and harmful health effects of phytoestrogens in humans and other animals. These findings, including effects on both reproductive as well as on general health, together with the realisation of presence of multiple such compounds in our everyday diet have led to the research on yet another important concern of their possible synergistic effects.

The piece of investigation presented in this book is one of such endeavour to assess the synergistic effects of two most potent phytoestrogens viz., Genistein and Coumestrol. Both in-vivo and in-vitro effects of these compounds have been studied using Ovariectomized Albino Mice and Recombinant Yeast Cells containing gene for Estrogen Receptor respectively. Interestingly, the findings revealed synergistic effects of the compounds for certain parameters while antagonistic effects for others either in the presence or absence of estrogen.

Dr. Nitu Debnath

DEDICATION

Dedicated to

My Parents

Late Nishikanta Debnath

&

Smt. Shefalika Debnath

ABSTRACT

Phytoestrogens are diverse group of naturally occurring phenolic, non-steroidal compounds that are natural components of certain plant foods. Although, they possess structural similarity to mammalian endogenous estrogen (17β-estradiol) and have affinity for binding estrogen receptors, they are estrogenically much less potent than natural estrogens. Dietarily potent phytoestrogens like Genistein and Coumestrol have captured much attention in recent years due to their genomic as well as non-genomic mechanism of actions and their differential interaction with estrogen receptors (ERα & ERβ) and transactivation which mediate comparable estrogenic activity. In the present study, the combinatorial effects of these two phytoestrogens in the presence or absence of endogenous/exogenous estrogen E_2 have been taken under consideration, through *in vitro* and *in vivo* assays.

The principal focus of the present investigation was to evaluate the combinatory effects using *in vivo* utero-vaginotrophic bioassays in female C3H/He albino mice following 3 d subcutaneous administration of pure Genistein and Coumestrol compounds and taking 17β-estradiol as positive control. Additionally, however, combinatorial estrogenic activity of combination was also tested in one of the highly estrogen-sensitive *in vitro* bioassays using recombinant yeast cells to gain insight into the combinatory/synergistic estrogenic behaviour of the tested compounds in a more specific and controlled system. The results showed a dose-dependent induction (nM) and synergistic inhibition (mM) of β-galactosidase activity.

Combinatorial effects on changes in uterine histology and other estrogen-sensitive endpoints like increase in wet weight, fluid imbibition, luminal epithelial height, stromal gland number and uterine total protein were measured. To gain better understanding of the molecular mechanism of action, combinatorial effects on expression of uterine estrogen-regulated genes like estrogen receptors (ERα and ERβ) and progesterone receptor (PR) were investigated using Real Time PCR. Some of these combinatorial effects were also compared with that in immature and ovary-intact mice. Antiestrogen fulvestrant (ICI, 182,780) pretreatment was done to assess whether the uterotrophic activities are mediated through ERs.

The vaginal estrogen sensitive parameters included increase in vaginal epithelial height, vaginal epithelial proliferation and vaginal epithelial cornification, in addition to changes in histology. Effect on estrous cyclicity was studied following vaginal smear method described by Stockard and Papanicolaou (1917).

In agreement with other studies, the present investigation also produced evidences in favour of comparative estrogenicity of Gen and Coum in ovariectomized and ovary-intact and immature mice, which possibly, in part, be attributed to differences in status of estrogen and estrogen receptors. However, as expected, together, Gen and Coum modulated uterine estrogen-sensitive endpoints in an additive or synergistic manner in both ovariectomized and immature mice and significantly also

in ovary-intact adult mice. The synergistic or additive response on uterine weight in ovariectomized mice also reflected down-regulation of ERα and PR while up-regulation of ERβ. This study established that Gen and Coum-induced uterine growth are ER-dependent and also clearly identified the inverse association of ERα/ERβ ratio with uterine growth for all treatments. Pre-treatment with antiestrogen fulvestrant (Ful) reduced uterine wet weight induced by E_2, Gen, Coum and their combinations indicating ER-dependent activity.

Even though Gen and Coum themselves did not exhibit synergism with respect to all the vaginal estrogen-sensitive parameters, they, in presence of E_2, altered all the vaginal estrogenic endpoints in an additive or synergistic manner.

Keeping in view of the various established non-genomic/non-estrogenic mechanisms of action of these two phytoestrogens and numerous claims about their potential beneficial role in health and diseases, effects on some additional biochemical parameters were assessed. In the acute *in vivo* exposure regime, Gen and Coum exhibited partial synergism with respect to increase in serum total and HDL cholesterol, total protein, albumin and globulin with a shift of albumin/globulin ratio towards globulin. Compared to ovariectomized mice, the overall effects of combination were more significant in ovary-intact mice. Although synergistic reduction of serum glucose by Gen and Coum appear to be mediated in estrogen-like manner, their significant combinatorial reduction of liver glycogen content seemed to be mediated in an estrogen-independent manner, since, steroidal estrogens may increase tissue glycogen content, primarily through the stimulation of glucose transport into the muscle and liver cells.

ABBREVIATIONS

AF-1/2	Activation function-1/2
ANOVA	Analysis of variance
AR	Androgen receptor
AhR	Aryl hydrocarbon receptor
BERKO	Estrogen receptor beta knock-out
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CNS	Central nervous system
COT	Committee on toxicity of chemicals in food, consumer products and the environment
Coum	Coumestrol
CPRG	Chlorophenol red β-D-galactopyranoside
DBD	DNA- binding domain
DEPC	Diethyl pyrocarbonate
DERKO	Double estrogen receptor knock-out
DES	Diethylstilbestrol
dl	Deci litre
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
E_2	17β-estradiol
EACs	Endocrine active chemicals
EDTA	Ethylenediamine tetraacetic acid
EE	Ethinylestradiol
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
ERKO	Estrogen receptor alpha knock-out
ERE	Estrogen responsive element
FSA	Food Standard Agency
FSH	Follicle stimulating hormone
Ful	Fulvestrant (ICI 182, 780)
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GE	Glandular elithelium
Gen	Genistein
GnRH	Gonadotropins releasing hormone
H_2SO_4	Sulphuric acid
HCL	Hydrochloric acid
HDL	High density lipoprotein
hER	Human estrogen receptor

HRT	Hormone replacement therapy
hsp	Heat shock protein
ICI	Imperial cancer institute
IGF	Insulin like growth factor
IGFR	Insulin like growth factor receptor
i.m.	Intramuscular
KOH	Potassium hydroxide
LBD	Ligand binding domain
LE	Luminal epithelium
LH	Luteinizing hormone
LDL	Low density lipoprotein
LOAEL	Lowest observed adverse effect level
mRNA	Messenger ribonucleic acid
NaOH	Sodium hydroxide
NOEC	No observed effect concentration
OD	Optical density
OECD	Organization for Economic Co-operation and Development
OVX	Ovariectomized
P_4	Progesterone
PCB	Polychlorinated bisphenol
ppm	Parts per million
PR	Progesterone receptor
RBA	Relative binding affinity
RNA	Ribonucleic acid
rpm	Revolution per minute
s.c.	Subcutaneous
SERM	Selective estrogen receptor modulator
STEAR	Selective tissue estrogenic activity regulator
SEM	Standard error of mean
SHBG	Sex steroid hormone binding globulin
TCA	Trichloro acetic acid
TAF	Transcriptional activation function
TGF	Transforming growth factor
Tris	Tris(hydroxymethyl)aminomethane
TSH	Thyroid stimulating hormone
µg	Micro gram
UV	Ultra violet
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Acknowledgement

1. I offer my deepest sense of gratitude to my teacher and PhD research supervisor Prof. Jogen Chandra Kalita, Head, Department of Zoology, Gauhati University, Guwahati for his valuable suggestions, able guidance, and constant encouragement during the period of investigation and preparation of the thesis.
2. I am indebted to all my revered Teachers of Department of Zoology, Gauhati University for their encouragement, and extending all possible help and cooperation during my work in the Department.
3. I am grateful to Prof. Stuart Richard Milligan, Head, Reproductive Physiology and Rhythm Research Group, King's College, London for providing the recombinant yeast cell line as generous gift, without which carrying out of the *in-vitro* studies would not have been possible. In this connection, I am also thankful to Dr. Utpal Das, Research Scientist, All India Institute of Medical Science, New Delhi for receiving and helping me to collect the frozen cell lines.
4. I accord the contributions of all with sincere gratitude whose help, assistance and support at various phases of accomplishment of this piece of research work was critical. I convey my thanks and gratitude to Dr. Bhupen Sharma, Asssociate Professor, Department of Surgery and Radiology, Dr. Krishna Sharma and Dr. Rajib Sharma, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Prof. Hirendra Nath Sharma, former Head, Dr. Monuj Kumar Bharali, Assistant Prof., Mr. Pranjiv Goswami, Mrs. Mousumi Das and Ms. Anju Jaishi, Research Scholars, Department of Zoology, Rajiv Gandhi University, Arunachal Pradesh, Dr. Shekhar Chakraborty ,Vice-Principal, and Dr. Arabindo Das, Head, Department of Pathology, my friend Dr. Ratnadeep Nath of Silchar Medical College, Silchar, Prof. A.K. Handique and Prof. G.U. Ahmed, Dr. Jayanta Das, Senior Research Scholar of Department of Biotechnology, Gauhati University, Dr. Sankar Kumar Ghosh, Professor & Head(i/c), Department of Biotechnology, Assam University, Vice-Chancellor, Kalyani University, Dr. Bishal Dhar, Research Scientist, , Kalyani, West Bengal, , Kalyani University.
5. I sincerely acknowledge the financial support through both Major and Minor Research Projects from University Grants Commission, New Delhi and North-East Regional Office (NERO), Guwahati, without which it would not have ever been possible to initiate and complete different phases of experiments included under the present work.
6. I must take this opportunity to express my sincere thanks, love and regards to my family members, especially, Smt. Shefalika Debnath (Mother), Dr. Nabanita Debnath (Wife), Sri Nitin Debnath (Son) & Sri Niloy Debnath (Son) and other family members for their blessings, encouragement, whole-hearted & constant indirect support in my long journey of academic success.

7. Finally, I am extremely happy to profusely thank Mrs. Rajani Adam, the Editor and her entire Team of renowned Kripa Dristi Publication, Pune, Maharashtra for accepting my work and also making all necessary editings towards publishing the same in the form of this Book.

Dr. Nitu Debnath

INDEX

About the Book

This Book is a humble attempt on part of the author to present the findings of his PhD research in a form which enhance visibility of relevant scientific knowledge and information to larger community of students, teachers, researchers, health-workers & readers alike. Because, creating more and more awareness about various facets concerning consumption of plant-derived food and their plausible health effects and disseminating the scientific information to society at large through various means are essential to ensure healthy life in current context.

The detail background on Phytoestrogens, specific objectives of the present work, various established methods applied to meet the objectives, the important findings of the work, and finally the analysis of the findings in the context of recent relevant information are presented with best of effort in the Book as six separate Chapters.

Hope, this book will cater to the need of Students, Teachers, Researchers, Health workers, Dietician and health conscious people in general who will play pivotal role in achieving the ultimate goal of this scientific research.

About the Author

Dr. Nitu Debnath *is presently working as Assistant Professor in the Department of Zoology at Cachar College, Silchar, Assam. He completed his B.Sc. in Zoology with specialisation in Fish & Fishery from Karimganj College under Assam University, Silchar in 2001 and M.Sc. in Zoology with Specialization in Animal Physiology & Biochemistry from Gauhati University, Guwahati in 2004. He was awarded Late Prof. K.N. Sharma Memorial Gold Medal for securing 1st class 1st position in Post-Graduation. Dr. Debnath completed his Doctoral Research from the same University in 2014. He has 1 year of PG teaching and more than 15 years of UG teaching Experience in Zoology. He has also earned Post-Graduate Diploma in Bioinformatics (PGDBI) from Department of Life Science & Bioinformatics, Assam University, Silchar in 2016. His area of research interest includes but not limited to Environmental Estrogen, Phytoestrogens, Environmental Endocrine Disruption and Toxicology. He completed one Research Project under UGC and currently pursuing research work on "Phytoestrogen profiling of Edible Green leafy Vegetables & Legumes and their in-vivo effects" as Principal Investigator in Major Research Project funded under SERB-TARE Programme of DST, Govt. of India. He published many Research papers as well as Book chapters in National & International Journals and Books. He has also completed Post Graduate Diploma in Higher Education (PGDHE) from IGNOU in 2020. His other academic & administrative experiences and assignments include Coordinator/Nodal Officer, UGC sponsored Community College, Coordinator, DBT, Govt. of India sponsored Institutional Biotech Hub, and Coordinator, Internal Quality Assurance Cell (IQAC) of Cachar College, Silchar.*

KRIPA DRISHTI PUBLICATIONS

Price: ₹599

Kripa-Drishti Publications
A-503 Poorva Heights, Pashan-Sus Road, Near Sai Chowk,
Pune – 411021, Maharashtra, India.
Mob: +91 8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

ISBN: 978-93-94570-80-1

9 789394 570801