



Ain Shams University
Faculty of women for
Arts, Science and Education
Biochemistry and Nutrition Department

Using Some Fruits Rich in Polyphenolic Compounds for Managing Dry Eye in Experimental Animal Model

Thesis

*Submitted to Faculty of Women, Ain Shams University in
Partial Fulfillment for Master of Science Degree (M.Sc) in
Biochemistry and Nutrition*

By

Ethar Ahmed Mohamed Ebrahim El-hoseny

B.Sc. in Biochemistry and Nutrition
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University

Under Supervisors

Prof. Dr. Fatma Hassan Abd El-Razek

Professor of Nutrition
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University

Prof. Dr. Anhar Mohamed Gomaa

Professor of Biochemistry
Biochemistry Department
Research Institute of Ophthalmology

Dr. Bakinam Ali Mohamed

Lecture of Biochemistry and Nutrition
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University

2018

Acknowledgment

First of all and foremost, thanks to **Allah** almighty for giving me the strength to continue this work.

I would like to express my sincere gratitude to **Prof. Dr. Fatma Hassan Abd El-Razek**, Professor of Nutrition, Biochemistry and Nutrition Department, Faculty of women for Arts, Science and Education, Ain Shams University, for her support, helps guidance, planning the frame of the work and her stimulating view.

Many thanks are extended to **Prof. Dr. Anhar Mohamed Gomaa Shehab**, Professor of Biochemistry, Biochemistry Department, and Research Institute of Ophthalmology, for her meticulous supervision and continuous encouragement, my words stand to express how much I'm obliged to her for helping me to complete this study, she never denied me her valuable time and effort.

I would like to thank **Dr. Bakinam Ali Mohamed** lecture of Biochemistry and Nutrition, Biochemistry and Nutrition Department, Faculty of women Faculty of women for Arts, Science and Education, Ain Shams University, for her effort, guidance and valuable advice.

I wish to express my deepest thanks to **Prof. Dr. Mohamed Saad Al-Balkini**, Professor of Ophthalmology, Ophthalmology Department, and Research Institute of Ophthalmology.

I would like to thank **Prof. Dr. Laila Kamal** Professor of Histopathology, Histopathology Department, and Research Institute of Ophthalmology.

With a great pleasure, I would like to express my sincere gratitude to my **Mother, Father, Husband, Daughter, Sister and Brother** for their continuous encouragement, support and love.



Ethar Ahmed Mohamed

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَفَاكِ
رَبِّ زِدْنِي عِلْمًا

صَدَقَ اللَّهُ الْعَظِيمُ

Dedication

I would like to dedication this thesis to my family especially to my father and my mother for their constant, unconditional love and support throughout my entire life. There are not enough words I can say to describe just how important my mother and my father are to me, and what a powerful influence they continue to be...

Also this work is lovingly dedicated to my husband, daughter, sister and brother. I will never forget their effort towards me and also for their encouragement, support and love.

Abstract

Objective: The present study was performed to investigate the protective effect of blackberry and pomegranate as polyphenolic compounds for managing dry eye in experimental animal model.

Materials and Methods: The blackberry and pomegranate were dried in air oven. Forty eight male rabbits (1000-1300g) were divided into six groups: G1: normal control, G2: dry eye, G3: normal fed on blackberry, G4: normal fed on pomegranate, G5: dry eye feed on blackberry and G6: dry eye feed on pomegranate. Dry eye induced by atropine sulphate 1% drops (2 drops in each eye twice daily) for 2 months, blackberry or pomegranate was taken as 20% of the main diet. Polyphenolic compounds of blackberry and pomegranate were analyzed by using high performance liquid chromatography (HPLC). Tear production measured using Schirmer test and tear break up time (TBUT). Tears were collected from all groups using 5- μ L silanated microcapillary pipettes. At the end of experiments, rabbits were fasted overnight and blood was withdrawn. The levels of reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and interleukin-1 β (IL-1 β) were estimated in the tears and blood. In addition catalase (CAT) and superoxide dismutase (SOD) activity were determined in the blood. Cornea excised and examined by light microscope. Evaluation of tears protein fractions by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was also identified.

Results: Significant improvements were noticed in Schirmer I score and TBUT of dry eye rabbits fed on 20% dried blackberry or 20% dried pomegranate compared to dry eye group. There was a significant ($p < 0.05$) decrease in serum and tears IL-1 β , NO

and a statistically increase in the blood GSH, plasma CAT in dry eye rabbits fed on 20% dried blackberry or 20% dried pomegranate compared to dry eye rabbit group.

In conclusion: Supplementation with dried blackberry or pomegranate is effective against the dry eye syndrome by decreasing ocular inflammation and increasing antioxidant contents in tears of experimental animal model.

Key words: dry eye, polyphenolic compounds, atropine sulphate, blackberry, pomegranate oxidative stress, antioxidant

List of Contents

Subject	Page No.
List of abbreviations	I
List of tables	IV
List of figures	VI
Introduction	1
Aim of the Work	4
Review of Literature	5
Materials and methods	46
Results	73
Discussion	123
Summery	148
Conclusion	153
recommendation	154
References	155
Arabic summary	

List of Abbreviations

AAP	4-aminophenazone
ALP	alkaline phosphatase
ARVO	The Association for Research in Vision and Ophthalmology
CAT	Catalase
DE	Dry eye
DED	Dry eye disease
DHBS	3,5- Dichloro -2- hydroxybenzene sulfonic acid
DTNB	5, 5' dithio-bis-2-nitrobenzoic acid
ELISA	Enzyme linked immune sorbent assay
EP	Epithelial cells
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
GSHPx	Glutathione peroxidase
HCEP	Human corneal epithelium
HNE	4-hydroxynonenal
HPLC	High performance liquid chromatography
HRP	Horeseradish peroxidase
IL-1 α	Interleukin 1 α
IL-1 β	Interleukin 1 β
iNOS	Isoform nitric oxide synthase

KCS	Kerato-conjunctiviti ssicca
LASIK	Laser-assisted insitu kertomileusis
LDL	Low density lipoprotein
LOO•	Peroxyl radical
MAD	Malondialdehyde
MAPK	Mitogen-actived protein kinase
MGD	Meibomian gland dysfunction
MMPs	Metalloproteinases
mtROS	Mitochondrial reactive oxygen species
N3•	azide
NADP	Nicotinamide adenine dinucleotide
Na ₂ EDTA	Disodium ethylene diamine tetra acetic acid
NFκB	Nucler factor-kB
NO	Nitric oxide
•OH	hydroxyl
ONOO-	Peroxynitrite
OS	Oxidative stress
PHS	Physicians 'Health Study
ROO•	peroxyl
ROS	Reactive oxygen species
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sIgA	Secretory immunoglobulin A

SOD	Superoxide dismutase
SS	Sjögren's syndrome
TBA	Thiobarbituric acid
TBUT	Tear break up time
TMP	3, 3',5 ,5'-tetramethylbenzidine
TNF- α	Tumor necrosis factor- α
UV	Ultraviolet
Vit	Vitamin
WHS	Women's Health Study

List of tables

No.	Title	Page
1	Commercial diet constituent	47
2	Quantitative identification of polyphenolic compounds in blackberry by high performance liquid chromatography (HPLC)	75
3	Quantitative identification of polyphenolic compounds in pomegranate by high performance liquid chromatography (HPLC)	77
4	Mean $\pm$ S.D and percentage change of Schirmer test in normal control and dry eye with and without 20% dried blackberry groups	79
5	Mean $\pm$ S.D and percentage change of Schirmer test in normal control and dry eye with and without 20% dried pomegranate groups	80
6	Mean $\pm$ S.D and percentage change for TBUT in normal control and dry eye with or without fed on 20% dried blackberry groups	84
7	Mean $\pm$ S.D and percentage change for TBUT in normal control and dry eye with or without fed on 20% dried pomegranate groups.	85

No.	Title	Page
8	Mean $\pm$S.D and percentage change of blood reduced glutathione (GSH), blood superoxide dismutase activity (SOD), plasma catalase activity (CAT), serum malondialdehyde (MDA), serum nitric oxide (NO) and serum interleukin 1-β (IL-1β) levels for normal, dry eye and dry eye with and without blackberry groups.	92
9	Mean $\pm$S.D and percentage change of blood reduced glutathione (GSH), blood superoxide dismutase activity (SOD), plasma catalase activity (CAT), serum malondialdehyde (MDA), serum nitric oxide (NO) and serum interleukin 1-β (IL-1β) for normal, dry eye and dry eye with and without pomegranate groups.	93
10	Mean $\pm$S.D and percentage change of tear reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and interleukin 1-β (IL-1β) levels for normal and dry eye with and without blackberry groups.	103
11	Mean $\pm$S.D and percentage change of tear reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and interleukin 1-β (IL-1β) levels for normal and dry eye with and without pomegranate groups.	104

List of Figures

No. of figure	Name	Page
1	Anatomy of eye	6
2	Major etiological causes of dry eye	14
3	Mechanisms of dry eye	16
4	Tear composition and tear production	20
5	The relationship between mitochondrial reactive oxygen species (mtROS) and dry eye disease	29
6	Chemical structures of the different classes of polyphenols	35
7	Bioactive components in berries	39
8	Principal anthocyanins present in pomegranate	44
9	Standard curve of nitrite	63
10	Typical stander curve for il-1 β rabbit ELISA	67
11	Schirmer test in normal control and dry eye with and without 20% dried blackberry groups.	81
12	Schirmer test in normal control and dry eye with and without 20% dried pomegranate groups.	82

No. of figure	Name	Page
13	TBUT in normal control and dry eye with and without 20% dried blackberry groups.	86
14	TBUT in normal control and dry eye with and without 20% dried pomegranate groups.	87
15	Blood reduced glutathione level in all experimental groups.	94
16	Blood superoxide dismutase activity in all experimental groups.	95
17	Plasma catalase activity in all experimental groups.	96
18	Serum malondialdehyde level in all experimental groups.	97
19	Serum nitric oxide level in all experimental groups.	98
20	Serum interleukin1 β in all experimental groups	99
21	Tears reduced glutathione level in all experimental groups	105
22	Tears malondialdehyde level in all experimental groups.	106
23	Tears nitric oxide level in all experimental groups.	107
24	Tears interleukin 1 β level in all experimental groups.	108

No. of figure	Name	Page
25	Light micrograph of normal control rabbit's cornea fed on basal diet	109
26	Light micrograph of dry eye rabbit's cornea fed basal diet + atropine sulphate 1% drops	110
27	Light micrograph of rabbit's cornea fed on basal diet and 20 % dried blackberry	111
28	Light micrograph of rabbit's cornea fed on basal diet and 20 % dried pomegranate	112
29a	Light microscopic examination of dry eye rabbit's cornea fed on basal diet and 20% dried blackberry	113
29b	Light microscopic examination of dry eye rabbit's cornea fed on basal diet and 20 % blackberry	114
30	Light microscopic examination of dry eye rabbit's cornea fed on normal diet and 20 % dried pomegranate	115
31	SDS-polyacrylamide gel electrophoresis profile of tears protein fractions of different experimental groups	118
32	Densitograph of marker proteins	119
33	Densitograph of normal control group	120
34	Densitograph of dry eye group	120
35	Densitograph normal group fed on 20% dried blackberry	121