

Faculty of Women for Arts, Science and Education Biochemistry and Nutrition Department

Evaluation of Pumpkin (Cucurbita moschata) Pulp and Seeds Extracts on Gastrointestinal Ulcers Induced by Indomethacin in Rats

Thesis

Submitted for Faculty of Women, Ain Shams University In Partial Fulfillment for the Master Degree in Science in Biochemistry and Nutrition

By

Karima Yahia Gad Farag

B.S.c. in Biochemistry and Nutrition

Supervised by

Prof. Dr. Tahany El Sayed Kholief

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

Prof. Dr. Heba Adel Abd-Elhamid Barakat

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

Dr.Shimaa Abd EL-Aziz Elmasry

Lecturer of Biochemistry and Nutrition Biochemistry and Nutrition Department

Faculty of Women for Arts, Science and Education, Ain Shams University

2019

لْأُنْزَلِ مِنَ السَّمَاءِ مَاءً فَسَالَتُ أُوْوِيَةً بِقَرَرِهَا فَاحْتَمَلِ السَّبْلِ زُبِرًا رَابِيًا وَمِمَّا يُوقِرُونَ عَليْه في (النَّار (ابتغَاءَ مليَةٍ أُوْ مِتَاع زَبَرٌ مِثْلَه كَزلِكِ يَضْرِبُ (للهُ (ألحقٌ وَالبَاطل فَأَمَّا (الزَّبَرُ فَيَزْهَبُ جُفَاءً وَأُمَّا مَا يَنْفَعُ (النَّاسَ فَيَمْكُثُ فِي اللَّهُرِض كَنر لِكُ يَضْرِبُ (لَالَّهُ اللَّهُ عُثَالَ



First of all, all gratitude is due to Allah almighty for helping me and blessing this work until it has reached this end, as a part of generous help, throughout my life.

I would like to express my deepest gratitude to Prof.Dr.Tahany El-Sayed Kholeif, professor of Biochemistry ,Biochemistry and Nutrition Department ,Faculty of Women for Arts ,Science and Education, Ain shams University ,I had the honor to proceed this work under her instructive supervision, and guidance to enrich my study with her experience and knowledge throughout the steps of this work and for her great support ,hard work and flexibility.

I would like to express my gratefulness towards my guide Prof.Dr.Heba Adel Abdel -Hamid Barakat ,professor of Biochemistry ,Biochemistry and Nutrition Department ,Faculty of Women for Arts ,Science and Education, Ain shams University , for her excellent guidance and dedicated efforts , understand a number of problems and solve them sincerely, her keen interest and encouragement serve as a constant support and inspiration during the period of this work.

I would like to express my profound thanks to Dr. Shimaa Abdel-Aziz Elmasry, Lecturer of Biochemistry and Nutrition, Biochemistry and Nutrition Department, Faculty of Women for Arts, Science and Education, Ain shams University, for her supervision of this work in all its stages, continuous encouragement and support.

I would like to to express my deepest gratitude to Prof. Dr Kawkab Abd El Aziz Ahmed, Professor of Pathology. Department of Pathology. Faculty of Veterinary Medicine. Cairo University for her great effort in histopathological examination.

I would like to express my sincere gratitude to all my family members especially my mother and aunt for their support and encouragement and financial moral support to me step by step from the start until the end of this work.

➤ Karima Yahia Gad Farag

Tist of Contents

Subject Page	No.
List of tables	i
List of figures	ii
List of abbreviations	
Abstract	
Introduction	
Aim of the work	
Review of Literature	
1 - The gastrointestinal tract	
1.1 Stomach	
Histology of the stomach	
1.2 Small intestine	8
1.3 Large Intestine	10
1.4 Cecum	10
1.5 Colon	11
2 - Gastrointestinal Ulcer (peptic ulcer)	12
2.1 Types of Peptic ulcers	13
Gastric ulcer	
Duodenal ulcers (DU)	
2.2 Causes of Peptic ulcer	
Helicobacter pryloi (H.pylori) infection	
Non -Steroidal Anti-Inflammatory Drugs (NSAIDS)	
Effect of Alcohol	
Oxidative stressStress	
Fasting condition	
The Biological Effect of Indomethacin Compound	
2.3 Indomethacin induced peptic ulcer	

Tist of Contents (Cont.)

Subject Page No		
3 - Pumpkin (Cucurbita Moschata)	26	
3.1 Distribution and description of pumpkin:	26	
3.2 Chemical constituents and nutritive value of pump (fruit)		
Chemical constituents of Pumpkin pulp (fruit)	28	
Nutritive value of Pumpkin pulp (fruit)	29	
Chemical constituents of Pumpkin seeds	29	
Composition and medicinal properties of Pumpkin	Seed Oil . 31	
3.3 Role of pumpkin in protection from peptic ulcer	32	
Materials and Methods	39	
Materials	39	
1.1 Plant materials	39	
1.2 Chemicals	39	
1.3 Animals	39	
1.4 Diet	39	
Methods	40	
1. Preparation of the pumpkin pulp extract	40	
2. Preparation of the pumpkin Seeds oil extract	40	
3. Animal Trial	40	
4. Induction of Peptic Ulcer	41	
5. Experimental design	41	
6. Samples Collection	42	
7. Biological Measurements	42	

Tist of Contents (Cont.)

Subject	Page No.
8. Volumetric measurements	43
8.1 Measurement of Gastric Juice volume	43
8.2 Determination of gastric free and total acidity	43
9. Biochemical analysis in stomach and small intestin	e tissues 43
Preparation of gastric and small intestine homogen	nates43
9.1 Determination of Malondialdehyde (MDA) lev	vel 44
9.2. Determination of nitric oxide (NO) level	44
9.3. Determination of Reduced Glutathione (GSH)) content 45
9.4. Determination of catalase enzyme activity	46
9.5. Determination of myeloperoxidase (MPO) enzym	me activity 47
9.6. Determination of xanthine oxidase (XO) enzy	me activity 48
10. Histopathological examination	50
11. Statistical Analysis	50
Results	51
1. Influence of pumpkin pulp and/or seeds oi biological parameters in all experimental groups	
1.1 Feed intake	51
1.2 Body weight change	51
1.3 Feed efficiency ratio (FER)	55
1.4 Relative weight of stomach	56
1.5 Relative weight of small intestine	56
2. Influence of pumpkin pulp and/ or oil seeds extr volume, total and free acidity in all experimental g	
3. Influence of pumpkin pulp and/ or seeds oil e oxidative biomarkers, xanthine oxidase myeloperoxidase (MPO), in small intestine in al groups	(XO) and l experimental

	Influence of pumpkin pulp and /or seeds extracts on the anti- oxidant bio markers, reduced glutathione (GSH) and catalase (CAT), in small intestine and stomach in all experimental groups 65
1	Influence of pumpkin pulp and /or oil seeds extract on oxidative biomarkers, malondialdehyde (MDA) and nitric oxide (NO), in small intestine and stomach in all experimental groups 69
1	Influence of pumpkin pulp and /or oil seeds extract on microscopic examination of small intestine and colon tissues in all experimental groups
	6.1 Small Intestine Sections
	6.2 Microscopic Examination of Colon Sections
Discu	ssion84
	Effect of indomethacin on biochemical parameters of experimental animals
1	Influence of pumpkin pulp and/or seeds oil extracts on biochemical parameters in gastrointestinal ulcers induced in experimental rats
;	Effect of pre and post oral doses of consuming pumpkin (<i>Cucurbita moschata</i>) pulp and seeds oil extracts on colon and small intestine microscopic examination in indomethacin induced rats
	3.1 Small Intestine Sections
	3.2 Histopathological Examination of Colon Sections 107
Sumn	nary 109
Concl	usion & Recommendations113
	Conclusion
	Recommendations
Refer	ences
	الملخص العربي
	المستخلص

List of Tables

Table	No.	Title Page	No.
(1)		Bioactive components and their percentages pumpkin seeds (nutrient value per 100 g)	
(2)		Fatty acids composition of pumpkin seeds	36
(3)		Mineral content of pumpkin seeds	37
(4)		Amino acid composition of pumpkin seeds	38
(5)		Influence of pumpkin pulp and /or seeds oil extra on biological parameters in all experimental grou	
(6)		Influence of pumpkin pulp and /or seeds oil extracts on on gastric volume, total and free acidity in all experimental groups	
(7)		Influence of pumpkin pulp and /or seeds oil extra on xanthine oxidase and myeloperoxidase enzy activity in small intestine in all experiment groups.	me ntal
(8)		Influence of pumpkin pulp and /or seeds extracts on the anti-oxidant bio markers, reduced glutathione (GSH) and catalase (CAT), in small intestine and stomach in all experimental groups	
(9)		Effect of pumpkin pulp and /or seeds extracts malondialdehyde (MDA) and nitric oxide (Nevels in small intestine and stomach in experimental groups.	IO) all

Fig.	No.	Title Page N	0.
(1)		Structure of the alimentary tract	4
(2)		Structure of the stomach	5
(3)		Structure of the small intestine	9
(4)		The structure of the large intestine parts	11
(5)		Shape of peptic ulcer	14
(6)		(A) Healthy gastric mucosa: balance between mucosal aggressive and protective factors. (B) Gastric ulcer formation: imbalance between mucosal aggressive and protective factors.	
(7)		Microbial bacterium (H.pylori) shape	17
(8)		Helicobacter pylori and non-steroidal anti-inflammatory drugs have synergistic effects on gastric mucosal damage.	19
(9)		Structural formula of indomethacin	24
(10)		Incidence of peptic ulcer after induction by indomethacin	
(11)		Structure of ripe pumpkin	27
(12)		Shape of pumpkin seeds	30
(13)		Standard Curve for MPO determination	48
(14)		Standard curve for xanthine oxidase determination	49
(15)		Percent of change in feed intake (g) of all groups from PG group.	53
(16)		Percent of change in Body weight change (g) of all groups from PG group	
(17)		Percent of change in feed efficiency ratio (FER) of all groups from PG group	
(18)		Percent of change in relative weights of stomach and small intestine (g/100gb.wt) of all groups from PG group.	54

ii

(19)	Percent of change in all biological parameters of PG group from NCG group55
(20)	Percent of change in gastric volume (ml/100g) of all groups from PG group
(21)	Percent of change in total acidity (mEq/L) of all groups from PG group
(22)	Percent of change in free acidity (mEq/L) of all groups from PG group
(23)	Percent of change in gastric volume, free and total acidity of PG group from NCG group60
(24)	Percent of change in xanthine oxidase enzyme activity (ng/100mg) in small intestine of all groups from PG group
(25)	Percent of change in myeloperoxidase enzyme activity (ng/100mg) in small intestine of all groups from PG group
(26)	Percent of change in myeloperoxidase (MPO) and xanthine oxidase (XO) of PG group from NCG group64
(27)	Percentage of change in catalase enzyme activity (u/g) in small intestine and stomach of all groups from PG group
(28)	Percent of change in reduced glutathione content (mg/g) in stomach and small intestine of all groups from PG group
(29)	Percent of change in catalase (CAT) and reduced glutathione (GSH) in stomach and small intestine tissues of PG group from NCG group
(30)	Percent of change in malondialdehyde level (nmol/g) in small intestine and stomach tissues of all groups from PG group
(31)	Percent of change in nitric oxide (µmol/g)) in small intestine and stomach tissues of all groups from PG group71
(32)	Percent of change in malondialdehyde (MDA) and nitric oxide (NO) in the small intestine and stomach tissues of PG group from NCG group

(33)	Intestine section of rat from normal control group (NCG) showing normal healthy histological layers from mucosa, submucosa and musculosa with no detectable pathological changes (stained with Haematoxylin & Eosin X 100)
(34)	Intestine section of rat from pathogenic group (PG) showing inflammatory cells infiltration that associated with hemorrhage in the mucosal layer with signs of inflammation and ulceration (stained with Haematoxylin & Eosin X 400)
(35)	Intestine section of rat from pathogenic group (PG) showing marked coagulative necrosis of the mucosa with inflammatory cells infiltration (staind with Haematoxylin & Eosin X 100)
(36)	Intestine section of rat from PPG showing mucosal oedema and inflammatory cells infiltration (stained with Haematoxylin & Eosin X 400)
(37)	Intestine section of rat from PSG showing few inflammatory cells infiltration in lamina propria (stained with Haematoxylin &Eosin X 100)76
(38)	Intestine section of rat from PPSG showing group showing congestion of submucosal blood vessel (stained with Haematoxylin & Eosin X 100)76
(39)	Intestine section of rat from PTPG showing focal mucosal haemorrhage (stained with Haematoxylin & Eosin X 400)
(40)	Intestine section of rat from PTSG showing slight submucosal oedema with few inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)77
(41)	Intestine section of rat from PTPSG showing submucosal inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)
(42)	Colon section of rat from normal control group (NCG) showing healthy endocellular, subendocellular and mucosa layer with no detectable pathological changes (stained with Haematoxylin & Eosin X 100)

(43)	Colon section of rats from pathogenic group (PG) showing submucosal oedema and submucosal inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)
(44)	Colon of rat from PPG showing submucosal oedema and submucosal inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)
(45)	Colon section of rat from PSG showing slight submucosal oedema (stained with haematoxylin & Eosin X 100)
(46)	Colon section of rat from PPSG showing slight submucosal oedema and few submucosal inflammatory cells infiltration (stained with haematoxylin& Eosin X 100)
(47)	Colon section of rat from PTPG showing a slight submucosal oedema and few submucosal inflammatory cells infiltration as well as congested blood vessel (Stained with Haematoxylin & Eosin X 100)
(48)	Colon section of rat from PTSG showing slight submucosal oedema with few inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)82
(49)	Colon section of rat from PTPSG showing submucosal inflammatory cells infiltration (stained with Haematoxylin & Eosin X 100)

Tist of Abbreviations

Abbr.	Full term
AA:	Arachidonic acid
AAP:	4-aminophenazone
CAT:	Catalase enzyme
COX:	Cyclooxygenase
CVDs:	Cardiovascular diseases
DHBS:	3, 5 Dichloro-2-hydroxybenzene sulfonic
	acid
DSM:	Defatted seeds meal
DU:	Duodenal ulcer
FER:	Feed efficiency ratio
GIT:	Gastrointestinal tract
GS:	Glutathione synthetase
GSH:	Reduced glutathione
GU:	Gastric ulcer
H .pylori:	Helicobacter pylori
HDL-c:	High density lipoprotein - cholesterol
HRP:	Horseradish peroxidase
LDL-c:	Low density lipoprotein -cholesterol
MALT:	Mucosa- associated lymphoid tissue
MDA:	Malondialdehyde
MPO:	Myeloperoxidase
NCG:	Normal control group
NEDA:	N - (1-naphthyl) – ethylenediamine
NH ₂ Cl:	Monochloramine
OCI:	Hypochlorite ion

List of Abbreviations

PG: Pathogenic group

PGE₂: Prostaglandin –E₂

PPG Pretreated pulp group

PPSG: Pretreated pulp seeds group

PSG: Pretreated seeds group
PTPG: Post treated pulp group

PTPSG: Post treated pulp seeds group

PTSG: Post treated seeds group

PUD: Peptic ulcer disease
SOD: Superoxide dismutase

TA: Total acidity

TBA: Thiobarbituric acid

TNF: Tumor necrosis factor

XO: Xanthine oxidase

ABSTRACT

Peptic ulcer is a deep lesion penetrating through the entire thickness of gastrointestinal tract (GIT) and muscular mucosa that is usually located in the stomach or proximal duodenum. Pumkin contain active components such as flavonoids that shows antiulcerogenic activity and improve the healing of gastric mucosal lesions. Hence, these constituents may be responsible for reduction of gastric ulcer indices and antiulcer effect of pumpkin. The present work was undertaken to investigate the effect of pumpkin (*Cucurbita moschata*) aqueous extract and or ethanolic extract of seeds on indomethacin – induced ulcer in rats.

Ninety-six adult male albino rats Sprague-Dawely strain were divided into eight groups: group (1) normal control and group (2) pathogenic group that received indomethacin by oral administration (5 mg/kg b.wt /day) for 2 weeks. The other six groups were administrated indomethacin (5mg/kg b.wt/day) for 2 weeks along with either oral pre or post doses of pumpkin pulp and / or seeds oil extracts (400mg/kg.b.wt/day) twice weekly for another 2 weeks.

The results of this study showed that indomethacin administration to rats caused significant reduction in feed intake, weight change, FER and relative weights of small intestine and a significant increase in the gastric volume, free and total acidity. Indomethacin induced significant increase in the oxidative biomarkers (XO, MPO, MDA and NO) and decrease in the antioxidant enzymes (CAT and GSH), all of these results were confirmed by histopathological examination which indicated remarkable inflammation, hemorrhage, necrosis of small intestine and colon tissues of pathogenic rats. Oral administration of pumpkin pulp and /or seeds oil extracts in pre or post treatment doses caused ameliorative effect on biological, biochemical and histological changes.