A Histological Study to Evaluate the Efficacy of the Antidepressant Fluoxetine Versus the Anti-inflammatory Sulfasalazine in Experimentally Induced Colitis in Albino Rats

Thesis

Submitted for the Partial Fulfillment of M.D. Degree in Anatomy and Embryology

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2017

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Acknowledgment

- First of all, thank **Allah**, the most merciful to whom I relate any success in achieving any work in my life.

- I wish to express my profound thanks &appreciation to **Prof. Dr. Azza Salah El Din Soliman,** Professor of Anatomy and Embryology, Ain Shams University, for her endless patience & guidance. This work could not have reached its goal in a satisfactory way without her support. Starting from the main idea till reaching the final goal, she has stood as the motivating power of each aspect of this study. I have the honor to thank her, as I consider her to be my mother before becoming my professor. Her advices will continue to be a shining light for my road. To her I owe much more than I can express.

- I would like to express my gratefulness & sincere appreciation for **Prof. Dr. Dalia Fawzi Kallini,** Professor of Anatomy and Embryology, Ain Shams University, for her skillful scientific guidance, contributive comments, sincere effort & kind support that served much in the construction of this work. She devoted much of her time, advice and supplied me with everlasting help. Her cheerful spirit and her patience gave me the power to continue this work.

- My deepest thanks and gratitude are due to **Prof. Dr. Rania Ahmed Salah El Din,** Professor of Anatomy and Embryology, Ain Shams University, for her support & encouragement. I always see her as a model in terms of ambition, struggle and perseverance. Also, I would like to extend my thanks to **Dr. Haidy Farid Abd El Salam,** Assistant Professor of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, for her tender care and kind cooperation.

- I would like to thank **Dr. Sherif Adel Kamar,** Lecturer of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, for his valuable remarks & suggestions that helped me in this work.

- Special thanks to **Prof. Dr. Shahira Youssef Mikhail,** Head of Anatomy and Embryology department, Faculty of Medicine, Ain Shams University, for her continuous support and encouragement. I am most honored that my thesis will be discussed in her time and I hope that it will meet her expectations.

- I am also deeply grateful to **Prof. Dr. Salwa S. Tashin,** Professor of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, for her precious opinions, discussion and helpful criticism.

I am greatly indebted to members of anatomy department for their kind cooperation and help, especially Dr. Ahmed Farid for his role in statistical analysis, Dr. Maha Zakaria for her guidance regarding the image analysis, Dr. Marwa A. Abouelfotouh & Dr.Noha M. Gaber for their great help and Dr. Walaa A. El Fakharany for her support.

Also, I'd like to thank my old & lovely friend Raghda Sayed for her kind help in reviewing the language aspect of this thesis.

I can never forget the great role of my friend, Dr. A'ishah Husein, my companion of the struggle from the beginning of the study of medicine until now, who stood beside me and helped me coordinating the writing of scientific references.

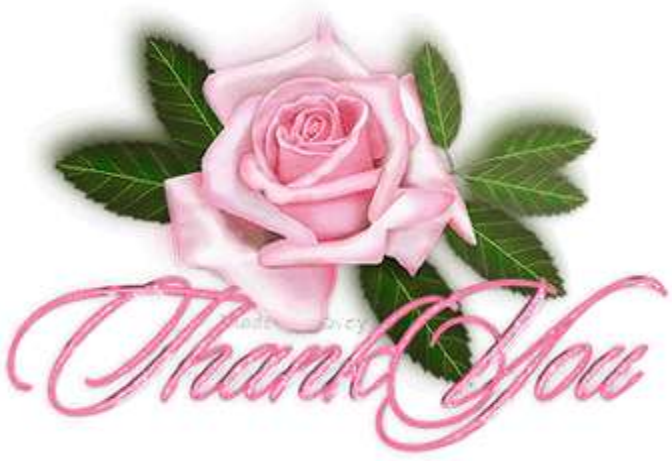
Shimaa Magdy Mohammed

Dedication

There are many times when silence speaks so much louder than words, but I would like to express my gratitude to all of my family members for their continuing love, support and encouragement. Im really thankful to my family, specially My parents, for their love & great support.

A big -Thank you-to my husband (Ahmad Fathy), the person who always believed in my ability to achieve all what I set out to accomplish. He helped me a lot and facilitated this journey to me and devoted every kind of help and support for me. I also dedicate this to My children (Omar) and (A'ishah), they are my inspiration and the reason I strive to achieve my best. Finally, I praise and thank My relatives and friends, who supported me through the ups and downs of this process

Im really thankful to all of you.



Contents

List of Abbreviations

AA	**:**	Acetic acid
AAIC	**:**	Acetic acid induced colitis
ASA	**:**	5-aminosalicylic acid
BM	**:**	Basement membrane complex
CAT	**:**	Catalase
CCCF	**:**	Corrupted colonic crypt fission
CD	**:**	Crohn's disease
CNS	**:**	Central nervous system
COX	**:**	Cyclo-oxygenase
CT	:	Connective tissue
DMH	**:**	Dimethylhydrazine
EC	**:**	Enterochromaffin cells
ECM	**:**	Extracellular matrix
EECs	**:**	Enteroendocrine cells
EIM	**:**	Extraintestinal manifestations
ENS	**:**	Enteric nervous system
FLX	**:**	Fluoxetine
GAG	**:**	Glycosaminoglycans
GC	**:**	Goblet cell
GIT	**:**	Gastrointestinal tract
GSH	**:**	Glutathion- reduced form
HPA	**:**	Hypothalamus-pituitary-adrenal

IBD	**:**	Inflammatory bowel disease
IE	**:**	Intestinal epithelium
IECs	**:**	Intestinal **epithelial** cells
iNOS^{+}	**:**	Inducible nitric oxide synthase
ISMC	**:**	Intestinal smooth muscle cells
LI	**:**	Large intestine
LP	**:**	Lamina propria
MALT	**:**	Mucosa-associated lymphoid tissue
MAP	**:**	Mycobacterium Avium Paraturbeculosis spp
ME	**:**	Muscularis externa
NO	**:**	Nitric oxide
NSAIDs	**:**	Non-steroidal ant1i-inflammatory drugs
QOL	**:**	Quality of life
ROS	**:**	Reactive oxygen specie
SC	**:**	Spinal cord
SEMF	**:**	Subepithelial myofibroblast
SERT	**:**	Serotonin reuptake transporter
SOD	**:**	Superoxide dismutase
SSRIs	**:**	Selcetive serotonin reuptake inhibitors
SSZ	**:**	Sulfasalazine
TNF-α	**:**	Tumour necrosis factor alpha
UC	**:**	Ulcerative colitis
5-HT	**:**	Serotonin, 5-hydroxytrptamine

Introduction

Inflammatory bowel disease (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are severe chronic inflammatory disorders of the gastrointestinal tract. The specific pathogenesis underlying IBD is complex, however the interaction of environmental factors with genetic susceptibility and immune-mediated phenomena may play important roles. There is limited information about the IBD in Arab community. The growing prevalence of this disease increases both economic and health care burden. Thus, better and more affordable treatment and eventually a cure is greatly needed ***(Guemei et al., 2008 and Low et al., 2013)***.

IBD adversely affects the quality of life and necessitates longterm dependence on effective drugs. Mesalazine, sulfasalazine and other 5-aminosalicylic acid (ASA) derivatives are considered currently drugs of choice for the management of most cases, while corticosteroids and immunosuppressants are retained for more severe forms of the disease. Although these drugs are effective the risk of their adverse effects is high, especially considering the chronic and relapsing nature of this condition.

Therefore, the search for new safer therapies continues ***(Abdel-Aziz et al.,2013).***

Acetic acid (AA)-induced colitis is a reproducible and simple model, sharing many characteristics with human colitis ***(Wang et al., 2013).*** The first report of this model was demonstrated by MacPherson and Pfeiffer where they instilled 10%–50% acetic acid into the rat rectum for 10 seconds, followed by flushing the lumen with saline three times. A diffuse colitis in an acetic acid dose-dependent manner was observed in these rats, with histopathological features including ulceration of the distal colon and crypt abnormalities. Subsequent modifications and optimization over years focused on varying the concentration of acetic acid and the contact time. The advantages of acetic acid-induced colitis are its low cost and the ease of administration ***(MacPherson and Pfeiffer 1978*** and ***Low et al., 2013)***

Sulfasalazine (SSZ) is used to treat the chronic human IBDs, UC and CD. It consists of one molecule of 5-aminosalicylic acid (5-ASA, mesalamine) coupled by an azo bond to one molecule of sulfapyridine. The azo bond allows uncoupling of the two constituent compounds in the lumen of the colon by the action of bacterial azo reductase

enzymes resulting in topical delivery of the compounds. It has been shown that the 5-ASA moiety of SSZ is the therapeutically active component in UC and CD and that the sulfapyridine moiety is inactive and causes most of the allergic and intolerant effects of SSZ ***(Vigna, 2014)***.

Recently, there is a great suggestion that psychiatric disorders could be one of the etiological factors of UC in some patients. There is some evidence that major depression in particular is accompanied by activation of the inflammatory system and that pro-inflammatory cytokines may play a role in the etiology of depression ***(Papadakis and Targan, 2000 and Kurina et al., 2001)***.

Among antidepressant drugs are selective serotonin reuptake inhibitors (SSRIs) that have a well recognized effect on depression and anxiety. Fluoxetine (FLX) is a SSRI of proven efficacy in major depressive disorders. This antidepressant exhibits higher safety and fewer side effects than other groups of antidepressants ***(Guemei et al., 2008*** and ***Koh et al., 2011)***. There are several reports about the analgesic and anti-inflammatory effects of antidepressant drugs; for instance, anti-inflammatory activity of fluoxetine was studied on the carrageenan-induced paw inflammation in the rat ***(Minaiyan et al., 2014)***. FLX was characterized

as a lipophilic weak base, which when administered orally experiences a direct contact with epithelial cells in the intestines. In these epithelial cells, it induces an increase in serotonin (5-HT) levels by blocking L-monoamine oxidase and serotonin reuptake transporters ***(Stopper et al., 2014).***

Aim of the work

The aim of the present work was to study the histological effect of the antidepressant drug (fluoxetine) versus the traditional anti-inflammatory drug (sulfasalazine) on induced colitis in albino rats.

Specific objectives:

Studying the colon histology by light microscopy using paraffin and semi-thin sections to:

(a) Assess the anti-inflammatory effect of sulfasalazine (SSZ), as a known effective treatment of human UC, in a model of acetic acid induced colitis in rats.

(b) Investigate the possible therapeutic effects of the antidepressant fluoxetine on the extent and severity of colitis induced by acetic acid in rats.

(c) To study the possible underlying mechanisms of action by which the drugs emerged their effects.

Anatomy of the colon

I- Human colon

The large intestine (LI) in humans consists of the terminal 1.0 to 1.5 m segment, which is about one-fifth of the whole length of the gastrointestinal tract [GIT] ***(Drake et al., 2010)***. Externally, the LI uniquely characterized by the presence of teniae coli and haustra, which are noticeable through the investing serosa and subserosal tissue. The fibers of the muscular layers of the LI are arranged into longitudinal and circular layers. The longitudinal fibers are present circumferentially through the length of the LI but are mainly concentrated into three flat bands called the teniae coli. The haustra, convexities of the circular layer, are transient and may be the result of structural and functional properties of the LI ***(Mills, 2012).***

II- Rat colon

The total length of the colon and rectum in rats is approximately 15 cm ***(Elwell and McConnell, 1990).*** Colon initially ascends (colon ascendens) rostrally from the cecum and then behind the right kidney it turnes in a transverse direction (colon transversum), finally going over