Chapter I

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) were commonly used for the treatment of rheumatoid diseases and to relief pain and inflammation due to their analgesic, antipyretic and anti-inflammatory properties. These drugs were used as prescription drugs and over the counter purchases **(Teoh and Farrell, 2003).**

The therapeutic value of NSAIDs in musculoskeletal pathologies or in pyretic and painful conditions was hindered by several side effects. The most severe side effect were both the stomach and the intestine **(Wolfe *et al.*, 1999) and (Thiéfin, 2005)**. Small intestinal ulceration was a frequent and potentially serious condition associated with nonselective cyclooxygenase (COX) I&II inhibitors NSAIDs including diclofenac **(LoGuidice *et al.*, 2010).**

The mechanisms underlying intestinal injury were unclear. Cyclooxygenase (COX) inhibition and the subsequent fall in prostaglandin synthesis, which is claimed as the main pathogenetic event of NSAID-induced gastric damage. It seemed to be less crucial in the etiology of intestinal injury. Many other mechanisms were likely to be relevant, such as uncoupling of oxidative phosphorylation, increased mucosal permeability, luminal bacteria invasion of gut wall, neutrophil-induced oxidative damage, intestinal hypermotility and microvascular injury **(Reuter *et al.*, 1997, Whittle, 2004).**

Another study added that the physical stimuli, chemical, inflammatory or mitogenic triggering stimulation of phospholipase A2 acted on the plasma membrane releasing arachidonic acid **(Mota *et al.*, 2010).**

In another study on human, it was reported that NSAID-induced permeability changes lead to inflammation of the small intestine in two-thirds of patients on long-term treatment with NSAIDs. The intestinal inflammation may persist for up to 16 months after withdrawal of NSAID. NSAIDs might expose the mucosa to luminal bacteria as well as other luminal content which might have aggressive effects and produce inflammation **(Bjarnason *et al.*, 1987)**.

***Moringa oleifera* lam (MO) (horse radish tree) (Moringaceae**) was a small sized tree ,Which was native to south Asia and also grows in tropical Africa **(Ghasi *et al.*, 2000).** Also, It can be cultivated in Egypt.

Various parts of MO were generally known for their multiple pharmacological effects including their anti-inflammatory effects **(Caceres *et al.*, 1992).** The extract of MO had been found to have potent

antioxidant action in vivo **(Ashok Kumar and Pari, 2003),** and in vitro **s**tudies **(Siddhuraju and Becker, 2003)**.

Accumulating evidence supported the protective effects of phenolic antioxidants from medicinal plants against oxidative stress-mediated disorders **(Soobrattee *et al.,* 2005).**

1.2-Aim of the work:

NSAIDS might be used in toxic doses by mistake or in high doses as a mandatory postoperative analgesia**.** The current study was evaluated the possible protective role of MO on the experimentally induced microscopical changes of duodenal mucosa of adult rats following administration of different high doses of DS

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Chapter II

REVIEW OF LITERATURE

2.1. Diclofenac Sodium

2.1.1-Name of the drug

Active ingredient: Diclofenac sodium

Chemical name: sodium-[0-[(2,6-dichlorophenyl)-amino] phenyl]-acetate

Molecular weight: 296.15

Molecular formula: C14H11Cl2NO2

Chemical Structure:

(http://www.newdruginfo.com)

2.1.2-Description

Diclofenac sodium is an odourless, yellowish-white, crystalline powder sparingly soluble in water.

In addition to diclofenac sodium, DICLOFENAC-GA tablets contain lactose,

calcium hydrogen phosphate, cellulose-microcrystalline, starch-maize, sodium starch glycollate, magnesium stearate, silica-colloidal anhydrous, methacrylic acid copolymer, triethyl citrate, talc-purified, titanium dioxide and iron oxide. **(www.ascentpharma.com.au).**

2.1.3-Pharmacology:

Diclofenac sodium (DS), Voltaren, is a nonsteroidal anti-inflammatory drug, which has analgesic and anti-inflammatory effects and widely used for treatment of a variety of rheumatoid disorders **(Aydin, 2003).** Nonsteroidal anti-inflammatory drugs (NSAIDs) have been prescribed extensively throughout the world. More than 70 million prescriptions for NSAIDs were written each year in the United States. With over-the-counter use included, more than 30 billion doses of NSAIDs were consumed annually in the United States alone. Most commonly ingested NSAIDs have few toxic effects, even when taken in significant quantities; however, with the numbers of both prescriptions and consumption of over-the-counter (OTC) NSAIDs increasing every year, so do the numbers of overdoses and NSAID-related complications reported to poison control centers around the country. Additionally, adverse events related to drug interactions, or exposure to vulnerable patients with disease states that predispose patients to NSAID toxicity, were common and may result in significant morbidity and mortality. NSAID toxicity in the setting of acute drug overdose as well as factors predisposing individuals to adverse effects from NSAIDs were described below **(Wiegand *et al.*, 2010).**

Nonsteroidal antiinflammatory drugs (NSAID) were among the most widely used drugs worldwide and represent a mainstay in the therapy of acute and chronic pain. However, their use is frequently associated with a broad spectrum of adverse effects, which were related to the inhibition of prostaglandin (PG) synthesis in tissues, PGs were responsible for physiological homeostasis. In the

early 1990s two structurally related isoforms of cyclooxygenase (COX) have been identified, namely COX-1, constitutively expressed in most mammalian tissues, and COX-2, which is usually undetectable under resting conditions and is rapidly induced at sites of inflammation in response to noxious stimuli. This has led to the theory that COX-1 isoenzyme produced PGs which exert house-keeping functions, including gastric mucosal defense and renal homeostasis, whereas COX-2 synthesizes detrimental PGs which were responsible for inflammation and pain **(Buttgereit *et al.*, 2001).**

2.1.4- Gastrointestinal Toxicity

Nonsteroidal antiinflammatory drug toxicity to the small intestine is common. Useful research tools have been developed to indirectly measure intestinal inflammation and permeability, but these were not generally available to the clinician, although enteroscopy and capsule endoscopy can be illuminating **(Fortun and Hawkey, 2007).**

Furthermore, small intestinal inflammation and ulceration do not only develop after chronic NSAID use but may also occur after short-term (7 days) therapy **(Bjornsson *et al.*, 2008).**
In another studies, there was up to 70% of patients on long term NSAIDs have evidence of increased intestinal permeability; inflammation, and bleeding in the small and large intestines **(Bjarnason *et al.*, 1987), (Sigthorsson *et al.*, 1998))**. The macroscopic appearance of small bowel lesions induced by NSAIDs includes single or multiple erosions or ulcers **(Agrawal, 1993)**. NSAID -induced strictures may be broad- based or diaphragm- like in the mid small intestine **(Hershfield, 1992).** NSAID use is associated with an increased incidence of small intestinal ulceration **(Agrawal, 1993)**.

2.1.5 Mechanism of action of NSAIDs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) can cause gastrointestinal mucosal damage, the risk of which appears to be related to both dosage and duration of therapy. Serious GI toxicity such as bleeding, ulceration and perforation can develop at any time, with or without warning symptoms, and occurs in approximately 1% of patients treated for 3 to 6 months and 2% to 4% of patients treated for one year. These trends continue with longer duration of use, although short-term therapy is not without risk. While agents that selectively inhibit cyclooxygenase-2 (i.e. COX-2 inhibitors) were generally thought to be associated with a reduced risk of GI toxicity compared to conventional NSAIDs, they have not been proven risk-free. Thus, therapy with all NSAIDs, including COX-2 inhibitors, should be prescribed cautiously in patients with a history of peptic ulcer disease and/or gastrointestinal bleeding. Caution is also advised if NSAIDs were prescribed to patients with other

risk factors such as oral corticosteroid or anticoagulant use, alcohol use, smoking, older age, and poor general health status **(Fortun and Hawkey, 2007).**

The mechanism of action that defines NSAIDs as a class was their ability to inhibit the COX activity of the enzyme prostaglandin G/H-synthase and thereby block the biosynthesis of prostaglandins ***(Vane et al., 1990)***. NSAIDs prevent the formation of prostaglandin H_2, the first committed step in the metabolism of arachidonic acid into a complex cascade of signaling lipids, such as prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2, and thromboxane, the principal prostanoid

metabolite in platelets (Fig. 1). Therapeutic concentrations of NSAIDs (usually in the low micromolar range) were not known to influence other pathways of arachidonic acid metabolism except indirectly by increasing the intracellular concentration of free arachidonic acid, which potentially causes shunting of arachidonic acid through other metabolic pathways ***(Vane et al., 1990)*.**

Fig (2.1): Arachidonic acid metabolism. The major metabolites of arachidonic acid produced by the cyclooxygenase (COX) and lipoxygenase (LO) pathways were indicated. Examples of tissues in which individual prostanoids exert prominent effects were indicated **in parentheses.** PGD_2 = prostaglandin D_2; PGE_2 = prostaglandin E_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; TXA_2 = thromboxane A_2; PGI_2 = prostaglandin I_2; HPETE = hydroxyperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid ***(Thun et al., 2002)*.**

Two distinct isoforms of prostaglandin G/H-synthase, designated COX-1 and COX-2, have been recognized since 1991 **(Smith et al., 1996) and (Xie et al., 2004)**. COX-1 is expressed constitutively in many tissues, and it plays a central role in platelet aggregation and gastric cytoprotection. Although COX-2 is expressed constitutively in the human kidney and brain, its expression is induced in many tissues during inflammation, wound healing, and neoplasia. COX-1 and COX-2 initiate the formation of biologically important prostanoids that coordinate signaling between thecell of origin (autocrine) and neighboring cells (paracrine) by binding to transmembrane G-protein-coupled receptors**(Morrow JD, 2001) .**

NSAIDs vary in their abilities to inhibit COX-1 or COX-2 at different concentrations and in different tissues. For example, aspirin is a relatively selective inhibitor of COX-1 in platelets when given at doses of 50–100 mg daily ***(Patrignani et al., 1982), and (FitzGerald et al., 1983)***. Most other conventional NSAIDs, such as ibuprofen, sulindac, and indomethacin, inhibit COX-1 and COX-2 to the same extent, whereas a new class of NSAIDs, selectively inhibits COX-2 ***(Peterson and Cryer, 1999)*** **and (Willoughby *et al.*, 2000).**

The use of NSAIDs is an important cause of gastropathy. It was always thought that only the non-selective NSAIDs were the causative agents, but the selective COX-2 inhibitors have now also been shown to be associated with untoward gastrointestinal side effects. NSAID-related ulcers require an individualized assessment of a patient's current disease state and medication use. More importantly, the patient should be assessed for his or her risk of gastrointestinal and cardiovascular toxicities. If the patient needed to continue on the NSAID, then an appropriate regimen should be sought, with the best possible efficacy, and lowest possible number of side-effects. New advances have been made in designing novel anti-inflammatory drugs, with reduced toxicity. Coupling of NSAID-moieties that

slowly release gastro-protective gaseous mediators, such as NO and H2S, appears to be a promising new approach to reduce the toxicity of these agents **(Schellack, 2012).**

The pharmacologic effects of NSAIDs were further complicated by the diverse functions of prostanoids in different tissues and by the variable effects of COX inhibition, depending on drug, dose, and clinical context. The formation of specific prostanoids varies across different tissues because of differences in the concentration of tissue-H_2. Furthermore, more than one G-coupled protein receptor may transduce different effects from the same prostanoid **(Wood *et al.*, 2001).**

Anti-inflammatory doses of NSAIDs (e.g., ibuprofen at a dose of 800 mg every 8 hours or naproxen at a dose of 500 mg twice daily) were therapeutic for patients with osteoarthritis or rheumatoid arthritis but can cause gastrointestinal ulceration, bleeding, or disruption of renal hemodynamics in susceptible patients **(Wood *et al.*, 2001).** Although, celecoxibs do decrease urinary excretion of prostacyclin 8

metabolites in normal subjects **(McAdam *et al.*, 1999), s**ome studies have shown that these drugs cause less mucosal damage in rats when acutely or chronically administered **(Keeble JE, 2002, Davies *et al.*, 1997) (Mizoguchi *et al.*, 2008).**

Selective COX-2 inhibitors chemically modified with the incorporation of a NO-donating group have been recently synthesized, that retain the therapeutic activity of COX inhibition and the gastrosparing effects of NO **(Chegaev *et al.*, 2007).**

In the recent years it has become clear that small gaseous molecules serve as endogenous mediators in the body; this is the case for NO, carbon oxide (CO) and H2S **(Fiorucci *et al.*, 2006)**. H2S is produced in several tissues and exerts many physiological functions; in the digestive system, this molecule is constitutively produced in the gastric mucosa from sulphur-containing aminoacids (cysteine) via the action of two enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE**) (Zanardo *et al.*, 2006).**

Other studies have shown that H2S is involved in the maintenance of mucosal integrity and in the regulation of blood flow, acting in concert with NO to protect the gastric mucosa from injury **(Fiorucci *et al.*, 2006).**

It was therefore hypothesized that deficiency of H2S synthesis might contribute to the pathogenesis of several GI disorders and, in particular, to NSAID-induced gastropathy. In line with this, the administration of NSAIDs, including aspirin, indomethacin, diclofenac and ketoprofen, resulted in a significant decrease in H2S synthesis **(Fiorucci *et al.*, 2006).**

Also, a H2S-releasing diclofenac derivative had been shown in rats to possess greatly reduced GI damaging effects as compared to the parent drug, despite a comparable antiinflammatory activity and suppression of PG synthesis **(Wallace *et al.*, 2007)**.

Recently, It was stated that gastric hypermotility played a primary role in the pathogenesis of NSAID-induced gastric damage. The response was related with PG deficiency due to COX-1 inhibition, occurring prior to other pathogenic events such as increased mucosal permeability. The ulcerogenic properties of NSAIDs required the inhibition of both COX-1 and COX-2, the inhibition of COX-1 upregulates COX-2 expression in association with gastric hypermotility, and PGs produced by COX-2 counteract the deleterious effect of COX-1 inhibition**).(Takeuchi, 2012).**