2. INTRODUCTION AND AIM OF THE STUDY

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used classes of medications to treat pain and inflammation. The very frequent use of NSAIDs is based on the facts that these agents are used in many indications. They alleviate the swelling, redness and pain of inflammation, reduce a general fever and cure headache, bodyache etc. so they are used in many indication like:

-Post operative & post traumatic inflammation
-Low back pain
-Arthritis
-Gout
-Spondylitis
-Inflammatory soft tissue rheumatism
-Prophylaxis purpose in CVS disorders

However, gastrointestinal complications associated with NSAIDs are prevalent, largely due to the frequent use of these agents. Adverse events associated with NSAIDs include minor side effects, such as dyspepsia, as well as serious complications, such as bleeding and perforation. Although the probability that any given individual user of an NSAID will suffer a serious gastrointestinal complication is fairly low, widespread patient exposure can translate into a major national health burden.

Table 5 Side Effects Shared by NSAIDs

Gastrointestinal ulceration and intolerance[a]
Blockade of platelet aggregation (inhibition of thromboxane synthesis).
CVS complications- (specially for COX-2 specific Inhibitors)
Inhibition of uterine motility (prolongation of gestation)
Inhibition of prostaglandin-mediated renal function[b]
Hypersensitivity reactions[c]

[a] Lesser side effects with nonacetylated salicylates or *p*-aminophenol derivatives.

[b] Of special importance for patients with decreased renal bood flow; retention of Na, K, and water (edema) can reduce effectiveness of antihypertensive regimens.

[c] More pronounced with aspirin than with nonacetylated salicylates.

Gastrointestinal complications are strongly associated with the use of conventional nonsteroidal anti-inflammatory drugs (NSAIDs) and are recognized as the most prevalent and severe cause of drug toxicity in the USA[61]. Millions of patients use NSAIDs for the relief of various types of arthritis pain, stiffness, and related symptoms. Selective NSAIDs such as cyclooxygenase (COX)-2 inhibitors were first introduced with the purpose of providing symptomatic pain relief along with lesser gastrointestinal risk. Recent studies indicated that COX-2 inhibitors were prescribed more often than NSAIDs in patients who are older, sicker, and have risk factors associated with NSAID gastropathy.[62] However, rofecoxib (Vioxx®; Merck & Co., Inc., Whitehouse Station, NJ, USA) was voluntarily withdrawn in September 2004; valdecoxib (Bextra®; Pfizer, Inc., New York, NY, USA) was withdrawn and a 'black box' warning was added for celecoxib (Celebrex®; Pfizer, Inc., New York, NY, USA) in April 2005; and a joint hearing of the US Food and Drug Administration Arthritis Committee and the Drug Safety and Risk Management Committee found that the use of COX-2 inhibitors is associated with increased risk for cardiovascular events.[63] These recent events have led many physicians to consider the use of traditional NSAIDs in combination with a proton pump inhibitor (PPI) to reduce the gastrointestinal side effects of NSAIDs. Indeed, major treatment guidelines recommend PPI prophylaxis in patients with a previous gastrointestinal event and in those at high risk for complications[64].

NSAID-induced gastrointestinal complaints are among the most commonly reported adverse events. Balancing these benefits and risks is an important clinical goal in the post Vioxx and post-Bextra era. Reducing the risk for gastrointestinal complications requires a thorough understanding of potential complications and underlying predisposing risk factors, which is a particularly important consideration in light of the fact that many individuals develop complications without antecedent warning signs or symptoms. This review provides updated information on traditional NSAIDs, including details regarding their efficacy and safety, and a discussion of the major risk factors that are commonly associated with gastrointestinal complications.

2.1 Spectrum of gastrointestinal risk

The association between NSAIDs and gastrointestinal erosions and ulcers is well established. The relative risk for experiencing serious adverse gastrointestinal events is approximately three times greater for NSAID users than for nonusers[65]. Furthermore, patients with rheumatoid arthritis (RA) are nearly twice as likely as those with osteoarthritis (OA) to suffer a serious complication from NSAID treatment.[66] Compared with RA, OA is a milder disease and requires lower doses of NSAIDs, which may explain why patients with OA appear to be at lower risk for gastrointestinal complications.

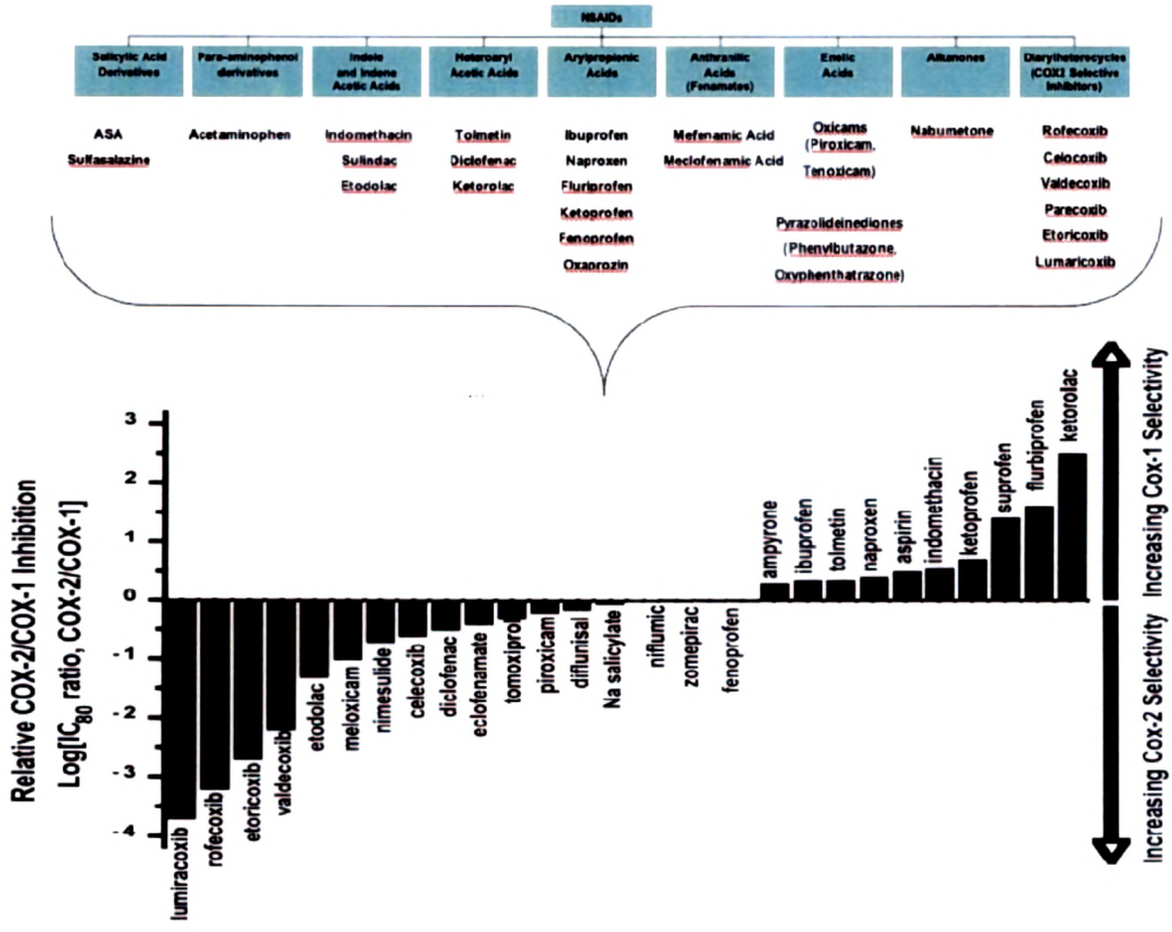


Figure 15 The 9 chemical groupings of NSAIDs are shown, along with key compounds in each class. Relative degree of COX-1 vs COX-2 selectivity is shown at the bottom of the figure from Warner and Mitchell. Common therapeutic

effects (in varying degrees of activity) for NSAIDs are antipyretic, analgesic, and anti-inflammatory. Common side effects (in varying relative frequencies) are gastrointestinal ulceration, inhibition of platelet aggregation, inhibition of uterine motility, inhibition of PG-mediated renal function, and hypersensitivity reactions.

NSAID gastrointestinal damage is mediated through several mechanisms that compromise mucosal integrity. In addition, NSAIDs, particularly aspirin, inhibit platelet function even at low dosage, giving rise to bleeding that most commonly affects the gastrointestinal tract. The distinction between erosions and ulcers depends on pathological and endoscopic definitions, with ulcers defined as lesions that penetrate to the level of the submucosa (involving endoscopically evident depth) and erosions defined as lesions confined to the mucosa (without endoscopically appreciable depth). Ulcers give rise to major bleeding, perforation, or obstruction. NSAID-related gastrointestinal adverse events can be classified into three broad categories[67] 'nuisance' symptoms such as heartburn, nausea, dyspepsia, and abdominal pain; mucosal lesions (which may or may not be symptomatic), such as ulcers; and serious gastrointestinal complications, such as perforated ulcers and catastrophic bleeding. Nuisance or minor gastrointestinal side effects, including nausea, dyspepsia, anorexia, abdominal pain, flatulence, and diarrhea, are common and affect between 10% and 60% of NSAID users. Mucosal lesions are also common, with nearly half of all patients who take NSAIDs on a regular basis having gastric erosions and 15–30% having endoscopically detectable ulcers. The majority of these lesions do not cause significant symptoms[68,69]. Clinically significant upper gastrointestinal events occur in 3–4.5% of NSAID users annually. The majority of these events are symptomatic ulcers whereas a smaller percentage (approximately 1%) are clinically serious and associated with gastrointestinal bleeding, perforation, or obstruction[70,71]. Throughout the 1980s, the overall risk for hospitalization resulting from gastrointestinal complications was estimated at approximately 1% per year in persons taking NSAIDs[61]. Recent data indicate that the incidence has declined substantially, to 0.5%, as a result of a number of factors, including lower doses of NSAIDs, use of gastro protective agents (PPIs and misoprostol), and

the introduction of the selective COX-2 inhibitors[72]. However, patients taking NSAIDs are 6.45 times more likely to be hospitalized for a gastrointestinal complication than are nonusers[61,66](Table 5). The number of deaths associated with NSAID-induced gastrointestinal damage, as acquired from ARAMIS (the Arthritis, Rheumatism, and Aging Medical Information System), which included post marketing surveillance of more than 36,000 patients from 17 centers in the USA and Canada, are staggering and are comparable to mortality statistics for AIDS and other terminal diseases (Fig. 15) [66,71].

2.11 Magnitude of risk for gastrointestinal complications associated with NSAIDS

Although only a relatively small proportion of NSAID users actually develop major gastrointestinal complications, the importance of these complications is magnified by the widespread use of these agents, thus translating this proportion into a large absolute number of toxicities. More than 30 million individuals are estimated to take NSAIDs daily[66]. Over 111 million NSAID prescriptions were written in the year ending in August 2000[66].Additionally, more than 30 billion over-the-counter (OTC) NSAIDs are purchased annually.[73] The prevalence of at least a once weekly NSAID dose among elderly patients aged 65 years or older has been reported to be as high as 70% (original source[67]; primary source.[74] This is particularly significant when it is considered that increasing age is an independent risk factor for gastrointestinal complications. Several risk factors are known to increase substantially an individual's risk for NSAID-induced gastrointestinal events[67]. These include a history of ulcer, presence of *Helicobacter pylori* infection, use of more than one NSAID (including aspirin), use of high-dose NSAIDs, concurrent anticoagulant or corticosteroid use, a serious underlying disease, and age greater than 75 years. The severity of RA may also be directly related to an increased risk for gastrointestinal events. In contrast, dyspepsia and other upper gastrointestinal symptoms do not reliably predict the development of upper gastrointestinal events[67].

The role played by *H pylori* in the development of gastrointestinal complications remains a subject of controversy. In a recent meta-analysis of 25

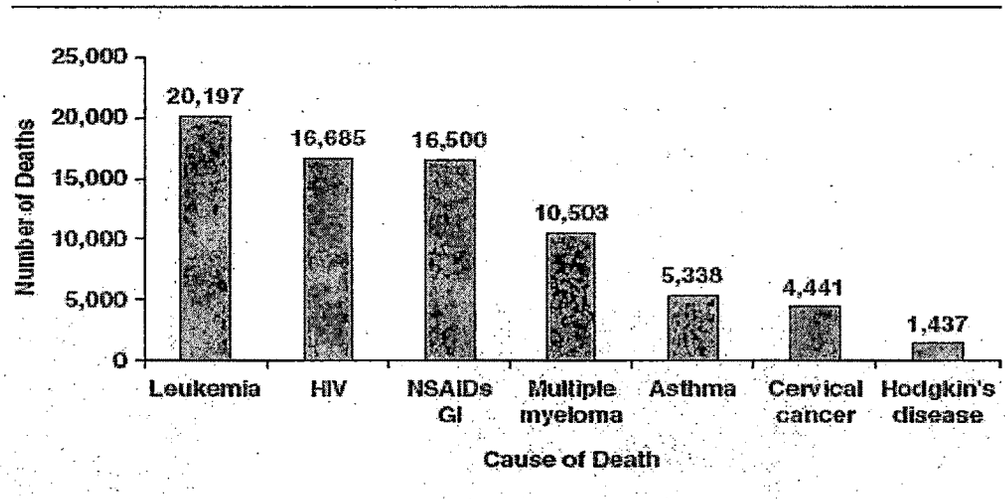
studies[75], *H pylori* infection in NSAID users was associated with a 3.53-fold increased risk for peptic ulcer disease above the risk associated with NSAID use alone. The use of NSAIDs and *H pylori* infection increased the risk for ulcer bleeding 4.85 fold and 1.79-fold, respectively; the risk for ulcer bleeding increased to 6.13-fold when both factors were present. Therefore, although both NSAIDs and *H pylori* independently confer increased risk, these findings also a synergistic interaction between these two factors, which leads to increased incremental risk. The highest relative risk for gastrointestinal events is associated with a history of complicated ulcer or multiple NSAID use (Table 6) [65, 67,76-78]. In one study, in which 1457 patients with a history of bleeding were compared with 10,000 control individuals, patients on multiple NSAID regimens were nine times more likely to experience upper gastrointestinal bleeding than were control individuals[79]. The correlation between multiple NSAID use and upper gastrointestinal bleeding should be placed in a broader perspective, not only because of the widespread use (and considerable under reporting) of OTC medications but also because patients do not recognize the potential complications that can develop with NSAIDs. A survey of more than 800 people found that approximately 65% said that they suffer gastrointestinal symptoms before the onset of a gastrointestinal event, despite considerable evidence that serious gastrointestinal complications occur in asymptomatic patients[80].

Many patients taking OTC NSAIDs and/or aspirin or other drugs do so without their physician's knowledge or approval, and are unaware of the increased relative risk for experiencing a gastrointestinal related event. This widespread use of NSAIDs underscores the importance of a thorough assessment of potential gastrointestinal risk in patients who are administered these agents for the management of pain and inflammation. Obtaining accurate information regarding concomitant medications from patients taking NSAIDs may also help to identify those at additional risk from concurrent multiple NSAID, anticoagulant, or corticosteroid use.

Table 6. NSAID use and GI complications in OA and RA patients

Gastrointestinal complications in osteoarthritis versus rheumatoid arthritis			
	OA hospitalizations	RA hospitalizations	RA deaths
Number of patients	1283	3883	2921
Person years of observation	3234	19,961	2,224
Person years taking NSAID	2199	15,638	8471
Number of GI events	19	228	25
Number of GI events while taking NSAID	16	205	19
Rates/year (%) while taking NSAID	0.73	1.31	0.22
Rates/year (%) while not taking NSAID	0.29	0.19	0.05
Relative risk while taking NSAID	2.51	6.77	4.21

GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug; OA, osteoarthritis; RA, rheumatoid arthritis. Reproduced with permission from



Deaths associated with NSAID induced gastrointestinal damage versus other causes. GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug. Data from Singh and Triadafilopoulos

Figure 16. NSAID induced GI damage deaths versus other causes.

Table 7. Risk factors for NSAID associate ulcer.

Risk factors for aspirin and NSAID associated ulcer complications, in order of relative importance	
Rank	Risk factor
1	Personal history of complicated ulcer disease
2	Concurrent use of more than one NSAID (including aspirin)
3	Use of high doses of NSAIDs
4	Concurrent use of an anticoagulant
5	Personal history of uncomplicated peptic ulcer disease
6	Age >70 years
7	Concurrent use of steroids

2.12 Assessment of gastrointestinal risk factors

It is imperative that clinicians carefully screen patients for risk factors for gastrointestinal complications due to NSAID use. Although dyspepsia is a frequent side effect of NSAIDs use, it is not – in contrast to common perception – an accurate predictor of gastrointestinal complications[66,67]. Approximately 15% of patients experience dyspepsia during NSAID therapy, but such symptoms correlate poorly with the severity of mucosal injury. As many as 50% of patients with dyspepsia have mucosa that appears normal on endoscopic examination. In fact, the majority of patients who develop gastrointestinal complications do so without any antecedent symptoms[81], further highlighting the importance of thorough risk assessment in patients receiving NSAIDs. Some advocates of *H pylori* screening recommend that *H pylori* eradication be considered in chronic NSAID users at average risk for gastrointestinal complications, as well as in chronic NSAID users at increased risk for gastrointestinal complications [82]. However, patients with gastric and duodenal ulcers may or may not have evidence of *H pylori* infection[83], and *H. pylori* eradication may reduce but not completely eliminate the risk for recurrent ulcers or complications[84]. Therefore,

this issue remains controversial. It should be noted that the period of greatest risk to an individual taking NSAIDs occurs during the first 3 months of NSAID therapy. Although the underlying mechanism for this is not well understood, one theory holds that gastric mucosa adapts to NSAID use over time[65]. However, clinical findings from a study in which 1600 individuals receiving NSAID therapy were followed for up to 15 years[66] demonstrated that the stomach does not adapt to NSAID use, and that the risk for complications continues over the long term[66]. Further elucidation of the mechanisms that are involved in early NSAID associated gastrointestinal events may help to ascertain which individuals are at greatest risk for early gastrointestinal events.

2.13 Comparison of gastrointestinal effects: aspirin, acetaminophen, and NSAIDs

The anti-inflammatory, analgesic, and antipyretic drugs are a heterogeneous group of compounds that share certain therapeutic actions and side effects, although they are chemically unrelated. Attempts to rank toxicity and efficacy have not been consistent. Earlier studies conducted by Henry and colleagues[85] established a comparative toxicity range for a select group of drugs (using ibuprofen as the reference comparator); for example, the relative risk (RR) for aspirin was found to be 1.6, for diclofenac it was 1.8, for naproxen it was 2.2, and for ketoprofen it was 4.2. A more recent nested case controlled analysis[86] showed that aspirin was associated with a RR of 2.9 for uncomplicated peptic ulcer, compared with a RR of 4.0 for nonaspirin NSAIDs. Low-dose aspirin and NSAIDs are among the most widely used drugs worldwide. Because of their anti-inflammatory and analgesic (NSAIDs) or antiplatelet (low-dose aspirin) effects, these drugs can benefit patients substantially but at the cost of increased risk for gastrointestinal complications. The benefits of low-dose aspirin in the prevention of myocardial infarction and vascular events are well established[87]. The American Heart Association recommends the use of low-dose aspirin (75–160 mg) in patients whose 10-year risk for a cardiovascular event is 10% or greater, except in persons at high risk for gastrointestinal bleeding or hemorrhagic stroke[28]. Long-term use of aspirin in the prevention of cardiac disease, especially

in elderly populations, is of increasing concern because it is widespread and is known to cause irritation and injury to the gastrointestinal tract [89]. Recent clinical findings suggest that no aspirin regimen is free from risk for upper gastrointestinal bleeding[90]. Even lose-dose aspirin is associated with a significant increase in upper gastrointestinal bleeding. In a Danish registry of 27,694 users of low-dose aspirin (100–150 mg) the incidence of upper gastrointestinal bleeding upon admission to the hospital was 2.6-fold greater than in the general population[90] . The risk for upper gastrointestinal bleeding (incidence ratio 5.6) is even greater among patients taking other NSAIDs in combination with aspirin and is not dose dependent[90,91]. In contrast to aspirin, the use of low-dose acetaminophen (<2000 mg) is not associated with an increased risk for upper gastrointestinal complications. There are limited data that suggest that doses greater than 2 g are associated with an increased risk for gastrointestinal bleed or perforation by a factor of 3.6[92]. *In vitro* analyses of human whole blood assays suggest that acetaminophen is a weak nonselective inhibitor of both COX-1 and COX-2[93].

Currently, the American College of Rheumatology (ACR) recommends acetaminophen as first-line treatment for OA of the knee or hip. This is largely because of the perception that acetaminophen is safer than NSAIDs[94,95]. However, the selection of treatment depends on a balance of factors, including efficacy, safety, tolerability, availability, cost, and patient acceptance. The recent emergence of coxibs has raised further questions regarding the role of selective NSAIDs in the treatment of OA. Until recently, few comparison data were available with which to evaluate the relative efficacy and safety of acetaminophen and NSAIDs. Data from clinical trials demonstrated that celecoxib and diclofenac are both superior to acetaminophen in the treatment of OA[96, 97]. Furthermore, a survey of 1799 patients found that the majority of patients with OA (>60%) prefer NSAIDs to acetaminophen in the symptomatic treatment of the condition based on perceived better efficacy[98]. However, newer data from several meta-analyses and pooled analyses evaluating the comparative efficacy and safety of NSAIDs versus acetaminophen indicate that, although NSAIDs are slightly more effective

in relieving pain, acetaminophen is associated with fewer adverse reactions and less frequent gastrointestinal discomfort than are NSAIDs. In a meta-analysis of seven clinical trials performed by Lee and colleagues[99], the efficacy and safety of NSAIDs, including coxibs, in the treatment of symptomatic hip and knee OA were compared with those of acetaminophen. Lee and coworkers determined that NSAIDs are statistically superior to acetaminophen in reducing walking and rest pain; however, NSAIDs were also associated with an elevated, but statistically insignificant, risk for withdrawals due to adverse events (odds ratio 1.45, 95% confidence interval [CI] 0.93–2.27).

Based on the results from another meta-analysis of 10 randomized controlled trials (n = 1712), in which patients with symptomatic OA of the knee, hip and knee, or multiple joints were evaluated, Zhang and colleagues[100] reported that acetaminophen was significantly more effective than placebo (effect size 0.21, 95% CI 0.02–0.41) but that NSAIDs were better than acetaminophen for pain relief (effect size 0.20, 95% CI 0.10–0.30), clinical response rate (RR 1.24, 95% CI 1.08–1.41), and symptom relief (as measured using Western Ontario and McMaster Universities Osteoarthritis Index scores). The number of patients who preferred NSAIDs was more than twice the number of patients who preferred acetaminophen (RR 2.46, 95% CI 1.51–4.12). Although NSAIDs exhibited superior efficacy in the metaanalysis conducted by Zhang and coworkers[100], the excellent safety profile of acetaminophen must be weighed against the therapeutic benefits of NSAIDs. In this metaanalysis, NSAIDs were associated with more frequent gastrointestinal discomfort, including abdominal pain, gastrointestinal distress, nausea, vomiting, and dyspepsia, than was acetaminophen (RR 1.35, 95% CI 1.05–1.75). It should be noted, however, that the studies examined in this metaanalysis included only short-term trials in which serious gastrointestinal events, such as bleeding or ulcer complications, were not evaluated. In balancing the efficacy and safety data for acetaminophen and NSAIDs, Zhang and colleagues concluded that acetaminophen at 4 g/day demonstrates significant efficacy for pain relief in OA, and that the current ACR guidelines that recommend acetaminophen as first-line treatment for OA are

supported by the evidence. Overall, however, acetaminophen is not adequate therapy for the majority of patients with daily OA pain. Acetaminophen is generally thought to be safer than NSAIDs, although the therapeutic index is narrow and a single acetaminophen overdose with twice the highest labeled dose is associated with life-threatening acute liver failure [101,102]. There have been few case reports of acetaminophen-induced acute liver failure at therapeutic doses [103]. In a prospective study of acute liver failure at 17 tertiary care centers in the USA[104], involving 308 patients with liver failure over the course of more than 3 years, liver failure due to acetaminophen overdose accounted for 39% of all cases. Comparatively, viral hepatitis (A and B), a common cause of liver failure, accounted for only 12% of cases. Although acetaminophen overdose has replaced viral hepatitis as the most frequent putative cause of acute liver failure, it remains extremely rare given the ubiquitous use of this OTC analgesic. The evidence for decreased gastrointestinal risk in patients receiving COX-2 is reviewed extensively elsewhere. However, the data presented here provide a context for discussion of the benefits and risks of these agents. Endoscopic studies, meta-analyses of serious gastrointestinal adverse events from clinical trials, and outcome studies have demonstrated the improved gastrointestinal safety of coxibs compared with most NSAIDs studied. Concerns over cardiovascular risk, however, have cast a shadow over the perceived overall safety profile of chronic coxib therapy. In addition, in patients with RA who are at risk for serious adverse gastrointestinal complications, the ACR recommends the use of one of the following[108] low-dose prednisone instead of an NSAID; a nonacetylated salicylate; a highly selective COX-2 inhibitor; or a combination of NSAID and a gastroprotective agent. A safety issue related to NSAID and coxibs safety that is not fully addressed in such guidelines, which are now 3 years old, is that low-dose aspirin (75–300 mg/day) for the prevention of stroke and myocardial infarction is increasingly common, especially in older patients. Because the concurrent use of aspirin with NSAIDs is common, the potential impact on safety should be weighed in patients receiving both selective and nonselective NSAIDs. Data available to date suggest that the potential

gastrointestinal safety benefit of coxibs compared with NSAIDs is lost with aspirin cotherapy.

2.14 Cardiovascular risk associated with COX-2 inhibitors

Before the development of COX-2 inhibitors, the risks associated with NSAIDs precluded robust study of such agents for various preventive indications. These indications have included prevention of cancer, Parkinson's disease and Alzheimer's dementia. The perceived safety of patented COX-2 selective inhibitors generated the financial and ethical support for long-term exposure in a setting in which it was ethical to conduct a comparison with placebo. Previously, only symptomatic conditions were studied, and so long-term placebo control was not possible. Results of placebo controlled, long-term clinical trials with over 2 years of exposure have indicated that there is cardiovascular risk associated with rofecoxib. Similar trials using celecoxib have been less consistent. Somewhat unexpectedly, the single long-term, placebo controlled study of a nonselective NSAID, namely naproxen, has been preliminarily reported as suggesting that there is cardiovascular risk with this agent, although a final report is not yet available. There have been at least seven large pharmacoepidemiologic studies on this topic published since 2001, with over 5 million person years of exposure. However, it is not clear whether all COX-2 selective agents entail cardiovascular risk, whether nonselective agents entail risk, or what effect dose and duration have on magnitude of risk [105]. The Food and Drug Administration has requested that all NSAID and COX-2 selective agents carry warnings that the product may increase the risk for cardiovascular events [106,107].

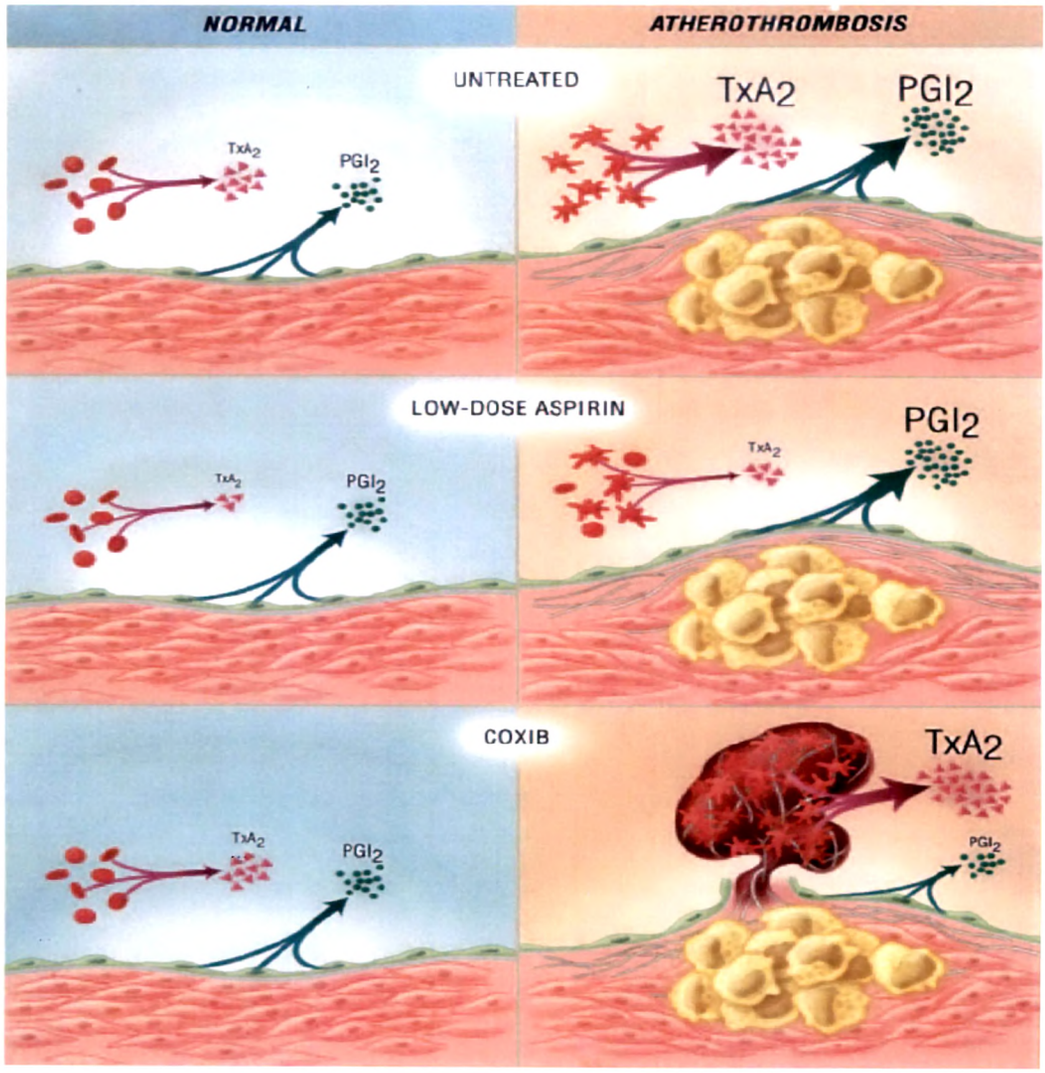


Figure 17. Consequences of COX inhibition for prostacyclin and thromboxane A2 production in normal and atherosclerotic arteries. Endothelial cells are shown as a source of prostacyclin (PGI2) and platelets as a source of thromboxane A2 (TxA2) under untreated conditions (top row) or treated with low-dose aspirin (middle row) or a coxib (bottom row) in the normal (left column) and atherosclerotic artery (right column) for comparison. COX-1 is the only isoenzyme expressed in platelets; endothelial cells express both COX-1 and COX-2. In the normal artery, the balance between PGI2 and TxA2 production favors PGI2 and inhibition of platelet-dependent thrombus formation. In the

atherosclerotic artery, both PGI2 and TxA2 production is increased, owing in part to increased platelet activation with compensatory PGI2 formation via both COX-1 and COX-2 in endothelial cell; the net effect is an imbalance favoring TxA2 production and platelet dependent thrombus formation. Low-dose aspirin selectively impairs COX-1–mediated TxA2 production in platelets restoring the net antithrombotic balance. Coxib use suppresses COX-2 dependent PGI2 production in endothelial cells, which has only a marginal effect on the net antithrombotic balance owing to the importance of COX-1 as a source of PGI2 in the normal state. In the setting of atherosclerosis, however, COX-2 plays a greater role as a source of PGI2 and more TxA2 is produced; thus, inhibiting COX-2 has a more profound effect on prostanoid balance, favoring TxA2 production and promoting platelet-dependent thrombosis.

Thus, it is important to reassess the ACR recommendations in the light of newer safety information. Co-use of NSAIDs and gastroprotective agents appears to be more attractive. Gastroprotective agents, which are considered to be effective in decreasing NSAID-associated gastrointestinal ulceration, include high-dose H2 blockers, PPIs, and the oral prostaglandin analog misoprostol. Although symptoms of dyspepsia often improve in patients treated with H2 blockers, their routine use is not recommended because of findings in 1921 patients from the ARAMIS cohort that prophylactic treatment with these agents may increase the risk for subsequent serious gastrointestinal complications. Numerous studies indicated that, when used in conjunction with NSAIDs, PPIs and the oral prostaglandin analog misoprostol significantly reduce gastric and duodenal ulcers in patients with and without a prior history of ulcers [108-112]. A recent study conducted by Chan and coworkers [113] raises the question regarding whether such a strategy is adequate in those who are at particularly high risk, based on a past history of upper gastrointestinal bleed. Despite these recent conflicting findings, the protective effects of these agents are well established [114]

Rofecoxib was withdrawn from the market following the findings of the Adenomatous Polyp Prevention On Vioxx (APPROVe) study.[115] This was a multi-centre, randomised, placebo controlled, double blind study designed to

determine whether or not rofecoxib prevented recurrence of colorectal polyps in patients with a history of colorectal adenomas. The study was terminated early due to the unexpected finding of a doubling of risk of CV events in the rofecoxib group compared with the placebo group (1.50 vs. 0.78 events per 100 patient years). This increased risk became apparent only after 18 months of treatment[55]. Previous studies may have missed this finding because outcomes were assessed at 1 year or less,[116,117] although a meta-analysis of randomised controlled trials and observational studies using rofecoxib found risk of MI to be increased after a few months of treatment[118,119]

The APPROVe study also found congestive heart failure and pulmonary oedema to be increased in the rofecoxib group, but this became evident at around 5 months. Use of rofecoxib is associated with a higher incidence of hypertension, peripheral oedema, and congestive heart failure compared with celecoxib and NSAIDs.[120-122]. This may contribute to the higher CV risk with chronic use of rofecoxib. Debate regarding the CV safety of COX-2 inhibitors arose in 2000 from the Vioxx Gastrointestinal Outcomes Research (VIGOR) study. This found a fourfold increase in risk of MI in patients taking rofecoxib (50 mg/day) compared to patients taking naproxen (incidence of MI, 0.4% vs. 0.1%).1 In contrast, the Celecoxib Long-term Arthritis Safety Study (CLASS) did not show a higher incidence of MI in patients taking celecoxib compared to patients taking diclofenac or ibuprofen. Differences in patient characteristics and concomitant use of aspirin were suggested to account for the differing findings between these studies.2 Rheumatoid arthritis (RA) patients have a higher CV risk and were included in the VIGOR study, while only patients with OA were enrolled in the CLASS study. Furthermore, 20% of patients in the CLASS study were using aspirin, while aspirin was not permitted in the VIGOR study. It was also suggested that naproxen, the comparator drug in the VIGOR study, may have a cardio protective effect, but more recent studies suggest that this is not the case[123,124].

2.21 CV risks with COX-2 inhibitors and NSAIDs

There is mounting evidence that increased CV risk is seen with other COX-2 inhibitors and may indeed be seen with traditional NSAIDs. The Adenoma Prevention with Celecoxib (APC) study was similar to the APPROVe study both in design and the decision to terminate early because of increased risk of CV events[65]. Death from CV causes, MI, stroke, or heart failure was higher in the groups taking celecoxib; 200 mg twice per day and 400 mg twice per day compared to the placebo group (7.8 and 11.4 vs. 3.4 events per 1000 patient years). Like the APPROVe study, this increased CV risk became apparent only after at least 12 months of treatment. Furthermore, both studies found increased CV risk with increasing doses, which supports findings from previous studies.[125] However, whether CV risk is increased with celecoxib is still debated; it has been suggested that rofecoxib has the higher risk.[126] A case controlled study with 1.4 million patients in Kaiser Permanente Health Insurance Scheme, California, found relative risk (95% confidence interval) from MI or sudden cardiac death for celecoxib 0.77 (0.60–0.99), naproxen 1.11 (0.96–1.30), rofecoxib 25 mg 1.02 (0.71–1.46), and rofecoxib >25 mg 5.04 (0.94–27.06).[126]

Evidence for CV risk with meloxicam is more limited. In a British cohort of patients, users of rofecoxib were found to have a higher incidence of cerebrovascular events compared to users of meloxicam (0.48% vs. 0.27%; relative risk 1.68), but not in CV events.[127] There were similar findings when comparing celecoxib with meloxicam (incidence of cerebrovascular events 0.39% vs. 0.27%; relative risk 1.66).[128] However, the cohort size was small, data on concomitant use of aspirin was incomplete, and there was no comparison made with patients using traditional NSAIDs or on no treatment.

Intravenous parecoxib and/or oral valdecoxib given postcoronary artery bypass graft surgery has been found to increase the risk of CV events. Myocardial infarct, cardiac arrest, stroke, and pulmonary embolism were more frequent among the patients given parecoxib and valdecoxib than among those given placebo (2.0% vs. 0.5%; relative risk 3.7). This study also highlights the

risk of using COX-2 inhibitors in high CV risk patients, these patients being excluded from previous randomised controlled trials.[129]

As demonstrated by the APPROVe and APC studies, large long term trials are required in order to answer the CV question. However, pooled data from several smaller studies is all that is available for valdecoxib and etoricoxib, with no significant increase in CV events found.[130] More recently however, the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) was a large study designed to look at CV risk with lumiracoxib. Patients with OA were randomised to take lumiracoxib, naproxen or ibuprofen, with the primary endpoint being MI, stroke, and CV death. There was no difference in CV events when lumiracoxib was compared with naproxen and ibuprofen, irrespective of aspirin use. Of clinical significance, the gastrointestinal benefits of lumiracoxib were lost when used together with aspirin, as has also been demonstrated with celecoxib in the CLASS study.

Traditional NSAIDs have also been implicated. Recent case control studies suggested an increased risk of MI with the use of diclofenac, ibuprofen, Indomethacin, sulindac, piroxicam, and meloxicam.[131] The highest risk was associated with the use of Indomethacin, with adjusted odds ratio (95% confidence interval) of 1.71, 1.35–2.17. Although these observational post marketing cohorts are useful to investigate the use of medications in 'real life' practice, further prospective studies designed to answer the CV question for NSAIDs are required.

2.22 How might COX-2 inhibitors and NSAIDs increase CV events?

2.221 Pro-thrombotic vascular environment

Selective inhibition of COX-2 could theoretically predispose to a pro-thrombotic vascular environment. Cyclo-oxygenase-1 (COX-1) and COX-2 isoenzymes catalyse the conversion of arachidonic acid to prostaglandins. COX-1 is the main source of production of thromboxane A-2 (TXA-2) which mediates platelet aggregation and vasoconstriction. COX-2 is the main source of prostacyclin (PGI-2) which has vasodilating, anti-aggregatory and antiproliferative effects. Therefore, selective inhibition of COX-2 causes suppression of PGI-2

without affecting TXA-2, and theoretically could predispose to hypertension and thromboembolic events.[132] Higher COX-2 selectivity and longer half life of rofecoxib compared to celecoxib has been proposed as the reason for higher CV risk associated with rofecoxib. However, this is probably less important given that etoricoxib and lumiracoxib have several fold higher COX-2 selectivity, and etoricoxib a longer half life than rofecoxib.

2.222 Renal impairment and hypertension

Both COX-2 inhibitors and NSAIDs predispose to renal impairment, hypertension and peripheral edema, and it is possible that these may contribute to increase CV risk.[133,134] Rofecoxib is associated with a higher incidence of hypertension, peripheral edema, and congestive heart failure compared with celecoxib and NSAIDs. It has been suggested that the higher rates of hypertension seen with rofecoxib may be related to its metabolism by cytoplasmic reductases. The other four COX-2 inhibitors are metabolised via the cytochrome P450 enzymes. Vasoactive hormones, including aldosterone, are also metabolised by cytoplasmic reductases, so it is plausible that rofecoxib competitively inhibits metabolism of these hormones.[135]

2.223 COX-2 inhibitors and CV risks

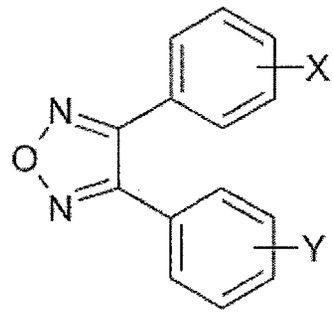
There are theoretical mechanisms and some evidence that COX-2 inhibition may have a beneficial effect on vascular endothelial function. Inhibition of COX-2 may decrease vascular inflammation, mononuclear cell infiltration, improve nitric oxide availability, enhance plaque stability, and decrease atherosclerosis progression.[136] Improvements in abnormal vascular endothelial function with celecoxib have been shown in patients with hypertension and with coronary artery disease already taking aspirin and statins.[137,138] Furthermore, in patients with coronary artery disease, high sensitivity C-reactive protein (HsCRP) and oxidised-LDL (Ox-LDL) decreased after treatment with celecoxib.[138] Improved vascular endothelial function has not been found in studies using rofecoxib[139-141] or parecoxib.[142] However, it is likely that mechanisms which increase thrombogenicity outweigh any beneficial effects on vascular endothelial function.

2.3 AIM OF THE PRESENT WORK

The main mechanism of action of NSAIDs is the inhibition of the enzymes possessing cyclooxygenase (COX) activity, which are involved in the formation of prostaglandins and thromboxanes from arachidonic acid contained in cellular membranes[62]. Prostaglandins, in addition to being most important factors of the inflammatory reactions, also exert specific physiological functions in several organs, the kidney and gastro-intestinal system in particular, inhibition of their synthesis is not devoid of side effects[80]. The major drawback of NSAIDs which limits its use is inhibition of the COX-1 which causes gastric ulceration. The discovery of two enzymatic isoforms of COX, COX-1 and COX-2, encoded by two different genes, has generated much hope of developing a more specific and safe approach in the treatment of inflammatory diseases[81], but that was only partially fulfilled since emerging information has challenged some aspects of the original hypothesis and the clinical studies have indicated only a relatively small improvement in the tolerability in GI system but simultaneously it increases CVS risks in several fold, the main reason behind the withdrawal of Rofecoxib-the potent cox-2 inhibitor from the market by Merck, USA in 2004. There are mounting evidences[17,18] that increased CVS risk is seen with other COX-2 inhibitors and may indeed be seen with traditional NSAIDs. Both COX-2 inhibitors and NSAIDs predispose to renal impairment, hypertension and peripheral edema.

Looking to the toxicities of existing anti-inflammatory drugs it is desirable to have new anti-inflammatory drug which will inhibit the prostaglandins and take care of above complications. The present study was aimed to explore the biochemical, toxicological and anti-inflammatory studies of newly synthesized compounds and some herbal drugs. Much care has been taken while designing and synthesis of the novel compounds to reduce the side effects e.g. through introduction of NO-donor group in the basic structure been found to be improved in terms of efficacy and safety. It has been hypothesized that replacement of less acidic or non acidic group in basic structure also helps to reduce the GI toxicity.

The scientific base behind the synthesis of 1,2,5 oxadiazole compounds and their N-oxides(Indian Patent . No. 109/MUM/2004)



1,2,5- oxadiazole

A wide variety of carbocycles and heterocycles can serve as templates for COX-2 inhibitors, *i.e.*, cyclopentene [SC-57666] [143] pyrazole [celecoxib [144], SC-58125[145]], furan [rofecoxib[146]], isoxazole [valdecoxib[147], paracoxib sodium[148]], and pyridine [etoricoxib[149]]. In an ongoing research program in our department on COX-2 inhibitors with improved biological profile, we synthesized [150] a series of 3,4-diaryl-1,2,5-oxadiazoles and 3,4-diaryl-1,2,5-oxadiazole *N*-oxides. The compounds are novel in that the diaryl heterocyclic (five membered ring) pharmacophore of the coxibs has been incorporated with the nitric oxide releasing group (1,2,5-oxadiazole *N*-oxide) into one single entity in the compounds synthesized. 1,2,5-oxadiazole *N*-oxides (furoxans) are reported [151] to be thiol dependent NO(Nitric oxide) donors, whose biological activity is produced by action on the soluble guanylate cyclasecyclic guanosine monophosphate (sGC-cGMP) pathway. Furoxans are considered to possess favorable bioactivity since they cause a slow release of NO resulting in longer duration of action without development of tolerance. Granik and Grigor [152] proposed the mechanism for the release of NO from 1,2,5-oxadiazole *N*-oxides. It is also reported that release of NO from a nitric oxide donor drug produces beneficial effects such as reduction in blood pressure and prevention of atherosclerosis[151]. NSAIDs possessing nitric oxide releasing capabilities are considered to be more promising drugs than coxibs as these would be devoid of potential cardiovascular side effects associated with coxibs[151]. Recently, a report has been published

discussing the synthesis of some monosubstituted 3,4-diaryl-1,2,5-oxadiazoles and *N*-oxides as selective COX-2 inhibitors, expecting them to be free from adverse cardiovascular effects [153], but we claimed synthesis of these compounds much earlier [150].

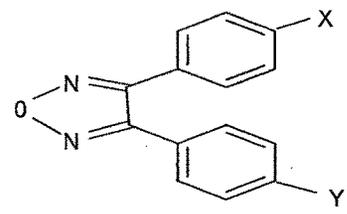
Looking to the gastrointestinal toxicity of NSAIDs and CVS complications of available COX-2 inhibitors, man kind is in need of safer and effective anti-inflammatory drug. To fulfill this need we tried to develop new drugs from three different sources, source 1 was to develop the preferential COX-2 inhibitor with nitric oxide moiety to reduce side effects, source 2 was to develop organo-metallic complex of Curcumin and vanadium metal, third was to try some herbal drugs extracts which have been used ancient time but their detailed pharmacology has not been done. The present thesis is an effort to develop safe and effective anti-inflammatory drug, a step forward to gift mankind the painless life.

The main aim of the present work was to evaluate 93 synthetic 1,2,5- oxadiazole compounds and 1 semi synthetic BCOV (Bis[Curcumino] oxovanadium) (synthesized in our medicinal chemistry division) and 9 herbal drugs for their preliminary biochemical studies (COX-2 and COX-1 *in vitro* inhibition assays). These compounds and herbal drugs which showed more inhibition of COX-2 and less inhibition of COX-1 were taken further for acute anti-inflammatory, chronic anti-inflammatory and toxicological evaluations including safety pharmacological studies.

2.31 Newly synthesized anti-inflammatory compounds

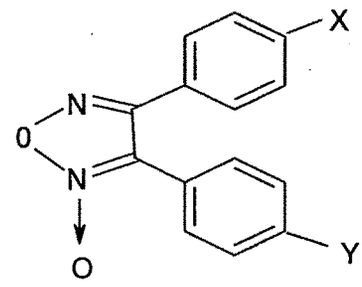
Taking care of above aspect Medicinal Chemistry Division, Pharmacy Department, M S University of Baroda, have synthesized following Compounds.

A)



X= -H, -Cl, -Br, -F, -CH_3, -OCH_3 , -NH_2, -NHAc, -$NHSO_2Me$, -NO_2, -SOMe, -SH, -SO_2NH_2,
Y = -H, -NO_2, -OCH_3

B)



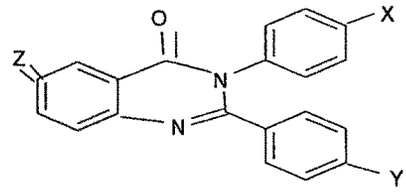
X= -H, -Cl, -Br, -F, -CH_3, -OCH_3 , -NH_2, -NHAc, -$NHSO_2Me$, -NO_2, -SOMe, -SH, -SO_2NH_2
Y = -H, -NO_2, -OCH_3

C)

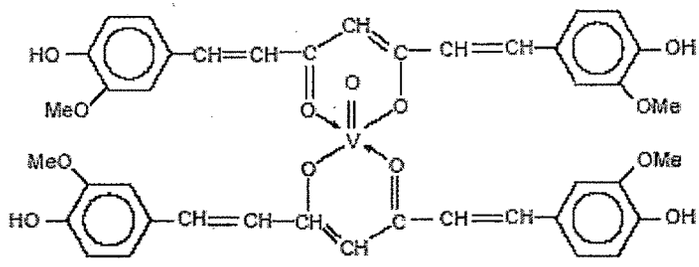
X= -H, -Cl, -Br, -F, -CH_3, -OCH_3 , -NH_2, -NHAc, -$NHSO_2Me$, -NO_2, -SOMe, -SH, -SO_2NH_2

Y = -H, -NO_2, -OCH_3

Z= -Cl, -Br, -NO_2, -NH_2, -NHAc, -$NHSO_2Me$



2.32 Vanadium complex with Curcumin- The organometallic complex



Structure of bis[curcumino]oxovanadium (BCOV).

As advancement in the synthesis of organometallic complexes, we have chelated the vanadium centre with Curcumin. Curcumin, an active constituent of curcuma longa, has been reported to have very good anti-inflammatory, antipyretic and analgesic activity. Furthermore, this complex have been reported for its anti dieabetic activity[154]. **Indian patent no. : 918 / MUM / 2001**

The concept of combining vanadium with a Curcumin ligand to form a compound that exhibits synergistic activity is appealing.

2.33 Herbal drugs

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can provide therapeutic effects. Several herbs can help to reduce high blood cholesterol level, reduce pain and inflammation provides protection against cancer, and stimulates the immune system. Furthermore, a diet in which culinary herbs are used generously to flavor food provides a variety of active phytochemicals that promote health and protect against chronic diseases. The discriminate and proper use of some herbal products is safe and may provide good therapeutic benefits. Herbal drugs may answer for chronic inflammatory condition like arthritis and inflammatory bowel disease because they are safe and effectively cure the disease from root level on long term treatment.

Following herbal extracts were obtained as a gift sample from Cherian Chemicals Baroda, India, and were tested for the various anti-inflammatory, safety pharmacology and toxicity testing models.

- **Banaba-*Lagerstroemia speciosa L*— leaf extract(BNB)**
- **Ashwagandha- *Withania Sominifera*-root extract**
- **Lodhra- *Symplocos racemosus*-root extract**
- **Sariva- *Hemidesmus Indicus*-root extract**
- **Arjuna- *Terminalia arjuna*-Bark extract**
- **Pomegranate- *Punica Granatum*-Fruit extract(PME)**
- **Bitter Melon- *Memordica Charantia*- fruit extract**
- **Tulsi- *Ocimum sanactum*- leaf extract**
- **Wheatgrass extract- *Elytrigia dasystachya*- whole plant extract**

2.33.1 Banaba-*Lagerstroemia speciosa L*— leaf extract

Lagerstroemia speciosa (Lythraceae), a southeast Asian tree more commonly known as Banaba, have been traditionally consumed in various forms by Philippinos for treatment of diabetes and kidney related diseases[155]. Scientists have identified different components of banaba to be responsible for its activity.

Using tumor cells as a cell model, corosolic acid was isolated from the methanol extract of banaba and shown to be an active compound. The ellagitannin Lagerstroemin was identified as an effective component of the banaba extract responsible for the activity. Penta-*O*-galloyl-glucopyranose (PGG) was identified as the most potent gallotannin. A comparison of published data with results obtained for PGG indicates that PGG has a significantly higher glucose transport stimulatory activity than Lagerstroemin. PGG exhibits anti-adipogenic properties in addition to stimulating the glucose uptake in adipocytes. The combination of glucose uptake and anti-adipogenesis activity is not found in the current insulin mimetic drugs and may indicate a great therapeutic potential of PGG.

2.33.2 Ashwagandha- *withania somnifera*-root extract

In Ayurveda ashwaganda is considered a rasayana herb, a herb that works on a nonspecific basis to increase health and longevity. This herb is also considered an adaptogen which is a nontoxic herb that works on a nonspecific basis to normalize physiological function, working on the HPA axis and the neuroendocrine system.[156] The roots and berries of the plant are used in herbal medicine. Ashwagandha in Sanskrit means "horse's smell", probably originating from the odor of its root which resembles that of sweaty horse. The species name *somnifera* means "sleep-bearing" in Latin, indicating it was considered a sedative, but it has been also used for sexual vitality and as an adaptogen. Some herbalists refer to ashwagandha as Indian ginseng, since it is used in ayurvedic medicine in a way similar to that ginseng is used in traditional Chinese medicine.

Seven American and four Japanese firms have filed for grant of patents on formulations containing extracts of the herb Ashwagandha. Fruits, leaves and seeds of the Indian medicinal plant withania somnifera have been traditionally used for the Ayurvedic system as aphrodisiacs, diuretics and for treating memory loss. The Japanese patent applications are related to the use of the herb as a skin ointment and for promoting reproductive fertility. The U.S based company Natreon has also obtained a patent for an Ashwagandha extract.

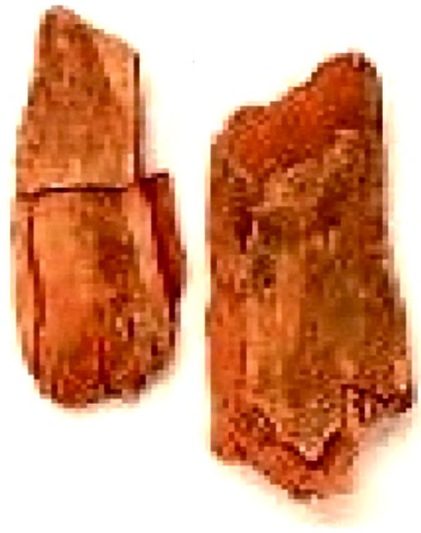
2.33.3 Sariva- *Hemidesmus Indicus*-root extract

Indian Sarsaparilla (*Hemidesmus indicus*) is a species of plant that is found in South Asia. It is used to make beverages and also used in traditional medicine. In Ayurveda it goes by the name of *ananthamoola* or *Anantmula*. It is also called the False Sarsaparilla. The plant enjoys a status as tonic, alterative, demulcent, diaphoretic, diuretic and blood purifier. It is employed in nutritional disorders, syphilis, chronic rheumatism, gravel and other urinary diseases and skin affections.[157] It is administered in the form of powder, infusion or decoction as syrup. It is also a component of several medicinal preparations.

Banaba-*Lagerstroemia speciosa L*

Ashwagandha- Withania Sominifera

Sariva- ***Hemidesmus Indicus***



Arjuna- ***Terminalia arjuna***

Pomegranate- ***Punica Granatum***

Bitter Melon- ***Memordica Charantia***

Wheatgrass- *Elytrigia dasystachya* **Tulsi- *Ocimum sanactum***

Chemical analysis of the root showed the presence of coumarins, volatile oil the chief component of which is p-methoxy salicylic aldehyde, two sterols and a pregnane glycoside.[157]

2.33.4 Arjuna- *Terminalia arjuna*-Bark extract

Terminalia arjuna is a medicinal plant of the genus *Terminalia*, widely praised & used by ayurvedic physicians for its curative properties in organic/functional heart problems like angina, hypertension, deposits in arteries etc. According to ayurvedic texts it also very useful in the treatment of any sort of pain due a fall, ecchymosis of all types, spermatorrhoea & sexually transmitted diseases like gonorrhea. Arjuna Bark (*Terminalia arjuna*) is thought to be beneficial for the heart.

Research suggests that Terminalia is useful in alleviating the pain of angina pectoris, and in treating heart failure and coronary artery disease. Terminalia may also be useful in treating hypercholesterolemia[158]. The cardioprotective effects of terminalia are thought to be caused by the antioxidant nature of several of the constituent flavonoids and oligomeric proanthocyanidins, while positive inotropic effects may be caused by the saponin glycosides. In

addition to cardiac effects, Terminalia may also be protective against gastric ulcers, such as those caused by NSAIDs.[159]

2.33.5 Pomegranate- *Punica Granatum*-Fruit extract

The Pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree growing to 5–8 m tall. The pomegranate is native to the region from Afghanistan, Pakistan, and Iran to the Himalayas in northern India and has been cultivated and naturalized over the whole Mediterranean region and the Caucasus since ancient times.

In preliminary laboratory research and human pilot studies, juice of the pomegranate has been found effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation, all of which are steps in atherosclerosis and cardiovascular disease. Tannins such as punicalagins have been identified as the primary components responsible for the reduction of oxidative stress which led to these risk factors.[160] Pomegranate has been shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme (ACE).[161]

Other research indicates that pomegranate juice may be effective against prostate cancer and osteoarthritis.[162] In 2007, six clinical trials in the United States, Israel and Norway have been approved to examine the effects of pomegranate juice consumption on parameters of prostate cancer or prostatic hyperplasia, diabetes or lymphoma.[162]

2.33.6 Bitter Melon- *Momordica charantia* - fruit extract

Momordica charantia is a tropical and subtropical vine of the family cucurbitaceae, widely grown for edible fruit, which is among the most bitter of all vegetables. English names for the plant and its fruit include bitter melon or bitter gourd, Bitter melon is also a demulcent and at least mild inflammation modulator, however, means that it rarely does have these negative effects, based on clinical experience and traditional reports.

Laboratory studies have confirmed that various species of bitter melon have anti-malarial activity, though human studies have not yet been published. Some claim bitter melon as "a cure for diabetes", although outside of anecdotal

stories scientific evidence for this claim is limited. Studies so far demonstrate improvement but not cure in some diabetic parameters.[163,164] It is widely grown in South and Southeast Asia, China, Africa, and the Caribbean.

2.33.7 Tulsi- *Ocimum sanctum*- leaf extract

Tulsi extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria. Traditionally, tulsi is taken in many forms: as an herbal tea, dried powder, fresh leaf, or mixed with ghee. Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes and in herbal toiletry. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects.

Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its significant amount of eugenol (1-hydroxy-2-methoxy-4-allylbenzene).[166] Studies have also shown Tulsi to be effective for diabetes, by reducing blood glucose levels.[167]. Tulsi also shows some promise for protection from radiation poisoning and cataracts.[168]

2.33.8 Wheatgrass

Wheatgrass refers to the young grass of the common wheat plant, *Triticum aestivum* that is freshly juiced and dried into powder for animal and human consumption. Both provide chlorophyll, amino acids, minerals, vitamins, and enzymes. Claims about wheatgrass' health benefits range from providing supplemental nutrition to having unique curative properties. Some consumers grow and juice wheatgrass in their homes. It is often available in juice bars, alone or in mixed fruit and/or vegetable drinks. It is also available in many health food stores as fresh produce, tablets, frozen juice and powder.

Proponents of wheatgrass claim regular ingestion of the plant can

- improve the digestive system
- prevent cancer, diabetes and heart disease
- cure constipation
- detoxify heavy metals from the bloodstream
- cleanse the liver

- prevent hair loss
- help make menopause more manageable
- promote general wellbeing.

There are limited but growing evidence in support of some of the above claims.[170,171,172]