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RESEARCH ARTICLE

STUDY COMPARISON EFFECT OF A NEW NAPROXEN MODULATED BY ADDITION OF ACTIVE GROUP AND A NAPROXEN ON THE GASTROINTESTINAL SAFETY AND ANALGESIC EFFICACY IN MALE MICE

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ABSTRACT

The first step of the research is the synthesis of a new agent derived from This is done by its conversion into phenyl hydrazine. At this point a number of products may be derived; addition of fructose results in the formation of X23, addition of D-ribose results in the formation of X3, whilst addition of C6H5CHO results in the formation of F57. Furthermore, addition of NaOH produces X2) Our study set out to investigate the analgesic activity of Naproxen (20 mg/kg body weight) and to compareit toa newly modified compound of naproxen by the addition of a chemical group. Analgesic activity done by use of hot plate. Healthy male albino mice (25-30 g) were obtained from animal houses (??) in groups of six in polypropylene cages. After adaptation the mice were randomly divided into six groups (six rats in each group) Group 1labeled as the control group, was given distilled water only, whilst mice from group 2 where given naproxen and 7-6(?? you mean 3-5) given new drugs. Modified naproxen compounds and naproxen drugs significantly ($p<0.001$) increase analgesic time per second (pain reflex) when compared to control. The Hot plate test useful in the elucidating centrally mediated ant nociceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.001$) reduced the pain as compare to the control group. The results of pharmacological tests performed in the present studies suggest that all new drugs possess potent analgesic activity. The second aim of our study was to carry out an investigation on the effect of new drugs(you mean the modified naproxen drugs?) on gastric tissue, the result show mice administer with X3 showed less ulcerative effect than naproxen by decrease acidity, and increase gastric juice than naproxen (do you mean; by decreasing acidity and increasing gastric juice as compared to naproxen). This finding may be grounds for further future research. (Examination of the existence of gastric ulcer in mice stomach under dissecting microscope revealed increase number and length of ulcer in mice administrated with alcohol. Groups administered with naproxen, X2, F57 also showed evidence of ulceration however less compared to groups administered with alcohol. Moreover number of ulcers where significantly less in X23 group, while X3 treated animal showed no evidence of ulcers or hemorrhage seen in stomachs after examined under dissecting microscope). Histopathological sections of stomach mice treated orally with 250mg /kg of naproxen,X2, F57 groups and X23 for 5 days show severe distractions of stomach mucosa, necrosis of intestinal villi with inflammatory cell infiltration in lamina properia and severe ulceration and necrosis of intestinal mucosa with congestion of blood vessels. The result of the X3 group was most prominent and revealed small intestine increase in number of goblet cell with increase amount of mucin seen in the lumen with mononuclear cell infiltration with lymphoid tissue that give good chance for increase immune tissue and resistant ulcer.

***Citation*:** Adnan. M Jassim, Sadiaq, A. Almansory, Sohaad J. Hadi, Ghasson A. Alkareem, 2015. "Study comparison effect of a new naproxen modulated by addition of active group and a naproxen on the gastrointestinal safety and analgesic efficacy in male mice", *International Journal of Current Research,* 7, (12), 23675-23687.

INTRODUCTION

Analgesic and anti-inflammatory drugs relieve pain, and reduces inflammation and fever. These agents are effective for pain joints, muscle and headache, but they are not effective in removing pain of visceral organs.

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This is due to potent inhibiting prostaglandins substances that are responsible for sensation to pain and inflammation as well as regulation of body temperature. High dose of drugs or prolonged use for long periods of time increase the riskforulcers to develop, gastric bleeding and gastro-duodenal injury (Wallace, 2008). Clinical treatment used for preventing NSAID- injury on gastro duodenal damage by traditional drugs as proton pump inhibitor such as omeprazole has been shown to effectively reduce gastro-duodenal damage (Wallace, 2012).

But recent animal studies suggest that suppression of acid secretion can lead to exacerbation of NSAID-induced small intestinal injury and bleeding **(**Scheiman**,** 2006 Wallace *et al*., 2011). There are several clinical studies that indicated intestinal damage in healthy volunteers taking NSAIDs with proton pump inhibitors (PPI's); one study showing increase elevation of a marker (which marker ?) of intestinal inflammation in patients taking PPIs (Fiorucci *et al*., 2002 and Kearney *et al*., 2006). Therefore it is important to evaluate the safety and efficiency of novel naproxen inmice that more closely resemble the patients who will be the major users of these drugs. New drugs should also be evaluated in combination with parent naproxen in regards to analgesia exacerbate induced GI damage. This is necessary in order to obtain more insight into the potential GI safety of drugs intended for use as treatments of inflammatory conditions.

NSAIDs= *Non-Steroidal Anti-Inflammatory* Drugs GI=

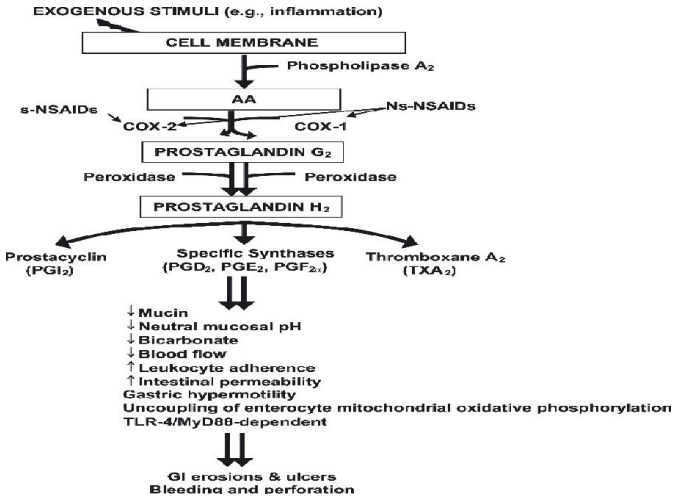


Figure 11. Effect of non steroidal anti-inflammatory drugs on Cycloo xygenase and prostaglandine with their effect on gastrointestinal tract function

Naproxen is (+)-2-(6-methoxy-2-naphthyl)-propionic acid. Its molecular formula is C14H14O3 and it has a molecular weight of 230.3. It's one of nonsteroidal anti-inflammatory drugs (NSAIDs) belong of propionic acid derivatives .

Mechanism of Action

Naproxen is a phenylpropionic acid derivative having analgesic effect, anti-inflammatory and antipyretic effect. Such effect is thought to be mediated by inhibition of the enzyme complex prostaglandin synthetase with consequent reduction in the synthesis of prostaglandins from arachidonic acid. Naproxen consider mild side effect than aspirin *and indomethacin. The mechanisms by which nonsteroidal anti-inflammatory* drugs (NSAIDs) induce gastrointestinal ulcer and bleeding is by destruction of the tissue surface barrier to gastric acid and cytotoxic effect on cell, on the other hand NSAIDs decrease *defense mechanism such as* Mucus, bicarbonate layer Blood flow, cell renewal, Prostaglandins, Phospholipids, Free radical scavengers. The mucus gel layer of the stomach and other part of the GItract possess non-wet table hydrophobic properties due to the synthesis and secretion of surfactant-like phospholipids, Lichtenberger *et al*., 2007).

Another study done on naproxen formulated with phosphatidylcholine Naproxen-PC show much lower GI injury and bleeding in two rodent model systems while Invariably anti-inflammatory and COX-inhibitory activity (Lichtenberger *et al*., 2008) (Lichtenberger *et al*., 2007). The addition of material assistance to the foundation material may alter the effectiveness of the qualities and material basis. Change may be a positive direction and thus reduces the harmful effect on GIT with potentiate analgesic and ant inflammatory properties (Solomon *et al*., 2005, Nussmeier *et al*., 2005, Zarraga and Schwarz, 2007).

Our program of maintaining the surface barrier of the GI tract, is quite different from the traditional way possessed by the pharmaceutical industry over the past contractin the development coxibs act on COX2 stimulate GI injury primarily by inhibiting constitutive COX-1 and that is away to exhaust the tissue of "cytoprotective" prostaglandins (Jüni *et al*., 2002, Budenholzer, 2002). Farkouh *et al*., 2007) indicates that NSAID results in minimum cardiovascular risk and provides extreme effective relief for arthritic patients. However since naproxen, especially at arthritic doses, is significantly toxic to the GI (Chan, 2006), our study aimed to improve Naproxen by increasing its analgesic effect and correct its side effects by modifying the parent naproxen by adding new compounds for formulation new drugs.

MATERIALS AND METHODS

Synthesis of new compound from naproxen

184//5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-4*H*-1,2,4-triazole-3-thiol A mixture of (0. 6g, 0.002 mole) of 182, and, 25 mL of NaOH (%4),was refluxed for (3) hrs. The reaction was checked by TLC. Using hexane: ethyl acetate (1.2), then it was added to cold water, was filtrated, HCl was added to filtrated solution and waited to give crystal which was recrystallised.

- Yield: (0.43gm), 71% ; m.p. = 260°C
- I.R(KBr): 3240 cm^{-1} (NH), 3077cm^{-1}(CH) *arom*, 1637 cm^{-1} (C=N),1604 cm^{-1} (C=C), 1164cm^{-1}(C=S),1288 cm^{-1} (C-O) asym.,1072 cm^{-1}(C-O)sym
- Anal. Calc. for $C_{15}H_{15}N_3OS$ (285.36): C 63.13, H 5.30, N 14.73.
- Found: C 62.95, H 5.38, N 14.51%

190- (E)-2-(6-methoxynaphthalen-2yl)-N-(2, 3, 4, 5-etrahydoxypentylidene) propanehydrazide

A mixture of 186 (0.3g, 0.00124mole), ribose sugar (0.195g, 0.0013 mole) in ethanol (20mL) was refluxed for 6 hrs. Then cooled, filtered and solvent removed under reduced pressure. The resulting solid was recrystallised from ethanol

- Yield: (0.268gm), 88% ; m.p.=88 -90 °C.
- I.R(KBr) : 3379cm^{-1} (OH) ,3209 cm^{-1}(NH),3062cm^{-1} (arom. CH), 2931 cm^{-1}(CH) aliph., 1656 cm^{-1} (C=O),1650 cm^{-1} (C=N). Anal. calc. for $C_{19}H_{24}N_2O_6$ (376.40):C 60.63; H 6.43; N 7.44
- Found: C 60.42; H 6.31; N 7.18% 194

(*E*)-2-(6-Methoxynaphthalen-2-yl)-*N*'-(1,3,4,5,6-pentahydroxyhexan-2-ylidene)propanehydrazide

A mixture of 186 (0.3g, 0.00124mole), fructose sugar (0.234g, 0.0013 mole) in ethanol (20mL) was refluxed for 6 hrs. Then cooled, filtered and solvent removed under reduced pressure. The resulting solid was recrystallised from ethanol

- Yield: (0.45gm) , 87% ; m.p.= 183-185 °C
- I.R(KBr) : 3379cm^{-1} (OH), 3301 cm^{-1}(NH) , 2931 cm^{-1}(CH) aliph., 1658 cm^{-1} (C=O),1635 cm^{-1} (C=N)
- Anal. calc. for $C_{20}H_{26}N_2O_7$ (406.43): C 59.10; H 6.45; N 6.8
- Found: C 58.78; H 5.89; N 6.68%

Animals

Thirty healthy albino male mice with weights that range from 25-30 grams were obtained from the animal house of Baghdad university. The mice where split into six groups (5 mice per group), and then placed in polypropylene cages. The animals were maintained at room temperature and under environmental conditions (approximately an 8-12hrs light and dark cycle). All the animals were acclimatized for 10 days to the animal house conditions prior to the start of experimental protocol. Pellets where mice's given diet, as wellas water *add libitum*.

Experimental I

After 7 days of adaptation the mice were randomly divided into six groups as follows:

- Group 1: Negative represent control group, this group was given distilled water only
- Group 2: After fasting overnight, ratsreceived single dose of drug (20 mg/kg) of Naproxen for analgesic effect.
- Group 3: After fasting overnight, rats were received (20 mg/kg) of X2 for analgesic effect.
- Group 4: After fasting overnight, animals were received (20 mg/kg) of F57 for analgesic effect.
- Group 5: After fasting overnight, animals were received (20 mg/kg) of X23 for analgesic effect.
- Group 6: After fasting overnight,animals were received 20 mg/kg of X3 for analgesic effect.

Hot plate method

The paws of mice are sensitive to heat induced by hot plate at temperatures 55°C to 60 °C but is resistant to skin damage. The hot plate, consists of an electrically heated surface. The standard drugs and other tested drugs were given orally after 60 minutes prior to the experiment, the animals are placed on the hot plate and then observations were recorded.

The temperature should not exceed 60 °C. Mice where placed on the hot plate by using cylinder small size. We recorded the time when mice responded to heat, which was suggested from licking, jumping or withdrawal of paws. Food and water was withdrawn nearly 12 hours prior to drug administration until completion of the experiment.

Experimental II (induced ulcers)

After 7 days of analgesic experiment mice of the same groups where given a high dose of naproxen and other chemical agent of alteration naproxen. Group 1served as control group and received vehicle distilled water, 1 ml/kg orally), group 2 received naproxen250mg/kg body weight, orally for 5 days other groups 3-6 received modified naproxen at 250mg/kg body weight, orally respectively for the period of 5 days. Mice were deprived of food, but not water, for 24h prior to the experiment. On the 6th day, 1 hour after the respective treatments the mice where anaesthetized with ketamine (80 mg/kg, intra-peritoneal).

Tissue sample preparation

Rats had been killed using overdose of ether, the abdominal wall was incised longitudinally. The stomach was then isolated and separated from other surrounding viscera by means of two incisions: The first was carried out proximally to the cardiac sphincter and the second was carried out distally to the pyloric sphincter of the stomach. After which the stomach was isolated and slightly inflated by injection of formalin through esophageal opening.

Then the inflated stomach was immersed in 10% neutral formalin for 10-15 mints for fixation of both the inner and outer layers of gastric wall. The stomach was opened along the greater curvature, rinsed briefly under continuous tap water then the mucosa was examined to determine the ulcer parameters by means of dissecting microscope.

Acidity of gastric juice

pH degree of gastric juice was estimated after collection of all juice by using pH meter apparatus.

Determination of gastric juice volume

Gastric juices were centrifuged at 500 rpm for 10-12 minutes, then separated and measured volume by use of insulin syringe (Smeeta and Subhash, 2013).

Histopathological examination

The stomachs of the scarified mice were taken and wash with normal saline then immersed in 10% formalin solution. The fixed specimens were then trimmed, washed anddehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded inparaffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain forexamination of the stomach as described by Carleton, (1979).

RESULTS

Different superscript letters in the same column indicate the presence of high significant differences below the level of probability 0.0001

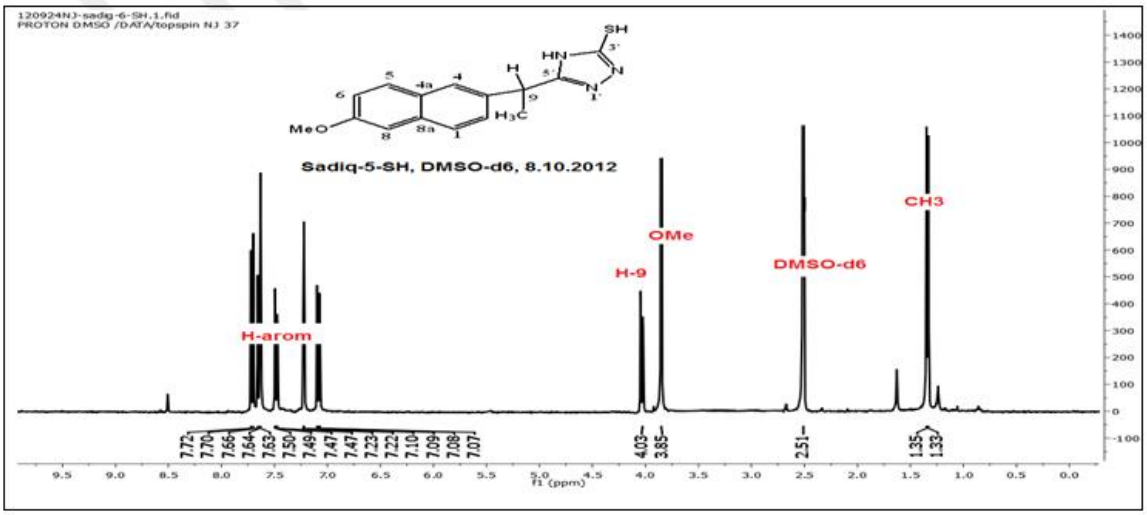


Figure1.1. NMR spectrum of the proton of the derivative

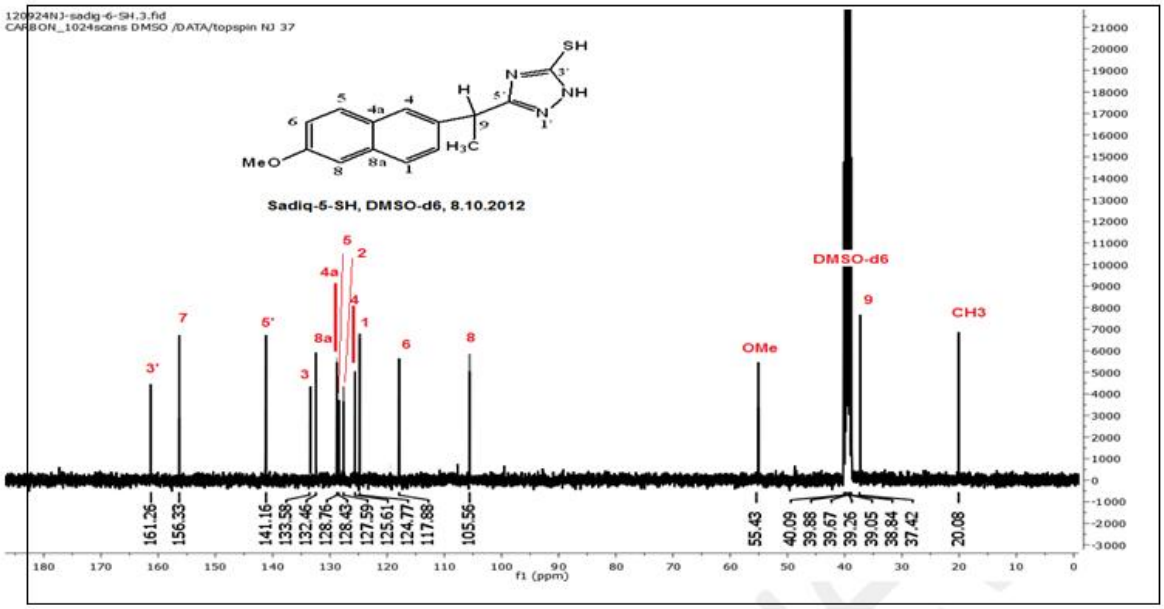


Figure1-2. Shows the NMR spectrum of the derivative X2 at 13- Carbone atom

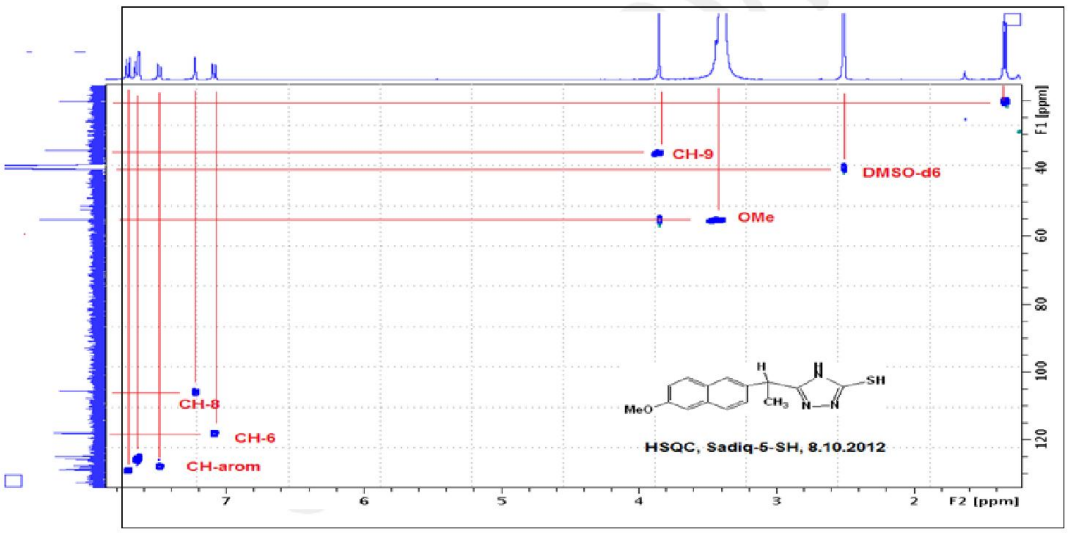
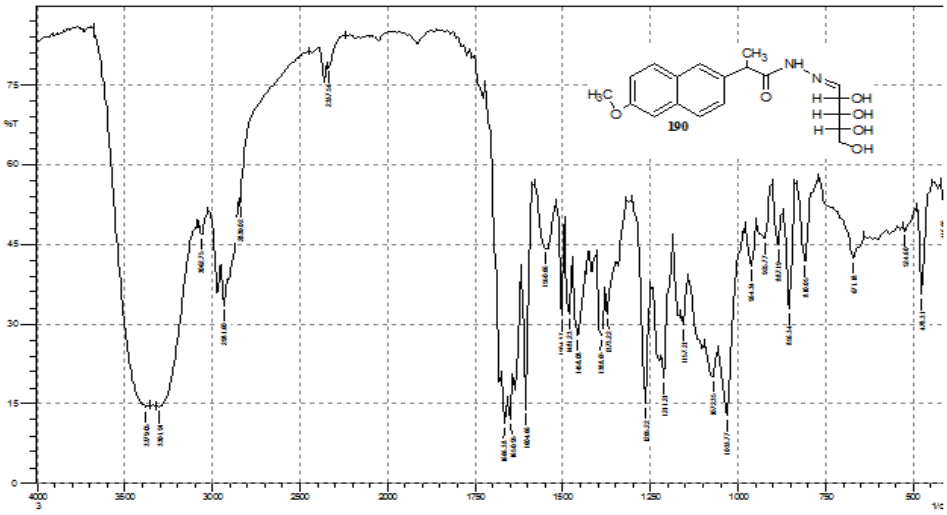


Figure 1.3. HSQC spectrum of the derivative X2 showed



(Figure 1−4) shows the FT−IR spectrum for

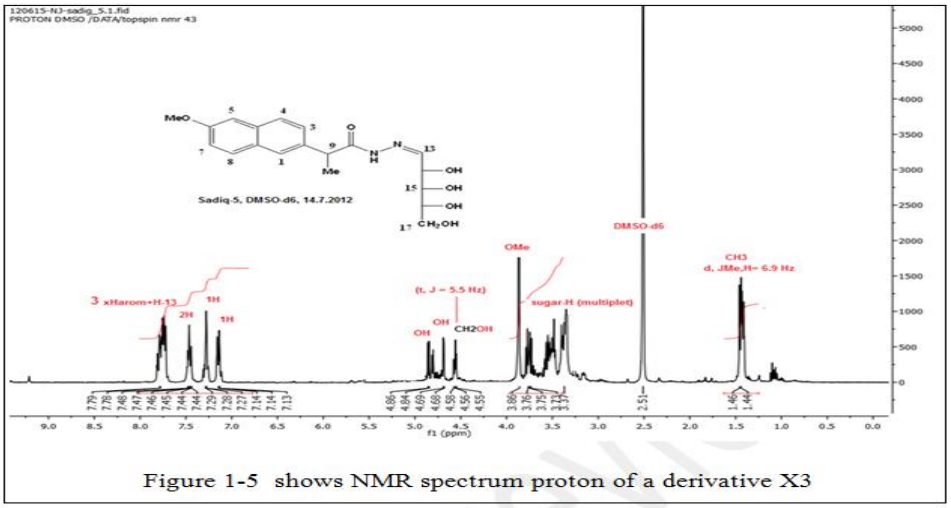


Figure 1-5 shows NMR spectrum proton of a derivative X3

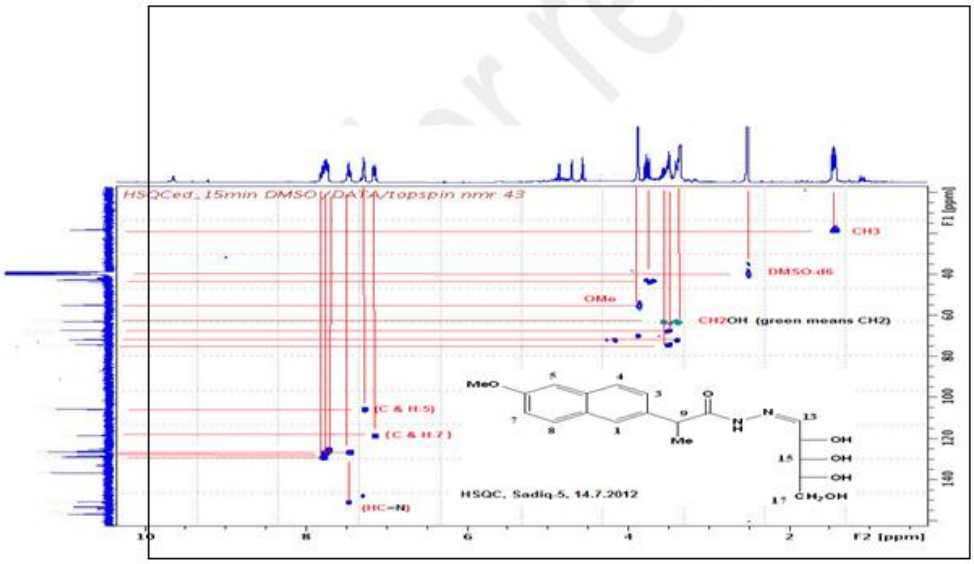


Figure 1.6. Explain HSQC spectrum of the derivative $_{X3}$

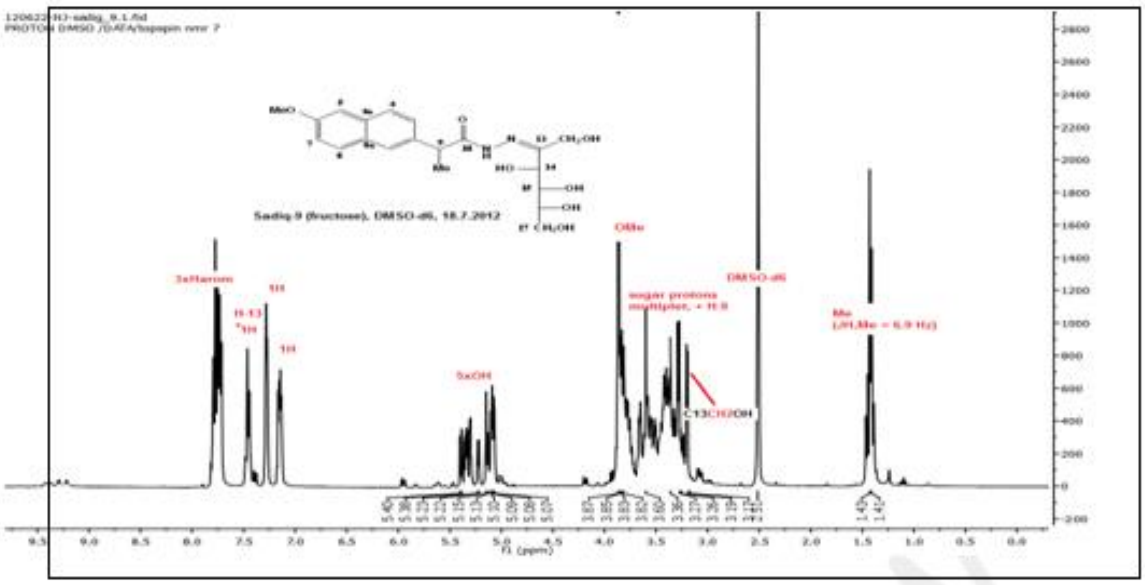


Figure1.8. Illustrates the NMR spectrum of nuclear Magnetizes of derivative X23

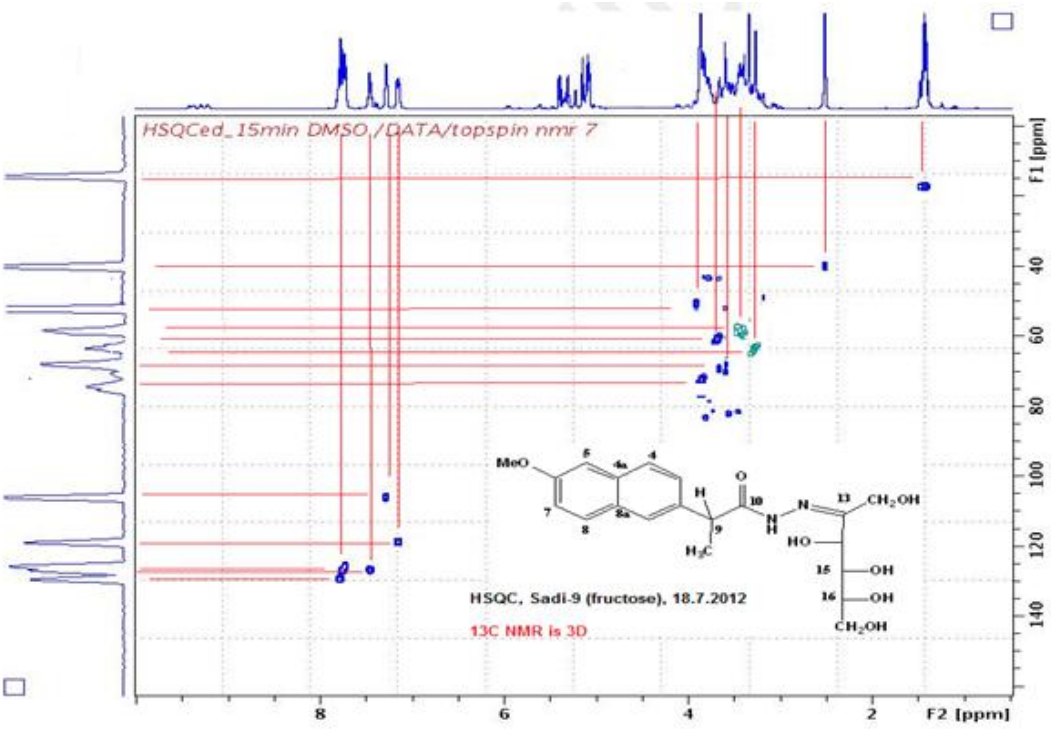


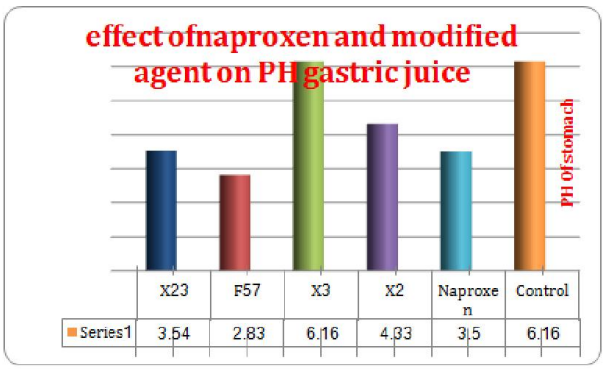
Figure 1.9. Illustrates the spectrum of HSQC for derivatives X23

Table 1. Evaluate Analgesic action by Hot Plate Method in male mice that orally given (Standard – naproxen (20 mg/kg), and other chemical agent of alteration naproxen at dose 20mg/kg orally

Treatment	Rate Pain reflex before analgesia in second	Pain reflex after analgesia in second	Gastric juice volume in ml	PH of stomach
Control	6.00 ± 0.51 A	6.66 ±0.88 D	1.00±0.04 A	6.16 ±0.30 A
Naproxen	6.33±0.71 A	25.83± 0.65 B	0.65±0.09 B	3.50 ±0.34 CB
X2	6.00 ±0.51 A	22.83±1.13 CB	0.60 ±0.08 B	4.33 ±0.21 B
X3	6.00 ±0.57A	43±4.62 A	0.98±0.05 A	6.16 ±0.40 A
F57	6.00 ±0.57A	26.00±1.39 B	0.37± 0.03 C	2.83± 0.30 C
X23	6.66 ±0.56 A	17.66±0.71C	0.40 ±0.03 C	3.54 ± 0.34 CB

Different superscript letters in the same column indicate the presence of high significant differences below the level of probability 0.0001

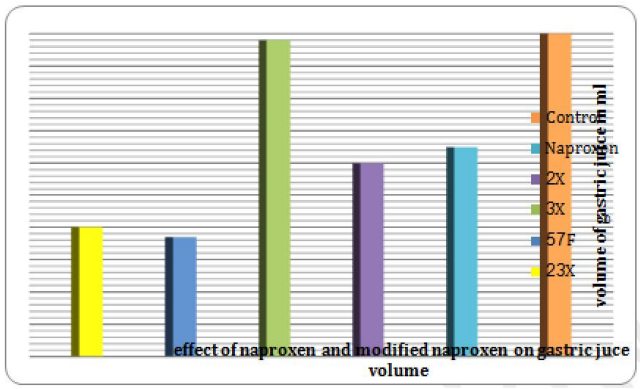
pH of gastric juice



Values of pH in mice treated with standard naproxen and different agent of modified naproxen in Table (1). Results demonstrated that control group show normal range at level 6.16 ±0.30.

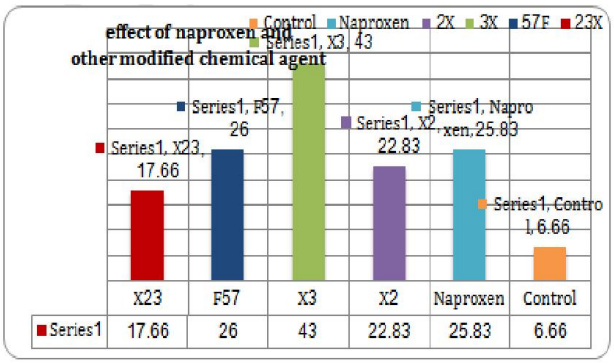
While groups given orally different agent of modified naproxen had significant decrease in pH value of gastric juice at $p<0.0001$ in X2 group (4.33 ±0.21) , F57 groups which reach at level very low (2.83± 0.30) and X23 also PH value decreased to(3.54 ± 0.34) . On the other hand group X3 Show value (6.16 ±0.40) near with control and there is no significant as compared with control at $p<0.0001$ and there is significant difference between X3 and other groups.

Volume of gastric juice in stomach



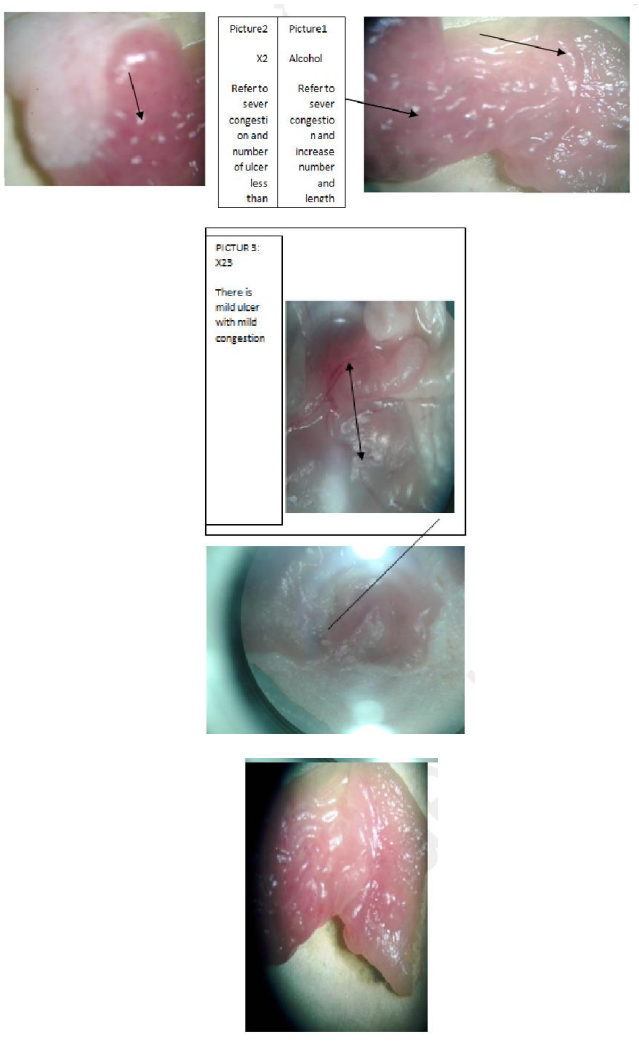
Volume of gastric juice (cm^3) in mice with standard naproxen and different agent of modified naproxen in Table (1). Results demonstrated that control group show normal range at level. The results showed that volume of gastric juice (cm^3) as mean±SE of group given orally (control group) was not significant decrease (1.00±0.04) at $p<0.0001$ as compared to naproxen other group X2, F57, X23 Respectively (0.65±0.09, 0.60 ±0.08, 0.37± 0.03 and 0.40 ±0.03).

Mice given orally X3 at a dose of 250mg/kg of b.wt. had a significant increase in volume of gastric juice to level (0.98±0.05) as compared to Mice given other chemical agent same dose, on the other aspect X3 reveal result degree very near from control and there is no significant differences between X3and control.

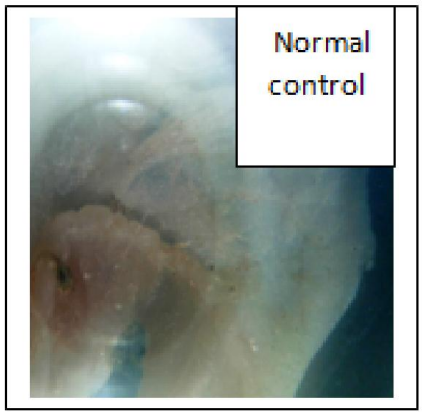


Analgesia activity of naproxen and other agent

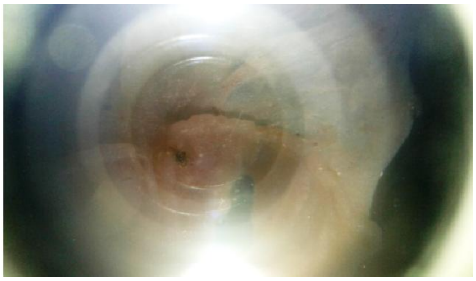
Animal produced paw licking and paw jumping in the control group, and *Animal* at all doses used in the study significantly inhibited the jumping and licking response in mice. Naproxen at 20mg/kg dose was significantly reduced the paw licking and paw jumping response (25.83± 0.65) when compared to the control (6.66 ±0.88). Other groups of agent also reveal degree of inhibition of pain for X2, F57, X23 Respectively, X3 treated show significant differences when compared to standard naproxen ,that analgesia degree reach to 43±4.62.



F57. Picture 5. there is sever congestion with multi ulcer area



PICTURE 6. X3 Showed very mild change



Naproxen showed sever ulceration and congestion picture 4

Macroscopically picture of stomachs after examined under dissecting microscope for determination of gastric ulcers

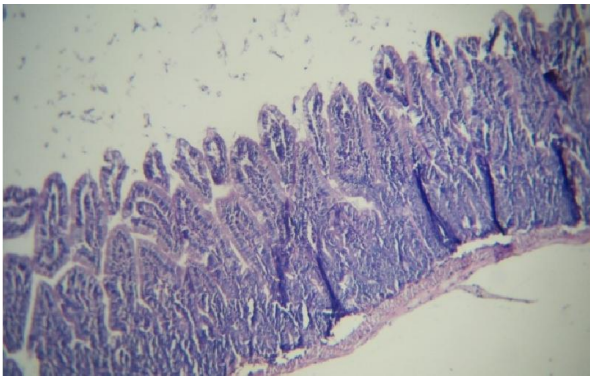


Figure 1. Histopathological section for control group ofmice small intestine showing the normal histological stricture. (H&E 400X)

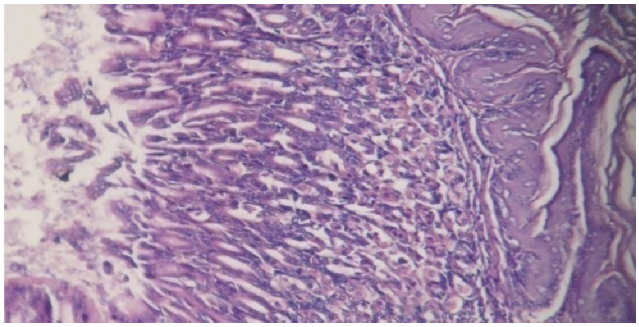


Figure 2. Histopathological section for control group of mice stomach showing the normal histological stricture. (H&E 400X)

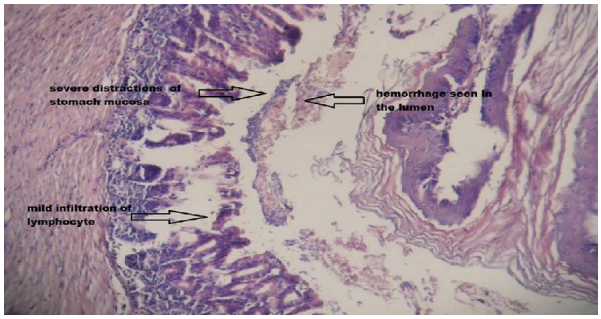


Figure 3. Histopathological section of stomach of mice treated orally with250 mg /kg of x23 for 5 day showing severe distractions of stomach mucosaand hemorrhage seen in the lumen with mild infiltration of lymphocyte. (H&E 400X)

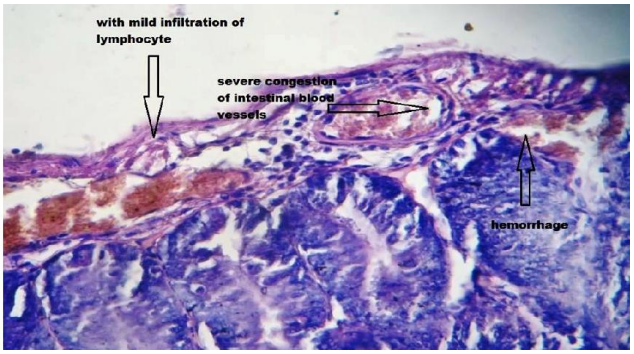


Figure 4. Histopathological section of small intestine of mice treated orally with250 mg /kg of x23 for 5 day showing severe congestion of intestinal blood vessels and hemorrhage with mild infiltration of lymphocyte (H&E 400X)

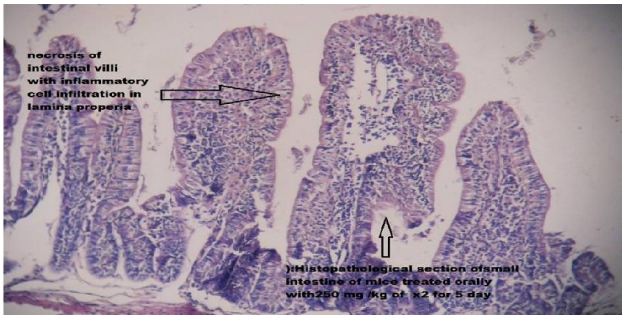


Figure 5. Histopathological section of small intestine of mice treated orally with250 mg /kg of x2 for 5 day showing necrosis of intestinal villi with inflammatory cell infiltration in lamina properia (H&E 400X)

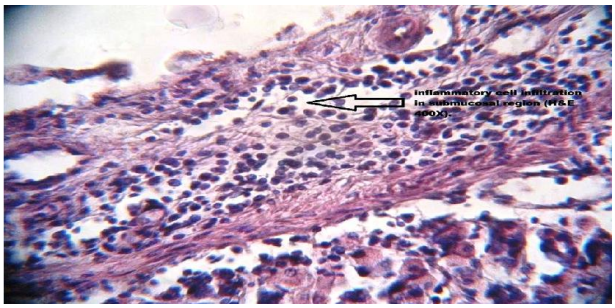


Figure 6. Histopathological section of stomach of mice treated orally with 250 mg/kg of x2 for 5 day for glandular region showing inflammatory cell infiltration in submucosal region (H&E 400X)

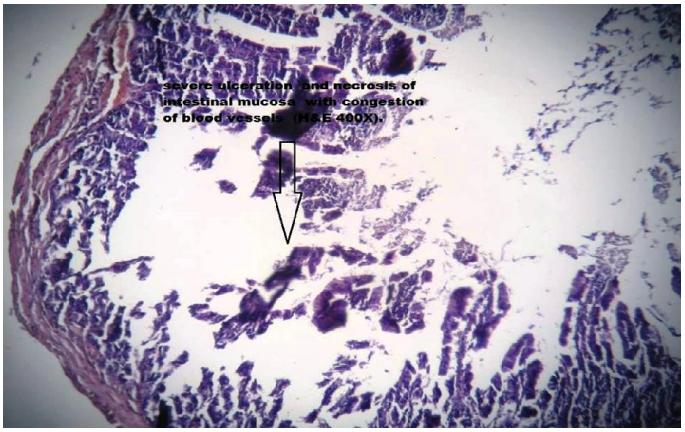


Figure 7. Histopathological section of small intestine of mice treated orally with 250 mg /kg of naproxine for 5 day f showing severe ulceration and necrosis of intestinal mucosa with congestion of blood vessels (H&E 400X)

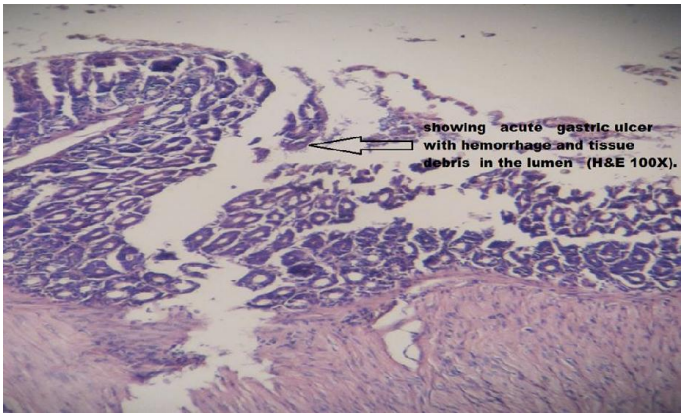


Figure 8. Histopathological section of stomach of mice treated orally with 250 mg /kg of naproxine for 5 days showing acute gastric ulcer with hemorrhage and tissue

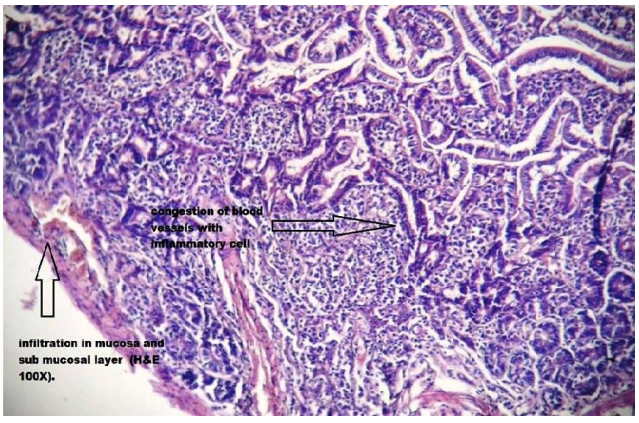


Figure 9. Histopathological section of small intestine of mice treated orally with 250 mg /kg of f57 for 5 daysshowing congestion of blood vessels with inflammatory cell infiltration in mucosa and sub mucosal layer (H&E 100X)

DISCUSSION

Our study was aimed to evaluate the efficacy of new synthetic chemical agents derived from naproxen by addition of chemical groups to standard naproxen.

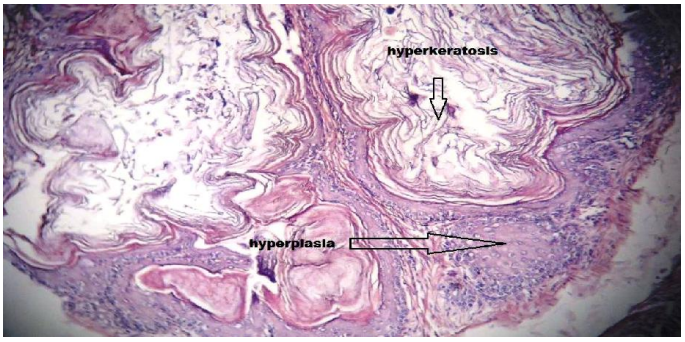


Figure 10. Histopathological section of stomach of mice treated orally with 250 mg /kg of f57 for 5 days for non -glandular region showing hyperplasia and hyperkeratosis (H&E 100X)

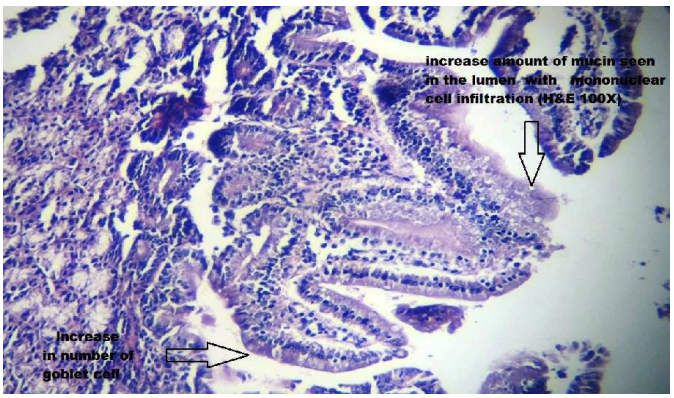


Figure 11. Histopathological section of small intestine of mice treated orally with 250 mg /kg of x3for 5 days showing increase in number of goblet cell with increase amount of mucin seen in the lumen with mononuclear cell infiltration (H&E 100X)

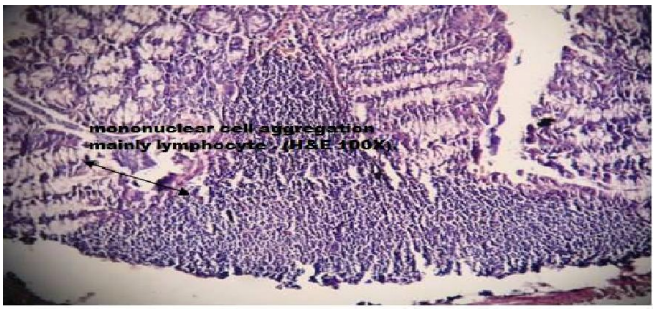


Figure 12. Histopathological section of small intestine of mice treated orally with 250 mg /kg ofx3 for 5 days showing mononuclear cell aggregation mainly lymphocyte . (H&E 100X)

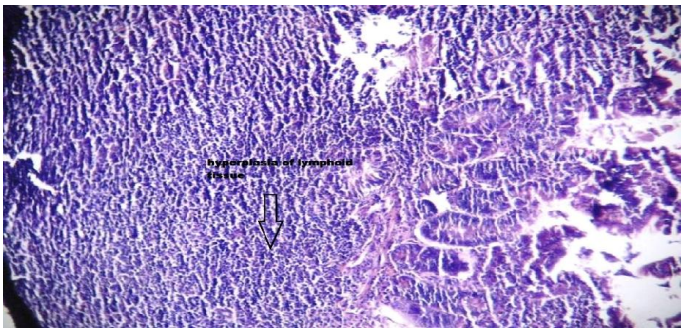


Figure 14. Histopathological section of small intestine of mice treated orally with 250 mg /kg of x3 for 5 days showing hyperplasia of lymphoid tissue. (H&E 100X)

By modifying the parent drug we hoped to generate new drugs that display properties that are more medically preferable than standard naproxen. Those being, the ability to prolong sedation and act as better good anti-inflammatory drugs with minimum side effects than the pure drug. The results (Table 1) demonstrated that there is no significant differences between all animal groups before analgesia. Our results show all formulations of drugs have analgesic activity similar or better than naproxen after induction analgesia and there is no significant differences between naproxen, X2and F57 (25.83± 0.65 , 22.83±1.13, 26.00±1.39) at $p<0.0001$. X3 group proved to be highly effective with better analgesic activity than naproxen to reach 43.±4.62.

Our data indicates that the formulation agent increasing analgesic activity was in agreement with (Lichtenberger *et al*., 2009) that indicate Naproxen Phosphatidylcholine (Naproxen-PC) revealed significantly anti-inflammatory and COX-inhibitory activity and less GI injury and bleeding in two rodent model systems. The results discussed above suggest that believed COX inhibitory activity may be greater for modified Naproxen than parent naproxen, it may have been highly effective to reduce pain sensation through potent inhibition of arachidonate cyclo-oxygenase and thus inhibition of the production of prostaglandins. There are two types cyclo-oxygenase (COX1 and COX2) that naproxen act on nonselective manner by inhibit both of them (Walter, 2010; Modi *et al*., 2012). Prostaglandins sensitize the nociceptive afferent nerve terminal to the mediator of pain. In the presence of PGE pain will be felt even with concentration of inflammatory mediator bradykinin that are too low to cause pain on their own. Naproxen are mainly active against this type of pain in which prostaglandins act to sensitize nociceptor via pain associated with tissue damage or inducer by heating (Wilcox *et al*., 2005; Boros *et al*., 2013). X3 group 6-methoxynaphthalen-2yl)-N-(2,3,4,5-tetrahydoxypentylidene).

Propanehydrazide, is produced by conversion of naproxen to phenyl hydrazine then addition D-ribose, that conversion may be increase potency of chemical compound leading to increase it activity. Prostaglandins and leukotrienes have important potentiate role in inflammation and pain (Melinda *et al*., 2013). The role of PG in pain through direct acting on G-protein – coupled R (via increase CAMP/PKA) to potentiate the tetrodoxine that restract Na channel and to produce pain. On the other hand leukotrienes stimulate leukocyte to produce sensitization of nocieptor (Boden *et al*., 2002; Koca, 2009). Mahendra *et al*., 2006 indicated that combination of 5-lipoxygenase inhibitors with naproxen potentiate the ant nociceptive effect of naproxen, reduce therapeutic dose and reduce side effect on GIT, Heart and renal that are popularly associated with NSAID. The new formulation of naproxen, especially X3 may be an addition to the inhibitor effect on COX, it may inhibit 5-lipoxygenase .Peptic ulcer is a common disease. Its incidence rates is about 10-12%. Pathogenesis of ulcers are produced when any factor causes an imbalance between the protective factors (Mucus, bicarbonate layer, Blood flow, cell renewal, Prostaglandins, Phospholipids and Free radical scavengers) and aggressive factors (Acid, pepsin, H .pylori, Bile salts and Drugs (NSAIDs) in the stomach (Del *et al*., 2003; Ojewole, 2004; Shaima *et al*., 2012). Our results demonstrated there is a sharp alteration in permeability gastric mucus through significant decrease volume of gastric juice (cm^3) in naproxen other group X2,F57,X23 respectively (0.65±0.09, 0.60 ±0.08 , 0.37± 0.03 and 0.40 ±0.03) while mice given orally X3 at a dose of 250mg/kg of b.wt. had a significant increase in volume of gastric juice to level (0.98±0.05) as compared to mice given other chemical agents same dose, on the other hand X3 revealed results where very close to that of the controls and there was no significant differences between X3 and control. Disturbances in gastric secretion, alteration in permeability, decrease of protective layer gastric mucin and the generation of free–radicals are identified as the pathogenic effects of pylorus ligation and naproxen-induced ulcer (Kandhare *et al*., 2011; Kandhare *et al*., 2012; Smeeta and Subhash, 2013). Radi and Khan, 2006 indicated in their study that orally used NSIADs cause GI erosion, ulcers, bleeding and in sever cases perforation occur at high dose that effect result from decreased gastric juice secretion, blood flow, increase acidity, leukocyte infiltration, and loss of these GI protective mechanisms.

The compound 6 methoxynaphthalen2yl) N (2,3,4,5tetrahy doxypentylidene) propanehydrazide advantages have good analgesic and not do not induce formation of ulcer sat high doses. This may be because of its inhibitory effect on gastric acid secretion or 'cytoprotective' activity by promoting local cell regeneration to maintain mucosal blood flow and increase local production of mucus. The result of PH parameter level where recorded as 6.16 ±0.40 this proves no existence of ulcer formation and results where very near to results seen by the control group and there is no significant as compared with control (6.16 ±0.30) at $p<0.0001$. There is significant difference between X3 and other groups. The Histopathological observations in this study showed no pathological changes in stomach tissue of control group mice. Negative groups in contrast to the stomach from ulcer induced group showed severe changes ulceration and hemorrhage.

Histopathological section of stomach of mice treated orally with 250 mg /kg of X23 for 5 days showed severe distractions of stomach mucosa. Hemorrhage was also observed in the lumen, along side mild infiltration of lymphocyte. Also there appeared severe congestion of intestinal blood vessels and hemorrhage (Figure 3 ,4) *.(E)-2-(6methoxynaphthalen-2-yl)-N-(1,3,4,5,6-pentahydroxyhexan-2-ylidene)propanehyrazide* has potent side effects on the gastrointestinal tract ,these side effects might be give as precaution to avoided orally uses .

5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thiol which represent group X2; showing necrosis of the intestinal villi along side inflammatory cell infiltration in lamina properia as well as infiltration of submucosal region in glandular prortion (Figure 5,6). Histopathological section of small intestine of mice treated orally with 250 mg /kg of naproxen for 5 days showed severe destruction of stomach represented by ulceration, necrosis of intestinal mucosa, congestion of blood vessels, in addition to acute gastric ulcer with hemorrhage and tissue debris in the lumen (Figure 7,8). These effects explain severity of naproxen in high doses potency of analgesic effect of chemical compound F57 is

high, histological study revealed congestion of blood vessels with inflammatory cell infiltration in mucosa and sub mucosal layer ,in addition to hyperplasia and hyperkeratosis of non - glandular region of stomach (Figure 9,10). The importunacy of chemical agent.

*(X3) :190- (E)-2-(6-methoxynaphthalen-2yl)-N (2,3,4,tetrahy doxypentylidene)propanehydrazide***,** have shown to have potent analgesic effect more than that of Naproxen and all other modified formulation. It has a slight effect on gastrointestinal area represented in the histopathological section of small intestine showing increase in number of goblet cell with increase amount of mucin seen in the lumen with mononuclear cell infiltration and mononuclear cell aggregation (mainly lymphocyte),with stimulation of immunity of area by hyperplasia of lymphoid tissue. This study reveals the difference in the severity of mucosal lesions that ranged from superficial lesions to that extending through the mucosa associated with alteration in the concentration of total serum antioxidant after exposure to salicylic acid with ethanol (Shaima *et al*., 2012).

Our results are in agreement with Elliott *et al*., 1988 who showed that administration orally of a single dose of naproxen of 250 mg/kg to rats lead to death in a period of seven days and abdominal adhesions, and small intestine necrotic foci accompanied with congestion and hemorrhage were observed when histopathological sections where analyzed .Peptic ulcers are resulted due to increase production of gastric acid and/or decrease in gastric mucosal protection mechanisms. There are many disturbances in stomach such as HCl and pepsin secretion , impaired in gastric mucosa, alteration in permeability and gastric mucus progress to destruction and generate of free–radical that potentiate induction ulcer by naproxen (Raygude *et al*., 2011; Kandhare *et al*., 2012; Gosavi *et al*., 2012, Smeeta *et al*., 2013). The stomach and duodenum contain gastric glands that secrete many agents such as mucus, hydrochloricacid (HCl), gastrin, somatostatin, acetylcholine, histamine, pepsinogen, pepsins, lipases, proteases for break down food particles so that mucosa layer always exposed to gastric acid that can harm living cells (Johnson *et al*., 2006).

Our result showed congestion of blood vessels with hemorrhage and inflammatory cell infiltration in mucosa and sub mucosal layer, these results here inline with McConnico *et al*., 2008 who indicates, when the safety of the GI barrier is disturbed, the rate of back-distribution of gastric acid and pepsin increases, leading to irritation, ulceration, inflammation and hemorrhage, therefore neutrophils and mast cells become activated and release leukotriens, free radicals, histamine, and proteolytic enzymes. These mediators of inflammation result in vasodilatation or vasoconstriction, increased vascular permeability and edema. In order to reduce the drugs side effect on the stomach and the intestine, and increase solubility and dispersion conversion of naproxen to phenyl hydrazine is necessary, followed bythe addition of D-ribose to produce 6methoxynaphthalen-2yl)-N-(2,3,4,5-tetrahydoxypentylidene) propanehydrazide. This conversion gives a potent analgesic effect, with disappear ulcerogenic effect. This result was in agreement with KUMAR and MISHRA's report in 2006 that indicated addition of polyethylene glycol and polyvinyl pyrrolidine to meloxicam showed significant increase in anti-inflammatory effect. In rodents paw oedema induced by carrageenan compared to those with meloxicam alone. Physical mixture and solid dispersion of some drugs possess best analgesic and anti-inflammatory properties with minimum ulcerogenic potential as compared to parent drugs. Moreover ATB-346 (a hydrogen sulfide- and naproxen-releasing compound) and NCX 429 (a nitric oxide- and naproxen-releasing compound) does not induce any significant gastrointestinal damage due to release of hydrogen sulfide and nitric oxide that act as mediators for mucosal defenseand has the ability to inhibit leukocyte activity (Rory *et al*., 2012).

Other studies suggest that pharmacokinetics ofATB-346 and NCX 429, enterohepatic circulation of drugs show very low levels of naproxen in the bile than parent naproxen. ATB-346 and NCX 429 do not posses free carboxylic acid residues, so would highly reduced local irritant characteristic compared to naproxen (Zanardo *et al*., 2006). Nagarsenker *et al*., 2000; Barzegar *et al*., 2002 have reported that solid forms of NSAND are less soluble in intestinal fluid and stay in lumen attached to the gastric wall for a longer period, thus generate a large concentration drugs that become very irritant to mucosa layer followed by ulceration occur. (Nambu *et al*., 1978; Barzegar *et al*., 2002). It is expected that in the formulation of drugs to phenyl hydrazine then addition of D-ribose, potentiate solubility, accelerate absorption and increase analgesic through increase bioavailability of drugs. Olmesartan medoxomil (OLM), orally administered, posses absolute bioavailability of only 26% due to the poor aqueous solubility, modulate complex with cyclodextrins (CD) has been reported to increase solubility due to complex formation of OLM with HP-β-CD was 5.26 fold for PM and 8.11 fold for KN. In PM and KN inclusion complex, about 100% within 15 min, while plain drug showed 92% dissolved in 60 min and total percentage of diffusion. After 6 hours of administration about 70.4% (for PM) and 79.9% (for HP-β-CD with the drug) and only 45.44% for OLM suspension so that increase in the solubility and the dissolution velocity give potent bioavailability while OLM, which is poorly bioavailable (Thakkar *et al*., 2012). Lichtenberger *et al*., 2008; Lanza *et al*., 2008 indicated that the therapeutic potency and efficacy of Ibuprofen-phosphatidylcholine (PC)-used for inhibition pain/ inflammation in patients with osteoarthritis as compared to that parent ibuprofen, for 6-week in patients with osteoarthritis result prove there is mild side effect on GIT in patient treated with PC than parent ibuprofen .Some studies showed that formulation of naproxen with PC in an oil-based soy lecithin formulation have a degree of protection against ulcerogenic effects rather than naproxen alone while posses analgesic effect and anti-inflammatory due to its intense COX inhibitory activity (Lichtenberger *et al*., 2008).

Histopathological section of small intestine of mice treated orally with 250 mg /kg of X3 showing proliferation of epithelial cell and hyperplasia of gut association lymphoid tissue that indicate to complete healing process. One other side that chemical X3 drugs may stimulate Toll-like receptors (TLRs) play a central role in mucosal innate immune regulation by production of cytokines (Rakoff-Nahoum *et al*.,

2004). TLRs play important role in control intestinal bleeding and protection from damage (Rakoff-Nahoum *et al*., 2004). The chemical reaction of sugar part in X3 with TLR-4 and its co receptor, MD-2, leading to liberation of pro -inflammatory cytokines. Number of date explain that a lack of TLR-4 or MyD88 Exacerbating intestinal damage to approximately 50% addition to limitation of chemokines, PGE2, cytokines, production. The decreased PGE2 appeared tobe mediated by COX-2 activation (Moses *et al*., 2009). Our study indicated addition of some compounds onto naproxen increases the potency and efficacy in most chemical alteration. X3 was notably considered the best group among the 6 groups in potency of analgesic without gastrointestinal damage. Therefore, we recommend to future studies to focusits research on the naproxen modification and its clinical use on human patients.

Conclusion

Our research concludes thats pharmacological tests performed in the present studies suggest that all new drugs possess potent analgesic activity but various from compound to other and demonstrate X3 naproxen derivative is more potent a worthy focus for future research and possible clinical implementation, due to its comparatively high efficacy and low side effects in comparison to other drugs tested in our study

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