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DEDICATION TO

My Husband and My sweet smile in my life Yassin & Farida

Vita

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Contents

List of Abbreviations

AAF	Acetyl aminofluorene
ACS	Acute coronary syndrome
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
ANOVA	Analysis of variance
AS	Atherosclerosis
AST	Aspartate amino transferase
ATO	Atorvastatin
ATV	Atorvastatin
B.WT	Body weight
BAX	Bcl_2-associated x protein
BCL-2	B-cell lymphoma 2
BUN	Blood urea nitrogen
CAMP	Cyclic adenosine mono phosphate
CAT	Catalase
CI-AKI	Contrast induced acute kidney injury
COQ10	Coenzyme Q10
COX	Cyclooxygenase
$COXIB_S$	Selective cyclooxygenase 2 inhibitors
DEFMO	Di fluromethyl ornithine
ED_{50}	Medium effective dose in 50%
EDTA	Ethyl Diamine Tetra Acetic acid end labeling

ER	Endoplasmic Reticlum
FBN	Fipronil
FSF	Fructus schisandrae aqueous extract
g/dl	Gram per deciliter
GE	Ginger extract
GFR	Glomrular filtration rate
GI	Gastro intestinal
GOT	Glutamic oxaloacetic transaminase
GPX	Glutathione peroxidase
GSH	Glutathione reduced
HB	Hemoglobin
HDL	High density lipoprotein
HFD	High fat diet
HS-CRP	High sensitivity c-reactive protein
I.M	Intra muscular
I.P	Intraperitoneally
I/R	Injury
IFN	Interferon
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
IONS	Inosine monophosphate dehydrogenase
LDL-C	Low density lipoprotein cholesterol
MAPK	Mitogen-activated protein kinase
MCH	Mean corpuscular hemoglobin

MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
MPO	Myeloperoxidase
MT	metalothionein
NAFLD	Non alcoholic fatty liver disease
NF-KB	Nuclear factorkappa beta
NO	Nitric oxide
NSAIDS	Non steroidal anti-inflammatory drugs
OX-LDL	Oxidized low density lipoprotein
PBC	Piroxicam-betacyclodextrin
PBS	Phosphate buffered saline
PCV	Packed cell volume
PGE_2	Prostaglandine E_2
PMN	Poly morph nuclear leukocytes
RBC_S	Red blood cells
ROS	Reactive oxygen species
ROSU	Rosuvastatin
Rpm	Revolution per minute
S.L	Sublingual
SD	Solid dispersion
SOD	Superoxide dismutase
SPSS	Satistical package for the social science
SV	Simvastatin

$TGF\beta_1$	Transforming growth factor beta1
TLC	Total leukocyte count
TNF-α	Tumor necrosis factor alfa
TUNEL	Terminal deoxynucleotidyl transferase-mediated dutp-biotin nick
USAN	United State Adopted Name
WBC_s	White blood cells

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1. INTRODUCTION

Piroxicam is one of the most commonly prescribed non-steroidal anti-inflammatory drug (NSAID) used mainly as analgesic, antipyretic, and anti-inflammatory **(Sahu, 2016).** Piroxicam is known to exert its action via suppressing prostaglandin synthesis by inhibiting cyclooxygenase enzyme through competitive antagonism with arachidonic acid **(Vane *et al*., 1998)**reducing the secretion of the gastro protective mucin which triggers ulcer formation **(Murray and Brater, 1993)**.Piroxicam has also reported to have deletrious toxic effects on liver and kidney tissues suggesting that piroxicam should be used under strict medical control to avoid such toxic effects**(Ebaid *et al*., 2007)**.

There is a cumulative evidence that that piroxicam exerts its hepatic and renal toxic effect through induction of oxidative stress and initiation of apoptotic mechanisms in the liver and kidney tissues **(Grosser *et al*.,2011)** through disruption of the redox hemostasis along with induction of oxidative damage. Several reports have suggested that the disruption of redox hemostasis during piroxicam poisoning is due to the increased production of reactive oxygen species (ROS).Endogenous antioxidant defense system (glutathione, GSH; glutathione peroxidase, GPx; superoxide dismutase, SOD; and catalase, CAT) plays a crucial role in scavenging the generated ROS. Therefore, when there is imbalance between prooxidants and antioxidants, the cell becomes susceptible to the oxidative stress indicated by lipid peroxidation, mitochondrial dysfunction, and DNA damage ended by initiation of apoptosis **(Lepetsos and Papavassiliou,2016).**

Rosuvastatin (ROSU) is a member of the statin family, which is comprised of anti-hyperlipidemic agents. ROSU inhibits 3-hydroxy- 3-

methyglutaryl coenzyme reductase **(Leite *et al.*, 2017)**. Independent of its lipid-lowering effects, ROSU also has also anti-inflammatory and antioxidant properties **(Maheshwari *et al.*, 2015; Selim *et al.*, 2017)**. Recently, ROSU was reported to provide protection against drug-induced nephrotoxicity **(Selim *et al.*, 2017)** and ischemia-reperfusion injury in the heart, intestine, and spinal cord through reduction of free radicals and up-regulation of antioxidant enzymes **(Die *et al.*, 2010; Maheshwari *et al.*, 2015).**

Therefore, this study was designed to investigate whether the ROSU could ameliorate the piroxicam-induced oxidative damage and apoptosis in liver and kidney tissues. This could be achieved by:

- Studying the effect of piroxicam and/or ROSU on liver biomarkers (AST & ALT & total protein, and albumin), kidney biomarkers (creatinine and urea), and lipid profile biomarkers (cholesterol and triglycerides).
- Studying the effect of piroxicam and/or ROSU on antioxidant biomarkers (GSH, CAT, and MDA) in liver and kidney tissues.
- Studying the effect of piroxicam and/or ROSU on histopathological changes in liver and kidney tissues.

Studying the effect of piroxicam and/or ROSU on the apoptotic mechanisms (Bax and Bcl-2) in liver and kidney tissues.

2. REVIEW of LITERATURE

2.1. Piroxicam:

2.1.1. Background:

Non-Steroidal Anti Inflammatory Drugs (NSAIDs) have been commonly used to reduce pain and inflammation in different arthritic and postoperative conditions due to their three major activities, viz., anti-inflammatory, antipyretic, and analgesic **(Hawkey, 1999)**.

The term "Inflammatory reaction", refers to the events, which occur in tissues in response to an invading pathogen. Inflammation is the response of the body to invasion by a pathogen (infection) or injury directed at destroying the pathogens and repairing the damaged tissues. The reactions are protective, but if inappropriately deployed, they are deleterious **(Cunningham and Lees, 1994)**.

Inflammation of various tissues is the most common problem faced by practicing veterinarians. Administration of anti-inflammatory agents to alleviate signs of inflammation is a standard therapeutic approach. Use of steroidal, non-steroidal and narcotic anti-inflammatory drugs is a major therapeutic approach for inflammatory diseases in animals as well as in human beings.

Numerous drugs have been discovered and used as antipyretic, analgesic and anti-inflammatory after the development of aspirin in the late 1800's. These drugs have been known as "Aspirin like drugs. The term NSAID was first applied to phenylbutazone after its introduction into clinical practice in 1949. Phenylbutazone and some other NSAIDs

are being used as therapeutic measures for pain, inflammation and fever in clinical veterinary medicine.

Vane (1998) mentioned that there are two COX enzymes: COX-1 and COX-2.The active site of COX kept in a narrow hydrophilic tunnel composed of an active inner site and protected by an outer area that is made up of three α helices. NSAIDs attach to these outer helices and temporarily inhibit the passage of arachidonic acid from reaching the active site and lead to the production of prostaglandins**(Garavito**,**1999).**

COX-2 was considered to be induced by inflammation and the presence of Pro inflammatory cytokines and mitogens.It has been recommended that the anti-inflammatory action of NSAIDs is due to the inhibition of COX-2 whereas COX-1 inhibition is associated with undesirable effects related to interference of the regulatory and protective mechanisms. **(vane *et al.*, *1998*;van *et al.*,1999).**

Piroxicam, the oxicam represent a potentially growing class of non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the benzothiazinone dioxide series of heterocyclic molecules. The term 'oxicams' has been adopted by the United State Adopted Names (USAN) Council to describe the relatively new enolic acid class of 4-hydroxy-1,2-benzothiazine carboxamides with anti-inflammatory and analgesic properties. The first member of this class, piroxicam was introduced in the United States in 1982 as Feldene (Pfizer) and gained immediate acceptance in the United States. Piroxicam (an acidic carboxamide) is a non-steroidal anti-inflammatory drug of the oxicam class used to relieve various painful and inflammatory conditions, specially as single largest group of NSAIDs associated with the palliation of symptoms rheumatoid

and osteoarthritis, ankylosing spondylitis and musculoskeletal disorders **(Roberts and Morrow, 2001)**.

More recently, it has received attention for its ability to reduce the size of tumors (transitional cell tumors and others) in dogs **(Mutsaers et al.,2003)** and colorectal and invasive bladder cancers **(Ronald, 2001)**.

2.1.2. Mechanisn of action of piroxicam

Funk (2001) explained anti inflammatory, analgesic and antipyretic properties of the aspirin-like drugs are due to Inhibition of prostaglandin synthesis. Also studied mechanism of action of NSAIDs. Inhibition of cyclooxygenase enzymes (COX-1 and COX-2) is the mechanisn of action of NSAIDs. Activation of COX-1 promotes release of eicosanoids involved in physiological processes (eg. thromboxane A3, prostacyclin or prostaglandin E2). Inhibition by NSAIDs of COX-1 results in side-effects (eg. gastrointestinal irritation). Inhibition of COX-2 reduces inflammation. Most currently available NSAIDs are more potent inhibitors of COX-1 than COX-2. NSAIDs that preferentially inhibit COX-2 reduce inflammation with less inhibition of the production of physiologically-active eicosanoids, so potentially reducing the risk of side-effects.

Churchill *et al.*, (1996) studied mode of prostaglandin synthesis inhibition by Piroxicam. Piroxicam is a potent inhibitor of prostaglandin biosynthesis. Experiments utilizing cell culture and microsomes derived from various sources have demonstrated that piroxicam is a selective inhibitor of the cyclooxygenase step of arachidonic acid metabolism. they reported little blocking activity at the phospholipase, thromboxane or prostacyclin synthetase and arachidonic acid lipoxygenase steps.

Andubhai, (2012) during a study on indomethacin and piroxicam showed that cycloxygenase inhibition significantly reduced tissue prostaglandin E2 synthetic capacity (indomethacin 96%, piroxicam 92%) but did not cause either macroscopic or microscopic mucosal injury. They suggested presence of a selective pathway for the uptake of intact proteins in gastric mucosa and the pathway is modulated by cyclooxygenase metabolites.

2.1.3. Pharmacological actions of piroxicam

2.1.3.1. Anti-inflammatory Activity

Monteiro-Riviere , (1996) evaluated the anti-inflammatory effect of piroxicam after oral and topical administration on an ultraviolet-induced erythema model in man by measuring the erythema area after UV injury on different sites of the back at 4, 6, 7, 8, and 24 hours post irradiation. They found that both treatments reduced the erythema size but the topical application produced a longer lasting erythema inhibition than the oral treatment.

Cronstein and Weissmann, (1995) reviewed targets for anti inflammatory drugs. Inhibitory effects of NSAIDs on neutrophil activation in vitro leads to the anti-inflammation. Neutrophils derived from the synovial fluid of patients with rheumatoid arthritis produced less oxygen radicle following 10 days of therapy with piroxicam than cells from normal volunteers who were given ibuprofen or piroxicam for 3 days. These cells failed to aggregate normally in response to chemoattractants.

Engelhardt (1996) studied acute anti-inflammatory activity was assessed in the rat carrageenan induced paw edema model. Paw edema

was induced by sub plantar injection of a 1 % solution of carrageenan. Paw volume was measured 3 hours after edema induction, when meloxicam and the other test substances like piroxicam, indomethacin, diclofenac, naproxen and acetylsalicylic acid were administered 1 hour before edema induction, there was a no difference in the anti-inflammatory activity between meloxicam and comparators tested.

Abd-allah et al., (2011) evaluated anti-inflammatory and analgesic effect of piroxicam loaded micro emulsion in topical formulations. The anti-inflammatory activity of the tested piroxicam formulations was evaluated using right hind paw oedema size of rats induced by carrageenan injection, while the analgesic effect was evaluated using Hot Plate method applied on mice.

Beyer et al., (2011) studied effects on muscle performance of NSAID treatment with piroxicam versus placebo in geriatric patients with acute infection induced inflammation. Piroxicam improves clinically relevant measures of muscle performance and mobility like EMS (Elderly Mobility Scale) scores, FR (Fatigue Resistance), GS (Grip Strength) and GW (Grip Work) in geriatric patients hospitalized with acute infection-induced inflammation.

2.1.3.2. Analgesic effect

Pain (algesia) that accompanies the inflammation and tissue injury probably results from local stimulation of pain fibres and enhanced pain sensitivity or lowered pain threshold (hyperalgesia), in part a consequence of increased excitability of central neurons in the spinal cord **(woolf et al.,2011)**.

Bliven et al., (1997) observed the effect of piroxicam on locomotor activity in rats with adjuvant-induced arthritis.

Chang et al., (2008) studied analgesic effect of piroxicam-betacyclodextrin in the treatment of acute pain of rheumatic disease. Piroxicam-betacyclodextrin (PBC) is a new formulation in which piroxicam has been complexed with beta-cyclodextrin, a cyclic oligosaccharide. This results in an increase in the rate of absorption of the active compound and consequently,in an earlier onset of analgesic action. PBC (Piroxicam-beta-cyclodextrin) is administered once daily. PBC has been used in the treatment of osteoarthritis. PBC showed rapid analgesic-anti-inflammatory action after the first administration in patients with active osteoarthritis.

Bianchi and Panerai, (2002) studied effects of lornoxicam, piroxicam, and meloxicam in a model of thermal hind paw hyperalgesia induced by formalin injection in rat tail. As clinical pain is characterized by hyperalgesia, they evaluated the effects of NSAIDs with similar chemical structures but different selectivities for cyclooxygenase (COX)-1 and COX-2 in a new behavioural model of central hyperalgesia in rats and assessed the effects of lornoxicam, piroxicam, and meloxicam on the reduction of hind paw nociceptive thresholds to thermal stimulation produced by a 10% formaldehyde (formalin) injection into rat tail. Each drug was administered intraperitoneally (i.p.) at its ED_{50} for the anti-inflammatory effect (namely the inhibition of carrageenan-induced hind paw oedema). At this dose (1.3 mg/kg, 1.0 mg/kg, and 5.8 mg/kg, respectively), lornoxicam, piroxicam, and meloxicam produced the same anti-inflammatory effect, did not modify thermal nociceptive thresholds, and significantly reduced the hyperalgesia.

Gramke et al., (2006) reported effect of Sublingual piroxicam for postoperative analgesia. They investigated in this randomized, double-blind study, whether sublingual (S.L.) piroxicam given before was more effective than that given after surgery.

Farshchi and Ghiasi, (2010) compared the analgesic effects of single dose administration of tramadol or piroxicam on postoperative pain after cesarean delivery. All patients were assessed at 0, 6, 12 and 24 hours post operation for pain degree, nausea and vomiting. There was no significant difference between the efficacy of tramadol and piroxicam injections. Pain intensity decreased markedly over time in both groups. Side effects were similarly minimal with all treatments. It might be concluded that IM injections of 20 mg piroxicam (single dose therapy) could relieve postoperative pain after cesarean section as well as tramadol and it could reduce opioid analgesic requirements with less adverse side effects during the first postoperative 24 h.

2.1.3.3. Anti-pyretic Activity

Regulation of body temperature requires a delicate balance between the production and loss of heat. The hypothalamus regulated the set point at which the body temperature is maintained **Devi et al.,(2003)**. In fever, this set point is elevated and NSAIDs promote return it to normal. Fever may be a result of infection or one of the sequelae of tissue damage, inflammation etc. A common feature of these conditions is the enhanced formation of cytokines such as interleukin -1s (IL-1s), interferons (IFN-α and s) and tumor necrosis factor - α (TNF- α). These cytokines increase the synthesis of PGE2 near the preoptic hypothalamic area, and PGE2 (via increase in cAMP) triggers hypothalamus to elevate body temperature by promoting increase in heat generation and decreases in

heat loss. NSAIDs suppress this response by inhibiting the synthesis of PGE2 **(Gordon *et al.*,2012).**

Piroxicam is an effective anti-inflammatory agent. It also exerts antipyretic and analgesic effects in experimental animals and man. It can cause gastric erosion and prolongs the bleeding time **(Grosser *et al*,2011).**

The evidence of this scenario includes the ability of prostaglandins, especially PGE2, to induce fever when infused into the cerebral ventricles or when injected into hypothalamus. The NSAIDs inhibit fever caused by agents that enhance the synthesis of IL-1 and other cytokines, which presumably cause fever by inducing endogenous synthesis of prostaglandins **(Insel, 1996)**.

2.1.4. Therapeutic uses of piroxicam

Pharmacological properties and therapeutic efficacy of piroxicam was reviewed in humen patients by **Childs *et al.*, (2007)**. Piroxicam 20 mg daily is comparable with aspirin 3 to 6 g, indomethacin 75 to 150 mg, phenylbutazone 400 mg, naproxen 500mg, ibuprofen 1200 to 2400 mg and diclofenac 75 mg in rheumatoid arthritis. In osteoarthritis, piroxicam 20 mg daily is comparable in efficacy with aspirin 2.6 to 3.9g, indomethacin 75mg, naproxen 500mg and fenbufen 600mg but is generally better tolerated than aspirin or indomethacin in human patients with arthritic diseases. Piroxicam 20mg was at least as effective as indomethacin 75mg in a study in ankylosing spondylitis. As with other non-steroidal anti-inflammatory drugs gastrointestinal complaints are the most frequently reported side effects and their frequency and severity appears to be dose-related.

Piroxicam is approved for the treatment of rheumatoid arthritis and osteoarthritis. It has anti-inflammatory, antipyretic and analgesic properties. It has also been used for the treatment of ankylosing spondylitis, acute musculoskeletal disorder and acute gout **(Grosser *et al.*, 2011)**.

Chen *et al.*, (2002) also determined a decrease in intestinal carcinogenesis in rats due to piroxicam intake and combination of piroxicam and DFMO (difluoromethyl ornithine).

Andubhai, (2012) had assessed the efficacy of 0.5% piroxicam applied for 15 days to the eyes of rabbit in which uveitis had been experimentally induced. The results demonstrate the capability of the drug to easily overcome the ocular barrier. On the 30^{th} day, a clear regression of the uveitis symptoms was observed.

Lascelles *et al.*, (2001) evaluated the relative clinical benefit of piroxicam and ketoprofen in cats with painful locomotor disorders. Both the drugs were found to be potent analgesics and well tolerated in cats, whatever, piroxicam was assessed to be significantly more palatable than ketoprofen.

Inhibitory effect of piroxicam on 2 acetylaminofluorene (AAF) induced hepatocarcinogenesis in male rats was studied by **(Cervello and Montalto, 2006).**They found a significant smaller incidence of liver cell tumors and the tumor multiplicity in rats receiving piroxicam (130 ppm in diet) along AAF, as campare to control.

Kohli and Kohli, (2011) evaluated effectiveness of piroxicam and ibuprofen premedication on orthodontic humen patients. Premedication with 20 mg of piroxicam results in significantly decreased pain

experienced, compared to premedication with 400 mg of ibuprofen or placebo. Usage of 20 mg of piroxicam 1 hour prior to separator placement is recommended.

2.1.5. Toxicity of Non-Steroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs available in the market suffer from side-effects like gastric ulceration, hepatotoxicity, renal toxicity, etc. Selective COX-2 inhibitors have been developed to reduce the side-effects, but unfortunately suffer from severe cardiovascular toxicity. As a result, several selective COX-2 inhibitors have been withdrawn from the market. **(Savjani and Suja 2015).**

2.1.5.1. Effect on physical and behavioral state

Acute toxicity studies conducted in rats and mice in which the animals expressed apathy, diarrhoea and emaciation at low dose levels and ataxia, rapid shallow respiration, flaccidity and diarrhoea at high dose levels with severe growth suppression in animals at all dose levels **(Andubhai, 2012)**.

2.1.5.2. Ulcerogenic activity

Carvalho *et al.*,(2011) studied incidence of gastric ulcers by indomethacin and piroxicam in rats. Comparative toxicity of indomethacin and piroxicam at low multiples of the human therapeutic dose was studied in inbred albino rats of both sexes. Using the drug-induced model, the two drugs were used to produce gastric ulcers in the rat. Both showed significant evidence of gastric ulceration measured by the ulcer index.

kumar and Mishara (2006) studied the ulcerogenic effect of Meloxicam,ulcerogenic potential of Physical mixture (PM) and Solid dispersion (SD) in rats by the method reported by **Nagarsenker *et al.*, (2000).**

Spellman (1992) reported that 7 day treatment of dog, suffering from osteoarthritis, with Piroxicam developed acute haematemesis and melena. Similarly gastrointestinal bleeding in 2 dogs associated with the use of naproxen and piroxicam, and phenylbutazone induced blood disorder in dogs **(Lium, 1994)**.

2.1.5.3. Effects on haematology

Bessone (2010) reported that piroxicam was associated with cholestatic jaundice and leucopenia. While aplastic anemia associated with piroxicam was reported by **Rawson *et al.*, (1998)**.

Effect of piroxicam therapy on granulocyte function and granulocyte elastase concentration in peripheral blood and synovial fluid of rheumatoid arthritis patients was studied by **Hartmann *et al.*, (2005)** and it was concluded that piroxicam may act at different sites on various PMN responses. Its effect on 02-generation and PMN elastase concentration in synovial fluid may have an important role in reducing destruction of arthritic joint tissue.

Koytchev *et al.*, (1994) in an open trial suggested a significant positive correlation between increasing serum concentrations of piroxicam and the degree of inhibition of platelet aggregation.

Velankar *et al.*, (1999) studied certain NSAIDs on various haematological parameters and toxic effect of long term use of NSAIDs in rats. They reported that the NSAIDs did not produce any significant

changes in total erythrocyte count, plasma protein concentration, MCV, MCH, and MCHC, however, the Hb concentration and PCV were reduced singnificantly after the administration of drug once daily for 7 days in rats.

Sharma (2002) studied the effects of NSAIDs on blood parameters and clinical observations following 10 day daily administration in dogs. They used aspirin 100 mg/kg b.wt. diclofenac sodium 15 mg/kg b.wt., ibuprofen 10 mg/kg b.wt., nimesulide 5 mg/kg b.wt. and serratiopeptidase 2 mg/kg b.wt. 1 capsule daily orally. He observed that drugs were studied, not produce any significant change in total erythrocyte count, total leukocyte count, differential leukocyte count, total plasma protein, platelets count and erythrocyte indices (MCV, MCH, and MCHC) when compared to control group. It was also revealed that aspirin reduced the concentration of hemoglobin significantly, while other drugs did not produce any significant effect on Hb concentration. The packed cell volume (g/dL) of RBCs was significantly reduced in the group treated with aspirin. Other drugs did not produce any significant change in packed cell volume.

Andubahi (2012) studied the efficacy of aspirin, diclofenac sodium, flubiprofen, nimesulide, piroxicam, serratiopeptidase and trypsine-chymotrypsin and their half dose combinations on adult albino rats. He evaluated the hematological parameters and he concluded that all individual drugs and their combinations, except nimesulide, serratiopeptidase, trypsin-chymotrypsin, nimesulide+serratiopetidase, nimesulide + trypsin-chymotrypsin and serratiopeptidase + trypsin-chymotrysin, induced follwing significant alteration in haematological and biochemical parameters. He revealed total erythrocyte count, Hb, and PCV significantly decreased as compared to control group. MCV was

significantly increased, MCH, MCHC and TLC did not change in any of the test group. Lymphocyte and monocyte counts significantly increased as compare to control group. While neutrophills, plasma protein and platelets were significantly decreased as compare to control group while eosinophils and basophils did not show any change as compared to control group.

Abatan *et al.*, (2006) reported the toxic effect of piroxicam (15 mg/kg), indomethacin (5 mg/kg), phenylbutazone (10 mg/kg), and aspirin (20 mg/kg), in rats. They found significant increase in the level of ALP except the increase by piroxicam which was not significant (P>0.05). Aspirin, indomethacin, and phenylbutazone caused increase in the level of serum enzyme AST. This increase was significant with the group given indomethacin. Furthermore, indomethacin also produced significant increase in levels of ALT . There were no significant changes in the levels of total protein and albumin in all the treatment groups. They have not found significant hematological changes win all the treatment groups except the group treated with indomethacin which showed significantly increased levels of total WBC.

Khoshnegah *et al.*, (2011) studied a comparative study of the long-term effects of piroxicam and ketoprofen on the gastric mucosa, kidney, liver and hematopoietic system of dogs. Fifteen mixed-breed healthy dogs of both sexes aged between 1 to 8 years were divided in three groups and treated with piroxicam (0.15 mg/kg IM), ketoprofen (1 mg/ kg IM) or placebo daily for 21 days. Although not statistically significant, the dogs receiving ketoprofen showed fewer and less severe lesions than the dogs in the piroxicam group. None of the dogs showed any clinical signs related to the gastric lesions. Serum biochemical and complete blood count parameters did not change significantly after NSAlD

administration. However, by day 14 a decreased number of platelets and prolonged bleeding time were detected in treatment groups compared with the control group. The clinical significance of this prolongation is unclear. This study suggested that ketoprofen and piroxicam produce mild lesions when administered to healthy dogs for 21 days and there is no difference between the two groups in the number and severity of lesions. There may be an indication that longer duration of drug administration may result in a greater number of gastric lesions. However, after long- term NSAID exposure (21 days in our study) gastric tolerance to the damage caused by NSAIDs will be developed.

2.1.5.4. Organ toxicity

Paterson *et al.*, (1992) mentioned that piroxicam induced submassive necrosis of the liver**.** Three cases of severe acute hepatitis have been reported in association with piroxicam. A fatal submassive necrosis that occurred in a 68 year old lady who had received piroxicam for 15 months is described. A 48 year old man who developed submassive hepatic necrosis six weeks after beginning piroxicam but was successfully treated with orthotopic liver transplantation is also reported. Piroxicam may induce submassive necrosis of the liver, probably as an idiosyncratic reaction.

Lipscomb *et al.*, (1998) carried out a double-blind placebo-controlled study of gastrointestinal tolerability of meloxicam and piroxicam. The effects of meloxicam and piroxicam on the gastroduodenal mucosa in healthy adults were determined. Observations suggested that meloxicam caused little acute damage to the upper gastrointestinal tract and piroxicam causes some acute gastric injury but such damage resolves in most subjects by 28 days.

Villegas *et al.*, (2002) compared the effects of two oxicams, preferential cyclooxygenase (COX)-1 or COX-2 inhibitors, on both gastric mucosa and some biological parameters (hematological, hepatic and renal) after subchronic administration (14 and 28 days) in rats. Equipotent doses of meloxicam (3.75 and 7.5 mg/kg) and piroxicam (5 and 10 mg/kg) were administered. Both drugs dose- dependently caused multiple gastric erosions and hemorrhage in rats after 14 and 28 days of administration. Treatment with meloxicam led to a higher gastric dam- age than with piroxicam on day 14 although these results were not significant. The levels of myeloperoxidase ac- tivity (as an index of neutrophil infiltration) were not changed compared with control after drug treatment. All the hematological parameters obtained after drugs administration for 14 and 28 days were in the range of nor- mal values, and a significant increase in platelet levels could be observed in the group treated with 5 mg/kg of piroxicam for 14 days. Aspartate aminotransferase (AST or GOT) increased significantly after 14 days, but after 28 days the values returned to normality. Creatinine and urea did not undergo significant changes except for the piroxicam 14-day 5 mg/kg group, in which uremia in- creased significantly over normal values. These results showed that meloxicam, a preferential COX-2 inhibitor, causes rates of gastric lesion comparable to those seen with traditional NSAIDs, without inducing important changes in biological parameters.

Abatan *et al.*, (2006) studied the toxic effect of piroxicam, indomethacin, phenylbutazone, and aspirin, in rats. Drugs used included indomethacin at 5 mg/kg; piroxicam at 15 mg/kg; aspirin at 20 mg/kg; and phenylbutazone at 10 mg/kg for 14 days. At the end of study detailed histopathological study was carried out and they reveled lungs of rats given indomethacin showed large focus of hemorrhage into the

interstitium and alveoli with mild periportal hepatic necrosis and kupffer cell proliferation.

Radi and Khan, (2006) reviewed the effects of cyclooxygenase inhibition on the gastrointestinal tract. COX- 1 is a constitutively expressed and found in most normal body tissues. COX-2 is expressed in normal tissues at low levels and is highly induced by proinflammatory mediators in the setting of inflammation, injury and pain. Inhibitors of COX activity include: (1) conventional nonselective nonsteroidal anti-inflammatory drugs (ns- NSAIDs) (2) selective COX- 2 inhibitors (COXIBs) and (3) COX-1 inhibitors. Non-selective NSAIDs at therapeutic doses inhibit both COX- 1 and COX- 2. The anti-inflammatory benefits of these drugs are primarily derived from COX-2 inhibition, while inhibition of COX-1 often elicits gastrointestinal (GI) toxicity. Therefore, COXIBs were developed to provide a selective COX-2 agent, i.e., one that at fully therapeutic doses demonstrated comparable therapeutic benefit to non-selective NSAIDs, without the attendant COX-1-mediated GI toxicities.

Ebaid *et al.*, (2007) studied piroxicam-induced hepatic and renal histopathological changes in mice. Animals were classified into a control group and 4 treated groups. Piroxicam was injected intraperitoneally using 0.3 mg/kg every day for four weeks. Each week a group of mice was sacrificed. Liver and kidneys were obtained for histological and histochemical examination. Liver sections appeared with inflammatory cellular infiltration, vacuolated hepatocytes, dilated sinusoids, and increased number of Kupffer cells. Kidney sections appeared with some cellular inflammations. The glomeruli were shrunk resulting in widening of the urinary space. Oedema and vacuolations were noticed in the

tubular cells. There was a positive correlation between these pathological changes and the increased treatment periods.

Sahu and Ghosal, (2007) studied pathological manifestations of piroxicam induced hepato-nephrotoxicity in mice. Mice were injected intramuscularly with piroxicam at a dose of 3.3 mg/kg either for one day or repeated for 10 days prior to their sacrifice. Control mice received intramuscular injection of saline (0.9%) for the specific experimental period. The animals were sacrificed at the end of the study. Liver and kidney tissue samples were taken for histology. Daily doses up to 10-day induced a pattern of parenchymal cell degeneration and necrosis along with evidence of swelling, mild fatty changes and sinusoidal dilatation in liver and kidney. Histological study showed atrophied glomerulous and change in Bowman's capsule along with renal papillary necrosis and stromal tissue proliferation. However one-day doses of piroxicam revealed mild degenerative changes only in liver. Results also indicate that changes in metabolic enzyme activities in serum substantiate histopathological observations during the repeated exposure period. It seems reasonable to speculate that toxicity of piroxicam for liver and kidney may relate to its metabolism and excretion of the drugs.

Bulman-Fleming *et al.*, (2010) evaluated the adverse events in cats receiving long-term piroxicam therapy for various neoplasms. Cats received daily piroxicam at doses of 0.13–0.41 mg/kg. Treatment duration ranged from 1 to 38 months. Treatment with piroxicam was found to significantly increase frequency of vomiting during the first month of therapy, though this was most significant for cats receiving concurrent chemotherapy. Piroxicam administration was not significantly associated with hematologic, renal or hepatic toxicities. Adverse events were not correlated with dosage. Adverse events were reported in 29% of

cats, and were generally mild and transient. This study indicated that long-term daily piroxicam is generally well tolerated in cats at conventional doses.

Saganwan and Orinya (2016) mentioned the acute toxicity signs of piroxicam at doses 207.5 mg/kg and above observed in the animals are torticollis, opisthotonos, somnolence, lethargy, diarrhea, gastroenteritis, generalized internal bleeding, anemia, congestion of the lung and liver, flaccid paralysis, cheesy lung, urinary incontinence, engorged urinary bladder, convulsive jerking of the limbs, lying in ventral recumbency, gasping for air, roaring, and death. Three out of six puppies died after being fed the carcasses of poisoned turkey, duck, and hen administered piroxicam at doses of 1000, 415, and 1000 mg/kg, respectively. White flaky cheesy materials observed in turkeys were also observed in the gastrointestinal content of the puppies. Paleness of carcasses, watery crop content, dryness of pericardium, gastroenteritis, intestinal perforation, and whitish pericardium were observed in broilers. There were effusions in thoracic and abdominal cavities as seen in all other carcasses poisoned primarily by piroxicam. Administration of atropine (0.02 mg/kg) led to survival of the remaining puppies. In conclusion, piroxicam is very to moderately toxic in monogastric animals.

2.2. Rosuvastatin:

2.2.1. Background:

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors and constitute the first-line drug treatment if exercise and a low-fat diet fail to correct hypercholesterolaemia. In this manner, statins substantially contribute to reduce morbidity and mortality in patients at the highest risk of cardiovascular events **(Vaughan and Gotto, 2004)**.

All available statins have similar pharmacology, established efficacy in terms of a dose-dependent beneficial effect on plasma cholesterol concentrations, and a comparable range and severity of adverse events. Atorvastatin and rosuvastatin are drugs with high cholesterol-lowering efficacy as compared with lovastatin, simvastatin, pravastatin, and fluvastatin, which have less cholesterol-lowering potency.

Apart from an intrinsic cholesterol-lowering effect, statins also exhibit anti-inflammatory, antioxidant, and plaque-stabilizing capacities that act in concert to prevent other than cardiovascular damage **(Vaughan and Gotto, 2004; Bedi *et al.*, 2016)**. In particular, statins may affect the kidneys via cholesterol-related and -unrelated mechanisms resulting in potential acute and long-term benefit on renal function **(Teshima *et al.*, 2010; Bedi *et al.*, 2016)**.

Rosuvastatin, as one of the statins family, is widely prescribed for dyslipidemia as a potent cholesterol-lowering drug **(Kumazaki *et al.*, 2013)**. Another trouble with statins is that they inhibit coenzyme Q 10 (CoQ10), which is one of the important mitochondrial enzyme system. It is also the main acceptor and donor of electrons in the mitochondrial membrane. This confirms mitochondrial dysfunction upon accelerating the ageing process. Accordingly, statins inhibit the formation of glutathione peroxidase (GPx), which is necessary to sustain cholesterol in the desirable un-oxidized state **(Rosenbaum *et al.*, 2013)**. Despite the wide utilization of statins to prevent cardiovascular disease, there are data assessing liver toxicity and muscular side effects characterized by rupture of muscle mass on increasing rosuvastatin therapy **(Bifulco, 2014)**.

2.2.2. Renal protective:

İşeri *et al.*, (2007) examined the protective effect of simvastatin against cisplatin-induced renal toxicity in rats. Cisplatin impaired kidney function as shown by increased BUN and serum creatinine concentrations and decreased creatinine clearance. This renal dysfunction was improved by simvastatin administration. Simvastatin decreased cisplatin-induced increase in myeloperoxidase (MPO) activity in the kidney but did not improve cisplatin-induced changes in renal MDA and GSH contents. Simvastatin attenuated cisplatin-induced increase in kidney tissues collagen content as indicator of fibrotic activity, and reactive oxygen metabolites. Kidney histological damages were completely reversed by simvastation.

Ozbek *et al.*, (2009) evaluated the effect of atorvastatin against gentamicin-induced nephrotoxicity in rats. Co-administration of atorvastatin prevented gentamicin-induced increases in blood urea nitrogen (BUN) and serum creatinine levels, reduction in calculated creatinine clearance values, reduction in renal tissue glutathione (GSH) levels and elevation of kidney malondialdehyde (MDA) and NO levels. Co-administration of atorvastatin also significantly prevented gentamicin-induced tubular and glomerular degeneration.

Fujieda *et al.*, (2010) evaluated the effect of pravastatin on cisplatin-induced nephrotoxicity. Cisplatin caused renal tubular damage with high renal MDA level. Pretreatment with pravastatin significantly improved cisplatin-induced renal dysfunction and proteinuria and attenuated cisplatin-induced immuonohistological changes in p53- and TUNEL-positive apoptotic cells in renal proximal tubular cells. Prophylactic administration of pravastatin also significantly prevented cisplatin-induced oxidative damage as shown by modulation in cisplatin-induced

changes in renal MDA and GSH levels and kidney tissue GPx expression and activity.

An *et al.*, (2011) evaluated the protective effects of high dose pravastatin administration shortly before cisplatin-induced acute nephrotoxicity. Pravastatin pretreatment significantly diminished cisplatin-induced increase in BUN and serum creatinine and histological damage to renal tubules. Cisplatin significantly increased renal MDA level, decreased kidney GSH level, and inhibited activities of antioxidant enzymes including CAT, SOD and GPx. All these oxidative changes significantly attenuated by pravastatin administration. Cisplatin increased kidney iNOS expression and peroxynitrite formation. These cisplatin-induced oxidative and nitrosative stimulations were partially suppressed by pravastatin. Pravastatin also reduced cisplatin-induced p38 MAPK activation in the kidney tissues. The authors concluded pretreatment with pravastatin can prevent cisplatin-induced nephrotoxicity via inhibiting oxidative and nitrosative stress.

Jabari *et al.*, (2011) evaluated the preventive effects of low, medium and high dose simvastation (2 mg/kg/d, 10 mg/kg/d, and 20 mg/kg/d respectively) against nephrotoxicity of low and high-dose gentamicin (50 mg/kg/d and 80 mg/kg/d respectively). Simvastatin administration started prophylactically several days before gentamicin injection and continued all over gentamicin administration days. Increasing simvastatin dosage up to 10 mg/kg/day dose-dependently improved gentamicin-induced changes in renal histopathology and function test.

Panonnummal *et al.*, (2011) evaluated the renoprotective effects of atorvastatin against vancomycin-induced acute kidney injury (AKI).

Atorvastation started several days before vancomycin injection and continued during vancomycin treatment and several days after that. Vancomycin decreased renal function as evidenced by increase in BUN and serum creatinine and decrease in creatinine clearance. Vancomycin also induced hypokalemia, oliguria, urinary sodium wasting, renal oxidative stress, and also some tubular and glomerular structural damages. Vancomycin-induced renal oxidative stress presented as increased renal tissue MDA, and decreased expression of antioxidant enzymes including SOD, CAT, and GHS in the kidney tissue. All vancomycin-induced renal functional, structural, and oxidative changes were mostly or completely prevented by atorvastatin administration. Authors concluded that renorpotective effect of atorvastatin against vancomycin-induced AKI is mediated through its antioxidant effect.

Mehany *et al*., (2013) investigated the protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. This was associated with a significant reduction in kidney glutathione (GSH), metallothionein (MT) contents and superoxide dismutase (SOD) activity. Furthermore inflammatory mediators such as myeloperoxidase (MPO) and tumor necrosis alpha (TNF-a) were increased. Renal damaged was also evidenced by the change in the kidney histopathological picture. Two weeks pre-treatment with vitamin E or atorvastatin before dichromate administration markedly improved its toxicity as indicated by reduction of serum urea and creatinine as well as improvement of kidney histopathological changes. Oxidative stress biomarkers such as renal MDA and nitric oxide contents were also decreased. Kidney superoxide dismutase activity was restored after pre-treatment with vitamin E. Furthermore, atorvastatin significantly reduced TNF-α content activity while vitamin E reduced TNF-α content. It could

be concluded that the ability of vitamin E as well as atorvastatin to ameliorate potassium dichromate-induced renal injury was associated with their antioxidant and anti-inflammatory properties.

Kose *et al.*, (2014) proved that the drug treatment of hyperuricemia and hyperlipidemia complications, Atorvastatin (ATV), which inhibits urinary protein, increases glomerular filtration rate (GFR) and has renal protective effects, and Rosuvastatin (ROSU) were found be suitable because they promote serum uric acid excretion.

Leoncini *et al.*, (2014) determined if in addition to standard preventive measures on-admission high-dose rosuvastatin exerts a protective effect against contrast- induced acute kidney injury (CI-AKI). The incidence of CI-AKI was significantly lower in the statin group than in controls. The 30-day incidence of adverse cardiovascular and renal events (death, dialysis, myocardial infarction, stroke or persistent renal damage) was significantly lower in the statin group. Moreover, on-admission statin treatment was associated with a lower rate of death or non fatal myocardial infarction at the 6-month follow-up.

Toso *et al.*, (2014) investigated whether the beneficial impact of high-dose rosuvastatin against contrast-induced Acute Kidney Injury (CI-AKI) in acute coronary syndrome (ACS) patients varied in relation to baseline high-sensitivity C-reactive protein (hs-CRP) levels. High-dose rosuvastatin administered on admission appears to exert more effective kidney protection in ACS subjects with higher baseline hs-CRP levels resulting in better short- and mid-term clinical outcome

Jaikumkao *et al.*, (2016) investigated the protective effects of atorvastatin against gentamicin-induced nephrotoxicity. Gentamicin-induced nephrotoxicity was confirmed by marked elevations in serum

urea and creatinine, kidney hypertrophy, renal inflammation, fibrosis, ER stress and apoptosis and attenuation of creatinine clearance. Atorvastatin pre and delayed treatment significantly improved renal function and decreased renal NF-κB, TNFαR_1, IL-6, iNOS and TGFβ_1 expressions. These results indicate that atorvastatin treatment could attenuate gentamicin-induced nephrotoxicity in rats, substantiated by the reduction of inflammation, ER stress and apoptosis. The effect of atorvastatin in protecting from renal damage induced by gentamicin seems to be more effective when it beginning given along with gentamicin or pretreatment.

Selim *et al.*, (2017) examined the protective effects of vitamin E (VIT. E) or rosuvastatin (ROSU) against amikacin (AMIK)-induced nephrotoxicity. The results showed that AMIK significantly increased serum levels of urea and creatinine. Meanwhile, serum levels of total protein and albumin were decreased. The kidney content of malondialdehyde was increased, whereas glutathione content and catalase activity were decreased. Tumor necrosis factor-α and nuclear transcriptional factor levels were increased. Conversely, administration of VIT. E and/or ROSU with AMIK ameliorated such damage and reduced DNA fragmentation, apoptosis, and necrosis. In conclusion, co-administration of VIT. E, ROSU, or their combination alleviated AMIK-induced nephrotoxicity.

2.2.3. Hepatoprotective effect:

Heeba and Abd-Elghany (2010) studied that combination therapy often takes advantage of complementary effects of different agents. This study investigated the combined effect of ginger extract (GE) and atorvastatin on lipid profile and on atorvastatin-induced hepatic injury. Rats were randomized into: control; GE (400 mg/kg); atorvastatin

(20 mg/kg) alone or with GE or vitamin E, and atorvastatin (80 mg/kg) alone or with GE or vitamin E. Administration of 80 mg/kg atorvastatin for 4 weeks had major hepatotoxic effect whereas the lower dose (20 mg/kg) seems to cause mild liver injury. Besides lowering serum total cholesterol and hepatic superoxide dismutase (SOD) and catalase (CAT), atorvastatin significantly increased serum aminotransferases, hepatic malondialdehyde (MDA) and nitric oxide (NO). Concurrent administration of GE and atorvastatin had the opposite effect. Histopathological study revealed that GE reduced liver lesions induced by atorvastatin. The results indicate that the ability of ginger to lower serum cholesterol and to decrease aminotransferases, MDA and NO is clinically important, because its chronic administration will neither lead to side-effects nor to hepatic changes as occurs with high atorvastatin doses. Therefore, combination regimens containing GE and low dose of statins could be advantageous in treating hypercholesterolemic patients which are susceptible to liver function abnormalities.

Ji *et al.*, (2011) employed a rat model of non-alcoholic fatty liver disease (NAFLD) to examine the therapeutic efficacy of dietary control and/or ATO treatment. Sprague-Dawley rats were fed with normal chow diet as normal controls or with high fat diet (HFD) for 12 weeks to establish NAFLD. The NAFLD rats were randomized and continually fed with HFD, with normal chow diet, with HFD and treated with 30 mg/kg of ATO or with normal chow diet and treated with the same dose of ATO for 8 weeks. Continual feeding with HFD deteriorated NAFLD and hyperlipidemia, treatment with dietary control, ATO or ATO with dietary control effectively improved serum and liver lipid metabolism and liver function. In comparison with ATO treatment, dietary control or combined with ATO treatment significantly reduced the liver weight and attenuated

the HFD-induced hyperlipidemia and liver steatosis in rats. Compared to ATO treatment or dietary control, combination of ATO and dietary control significantly reduced the levels of serum total cholesterol and low density lipoprotein cholesterol (LDL-C). However, the combination therapy did not significantly improve triglyceride and free fatty acid metabolism, hepatic steatosis, and liver function, as compared with dietary control alone. ATO treatment effectively improved NAFLD-related hyperlipidemia and inhibited liver steatosis, accompanied by modulating the expression of genes for regulating lipid metabolism. ATO enhanced the effect of dietary control on reducing the levels of serum total cholesterol and LDL-C, but not triglyceride, free fatty acid and hepatic steatosis in HFD-induced fatty liver and hyperlipidemia in rats.

Kocak *et al.*, (2015) investigated the hepatoprotective role of two different doses of simvastatin (SV) pretreatment in rats with experimental hepatic I/R injury in rats. Pretreatment with 5 mg/kg SV reduced malondialdehyde and nitric oxide levels and increased superoxide dismutase, glutathione peroxidase, and catalase activities significantly in I/R with 2.5 mg/kg SV compared with I/R group. In addition, SV decreased Kupffer cell activation, and hypoxia-inducible factor-1α and vascular endothelial growth factor protein levels. The results of this study suggest that 5 mg/kg SV pretreatment may be protective against hepatic I/R injury. This effect could be achieved by antioxidant and antiapoptotic activities.

Mousah *et al.*, (2016) assessed the protective effect of L-carnitine, atorvastatin, and vitamin A on progression of acetaminophen induced hepatotoxicity in rats. The rats in acetaminophen treated group, showed a significant elevation of serum ALT, AST, and ALP levels and a significant reduction of the GSH with a significant elevation of MDA

levels in liver compared to group-1. All of these results were ameliorated by administration of L-carnitine, atorvastatin, and vitamin A. L-carnitine, atorvastatin, and vitamin A have protective effects against acetaminophen induced hepatotoxicity.

Yu *et al.*, (2016) evaluated the protective effect of rosuvastatin treatment on the mechanism of oxidized low‐density lipoprotein (Ox‐LDL) in rats with liver fibrosis. Rats were divided into 3 groups: control group (A), obstructive jaundice models group (B) and rosuvastatin group (C). In groups B and C, the rat models were successfully established, and there were significant changes in the expression of Ox‐LDL and the three liver fibrosis indicators when compared to group A ($P<0.01$). However, the expression of Ox‐LDL and the three liver fibrosis indicators in group C were decreased compared with group B, while SOD increased and MDA decreased. The three liver fibrosis indicators were different in comparison to group B. Thus, there appeared to be an association between the expression of Ox‐LDL and liver fibrosis. Treatment with rosuvastatin could regulate the expression of Ox‐LDL and improve liver fibrosis in rat models with obstructive jaundice.

Wat *et al.*, (2016) determined if the combination use of Atorvastatin (AS) and *Fructus Schisandrae* aqueous extract (FSE) could (a) exert potent therapeutic effects not only on high-fat diet-induced hyperlipidemia, but also on hepatomegaly (enlarge of liver size) and hepatic steatosis (fatty liver); and (b) reduce side effects caused by intake of statin alone including increased incidence of elevated liver enzymes and liver toxicity in Sprague Dawley rats. These data suggested FSE has a potential beneficial effect on weight control and lipid metabolism in Sprague Dawley rats with diet-induced obesity, and the combination use

of FSE with AS could significantly prevent liver toxicity and anti-oxidative status induced by AS alone

Mansouri *et al.*, (2017) investigated the possible protection of pravastatin against hepatic oxidative stress and dysfunctions induced by doxorubicin in rats. Statins have beneficial effects on oxidative stress and inflammation. Pravastatin reduced the scale liver injury and protected liver functions and other biochemical parameters. Increase in MDA level associated with a reduction in antioxidant activities in the doxorubicin group was attenuated by pravastatin treatment. Results indicated that pravastatin has a protective effect on the liver against doxorubicin-induced hepatotoxicity in rats.

Abdel-Daim and Abdeen (2018) investigated the protective role of rosuvastatin (ROSU) and vitamin E (Vit E) against fipronil (FPN) induced hepatorenal toxicity in albino rats. The results revealed that FPN significantly increased serum levels of ALT , AST, ALP, LDH, cholesterol, urea, and creatinine. In addition, there were substantial increases in the liver and kidney contents of MDA and NO, along with significant decreases in glutathione, superoxide dismutase, catalase, and glutathione peroxidase. FPN also caused histological changes and increased the expression of caspase-3 in the liver and kidney tissues. However, administration of ROSU and Vit E alone or in combination ameliorated the FPN-induced oxidative damage and apoptosis, possibly through their antioxidant properties.

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Tested substances:

Piroxicam (Feldene®): It is dispensed as 2ml capacity injectable solution, Each 1 ml ampoul contains 20 mg piroxicam, It was obtained from Global pharmaceutical industries for Pfizer Egypt.

Rosuvastatin (Crestor®): It is dispensed as oral tablet, Each tablet contains 10 mg rosuvastatin. It was purchased from Astrazenca Group, 6th of October City, Giza, Egypt**.**

Rosuvastatin (Crestor®): An oral tablet, each tablet contains 20 mg rosuvastatin. It was purchased from Astrazenca group, 6th of October City, Giza, Egypt**.**

3.1.2. Experimental animals:

The present study was carried out on a total number of 25 white Albino male rats weighting 185-210 gm. Rats were obtained from Center of Laboratory Animal, Faculty of Veterinary Medicine, Benha University, Egypt.They acclimatized for one week prior to the experiment. All rats received standard laboratory balanced commercial diet and water *ad libitum*.

3.1.3. Material used for serum biochemical studies:

Special commercial diagnostic kits used for estimation of concentration of different biochemical parameters:

The special diagnostic kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, creatinine, urea, cholesterol and triglycerides were supplied from Centronic GmbH, Wartenberg, Germany.

3.1.4. Material used for oxidative cascade in liver and kidney tissues:

Special diagnostic kits of Glutathione reductase (GSH), Catalase enzyme activity (CAT) and Malondialdehyde (MDA) level, were supplied from Bio diagnostic company, Cairo, Egypt.

3.1.5. Reagents used for oxidative cascade in liver and kidney tissues:

1- Phosphate buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin.

2- Cold buffer (100 ml potassium phosphate, pH 7.0, containing 2 mlEDTA per gram tissue).

3.1.6. Chemicals for immunohistochemistry examination:

1- Formalin (10 %): from Middle East Company, Cairo, Egypt.

2- DAB,PBS ,H_2O_2 and Mayer's hematoxylin.

3- Anti BCL_2 antibody &Anti BAX antibody was obtained from Dako Corporation (Life Trade, Egypt).

4- ABC kit (Vector laboratories).

3.1.7. Chemicals for histopathological examination:

1- Formalin (10%): from Middle East Company, Cairo, Egypt.

2- Hematoxylin and Eosin (H&E) stain :from Middle East Company, Cairo, Egypt.

3.1.8. Equipment and apparatus:

3.1.8.1. Apparatus for hematological studies:

1- Automatic cell counter (H.A-Vet Clindiage, Belgium).

2- Centrifuge, Heraeus, W. Germany.

3- Microhematocrite centrifuge.

4- Clean dry weatherman tubes, without EDTA.

3.1.8.2. Apparatus for serum biochemical studies:

1- Spectrophotometer, JASCO 7800, uv/vis, JAPAN.

2- Clean and dry Eppendorf labeled tubes for serum preservation.

3.1.8.3. Apparatus for oxidative cascade:

1- Sonicator homogenizer.

2- Clean and dry Eppendorf labeled tubes for liver and kidney tissues preservation.

3- Cooling centrifuge Heraeus, W.GERMANY.

4- Refrigerator for preservation of samples.

3.1.8.4. Apparatus for histopathological imaging:

1- Slide microtome.

2- Light microscope: NOVEL, model XSZ-N107-1.

3.1.8.5. Apparatus for immunohistochemistry imaging:

1- Slide microtome.

2- Light microscope: NOVEL, model XSZ-N107-1.

3.2. METHODS

3.2.1. Experimental dose of piroxicam:

Piroxicam was administrated at a dose of 7 mg/kg b. wt intraperitoneally onece daily for 28 days according to **Abatan *et al.*, (2006)**.

3.2.2. Experimental dose of rosuvastatin:

Rosuvastatin was administrated at a dose of 10 mg/kg b.wt orally once daily for 28 days by stomach tube according to park et al .,(2009).

Rosuvastatin was administrated at a dose of 20 mg/kg b.wt orally once daily for 28 days by stomach tube according to **El Dawi *et al.*, (2013).**

3.2.3. Experimental design:

In the present study male albino rats were randomly assigned into 5 equal groups (5 rats each).

Group 1: Rats which served as the control was injected (I.p) with saline (the vehicle) once daily for 28 consecutive days.

Group 2: Rats in this group were received rosuvastatin (20 mg/kg b.wt.), orally once daily for 28 consecutive days.

Group 3: Rats in this group were served as piroxicam toxic control and were injected (I.p) (7 mg/kg b.wt.), once daily for 28 days.

Group 4: Rats in this group were received both piroxicam (7 mg/kg b.wt, I.p) and rosuvastatin (10 mg/kg b.wt, orally) once daily for 28 consecutive days.

Group 5: Rats in this group were received both piroxicam (7 mg/kg b.wt, I.p) and rosuvastatin (20 mg/kg b.wt, orally) once daily for 28 consecutive days.

Table (1): Experimental design:

Groups	N. of rats	Dose & rout of administration	Duration	Sampling
Group (1)	5	Saline solution once daily by i.p injection.	28 days	* Whole blood for erythrogram and leucogram. * Serum for biochemical parameters (ALT, AST, total protein, albumin, creatinine, urea, cholesterol and triglycerides. * Liver and kidney tissue specimens for oxidative cascade (GSH, CAT and MDA). * Liver and kidney tissue specimens for histopathological and immunohistochemical examination.
Group (2)	5	Rosuvastatin (20 mg/kg b.wt) orally once daily by stomach tube.	28 days	
Group (3)	5	Piroxicam (7 mg/kg b.wt) by I.p injection once daily.	28 days	
Group (2)	5	Piroxicam (7 mg/kg B.wt) by I.p injection once daily + Rosuvastatin (10 mg/kg b.wt) orally once daily by stomach tube.	28 days	
Group (5)	5	Piroxicam (7 mg/kg B.wt) by I.p injection once daily + Rosuvastatin (20 mg/kg b.wt) orally once daily by stomach tube.	28 days	

3.2.4. Sampling:

After 28 days, all rats were euthanized and blood samples, kidney and liver tissue were collected.

3.2.4.1. Blood samples:

Blood samples were collected by puncture of retro orbital plexus from 10 rats in each group after 8 weeks of experiment and divided as follow:

A- Blood received on disodium EDTA 10% solution (20 µl/ml blood) was used for hematological studies.

B- Serum obtained by blood collection in clean dry centrifuge tube. The serum was kept at -20 °C till used in the evaluation of biochemical studies.

3.2.4.2. Tissue specimens:

Liver and kidney were dissected out. Each organ was then washed with normal saline to separate the surrounding fat and connective tissues.

Samples from liver and kidney (1 gm) of 10 rats in each group were collected in labeled Eppendorf tubes at the end of the experiment. The samples were washed by physiological saline and kept in plastic bag separately then stored at -80°C for determination of oxidative cascade.

Other tissue samples (liver and kidney) were collected after 28 days of the experiment and fixed in 10% formalin for histopathological studies.

3.2.5. Hematological studies:

The hematological studies included erythrogram and leucogram were evaluated at the end of the experiment (after 28 days) directly by using automatic cell counter (H.A-Vet Clindiage, Belgium) which depends on both electrical and optical techniques according to Knapp et al., (1996).

The erythrogram include hemoglobin concentration, packed cell volume (PCV %) and total erythrocyte cell count.

3.2.6. Serum biochemical studies:

Serum AST, ALT, total protein, albumin, creatinine, urea, cholesterol and triglycerides were determined using diagnostic kits obtained from Centronic GmbH, Wartenberg, Germany. AST and ALT were estimated according to **Eidi *et al.*, (2006).** Urea was determined according to **Eisenwiener, (1976)** and creatinine according to **Allen, (1982).** While, total protein was determined according to **Gornall 1949** and albumin was determined according to **Doumas et al., (1971).** Cholesterol and triglyceides was determined according to **Ellefson and Garaway (1976); Buccolo (1973)**, respectively.

3.2.7. Preparation of liver and kidney homogenates:

The tissue was dissected and washed with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. One gram of each tissue was homogenized in 5 ml of 5-10 ml cold buffer (i.e., 50mM potassium phosphate, pH7.5 1mM EDTA) per gram tissue, using sonicator homogenizer. Aliquots of tissue homogenates was centrifuged by cooling centrifuge 4000 rpm for 20 min then stored at -20^{o}C till do biochemical analysis.

3.2.8. Detection of oxidative cascade:

Oxidative status was done by determination of the activity of reduce glotathione (GSH), catalase (CAT) and malondialdehyde (MDA), levels by using special diagnostic kits obtained from Bio diagnostic laboratory company, Egypt .the procdure was performed according to **Aebi, H., (1984)**, **Satoh, (1978) and Koracevic et al., (2001)** respectively.

3.2.9. Histopathological studies

Autopsy samples were taken from the liver and kidney of rats in different groups and fixed in 10% formalin for 24 hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 C in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparffinized (xylene) and stained by hematoxylin and eosin stains **(Banchroft, et al., 1996)** for histopathological examination by light microscope.

3.2.10. Immunhistochemical studies:

For immunostaining, liver and kidney sections were deparaffinized and dehydrated sequentially in graded ethyl alcohol. Next, the antigen retrieval was achieved by heating the slide in distilled water by autoclaving at 121°C for 5 min. After the endogenous peroxidase had been inactivated by immersing the slides in 3% H2O2 and washed 3 times in PBS, the slide was blocked in 5% bovine serum albumin blocking reagent for 20 min to reduce nonspecific reactions. Then, the slide was incubated with anti-caspase 3 primary monoclonal antibody

(1:100 dilution) at 37°C for 1 h followed by an incubation with avidin-biotin complex (ABC kit, Vector Laboratories) at 37°C for 45 min. The reaction product was visualized by treatment with 3,3-diaminobenzidine tetrahydrochloride (DAB), and the slide was counterstained with Mayer's hematoxylin. **Abdel-Daim and Abdeen(2018)**

3.2.11. Statistical analysis:

Statistical analysis was performed using SPSS (Version 20.0; SPSS Inc., Chicago, IL, USA). The significant differences between groups were evaluated by one way ANOVA using Duncan test as a post hoc. Results are expressed as mean ± SEM. $P<0.05$ was considered significant.

4. RESULTS

This study was conducted to evaluate the hepato and renal protective effect of Rosuvastatin against experimentally-induced piroxicam toxicity in rats.

4.1. Effect of rosuvastatin and/or piroxicam on hematological parameters:

In the present study, there was significant reduction in RBCs, Hb, and PCV and significant increase in WBCs following piroxicam administration at the dose rate of 7 mg/kg body weight once daily orally for 28 days when compared to control group. In groups (piroxicam+rosuvastatin 10 mg/kg & piroxicam+rosuvastatin 20 mg/kg), significant reduction in RBCs, Hb, and PCV and significant increase in WBCs when compared to control group. These results of hematology (RBCS, WBCs, Hb and PCV) were shown in **tables (1, 2, 3, 4)** and illustrated in **figures (1, 2, 3, 4)**, respectively.

Table (2): Effect of rosuvastatin and/or piroxicam on RBCs count (10^{12}/L) in blood of rats (n=5).

Groups	RBCs count (10^{12}/L)
Control Group (Saline, i.p.)	5.57 ± 0.13 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	5.33 ± 0.17 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	4.46 ± 0.50 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	3.74 ± 0.25 [bc]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	3.55 ± 0.15 [c]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (1): Effect of rosuvastatin and/or piroxicam on RBCs count (10^{12}/L) in blood of rats (n=5).

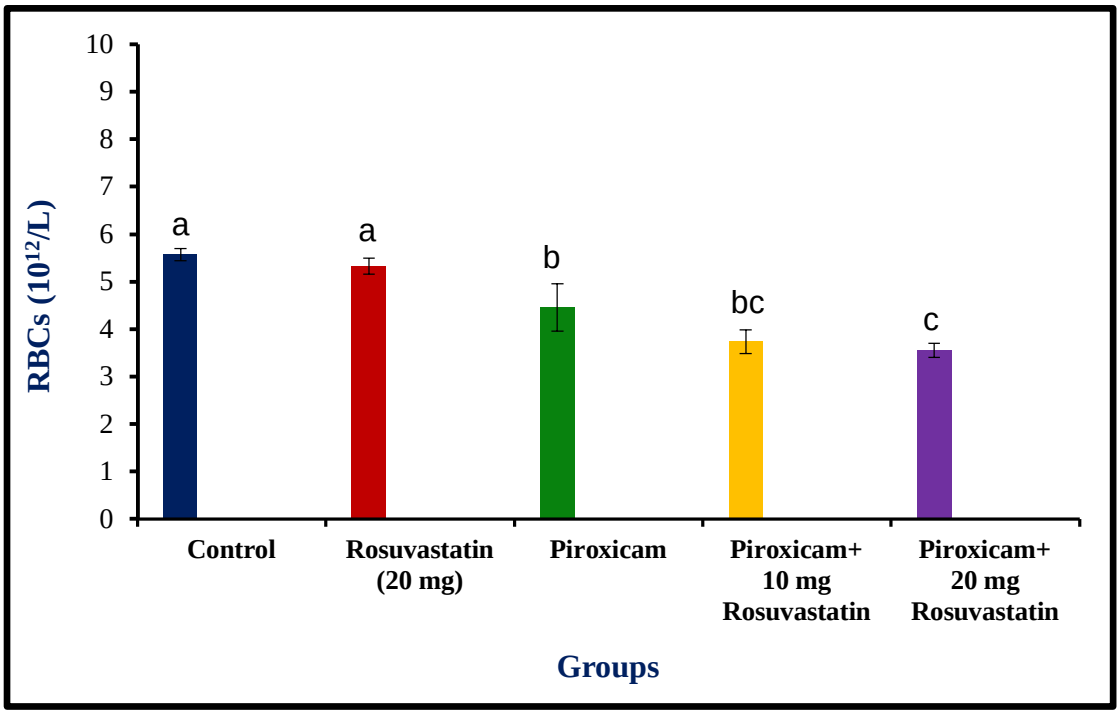


Table (3): Effect of rosuvastatin and/or piroxicam on WBCs count (10^9/L) in blood of rats (n=5).

Groups	WBCS count (10^9/L)
Control Group (Saline, i.p.)	11.62 ± 1.02 [c]
Rosuvastatin (20 mg/kg b.wt, p.o.)	17.87 ± 1.27 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	17.98 ± 1.14 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	18.47 ± 0.95 [b]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	21.75 ± 1.89 [a]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (2): Effect of rosuvastatin and/or piroxicam on WBCs count (10^9/L) in blood of rats (n=5).

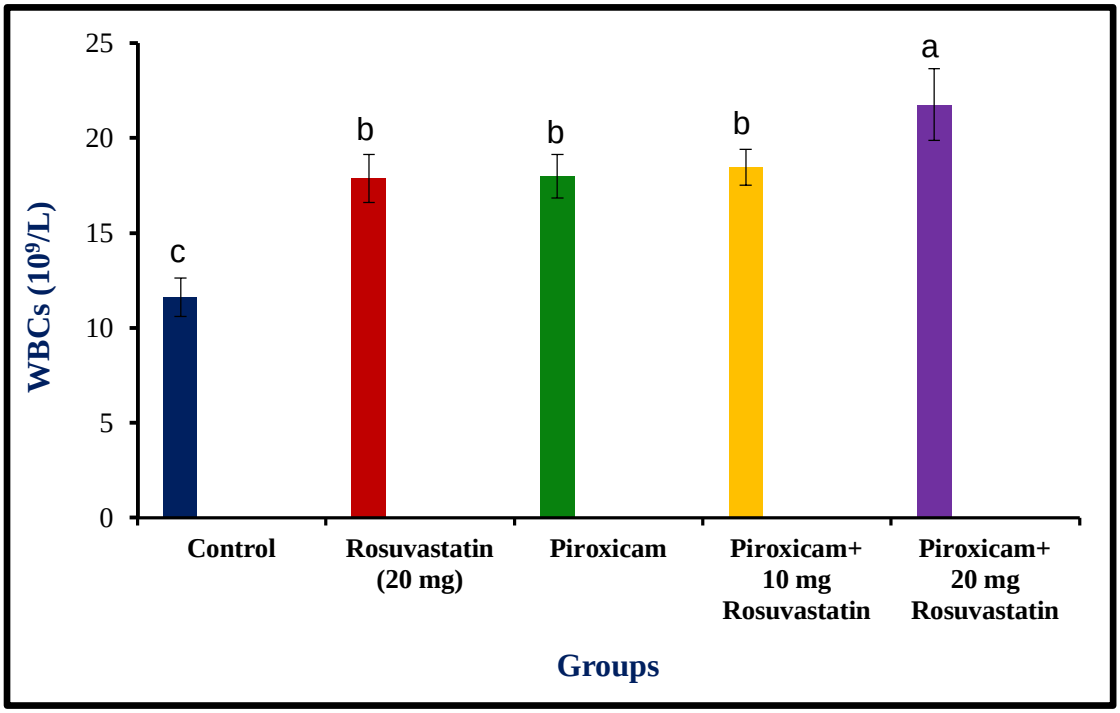


Table (4): Effect of rosuvastatin and/or piroxicam on Hb (g/dl) concentrations in blood of rats (n=5).

Groups	Hb concentrations (g/dl)
Control Group (Saline, i.p.)	11.88 ± 0.30 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	11.58 ± 0.53 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	9.22 ± 1.28 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	7.36 ± 0.76 [bc]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	5.84 ± 0.63 [c]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (3): Effect of rosuvastatin and/or piroxicam on Hb (g/dl) concentrations in blood of rats (n=5).

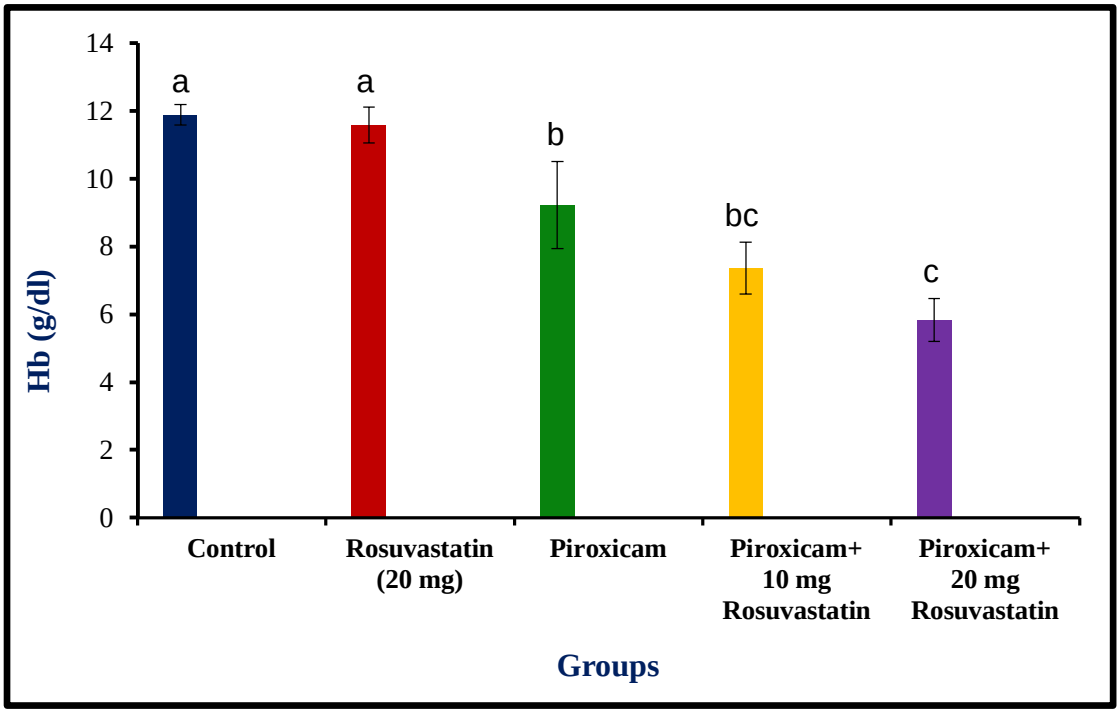
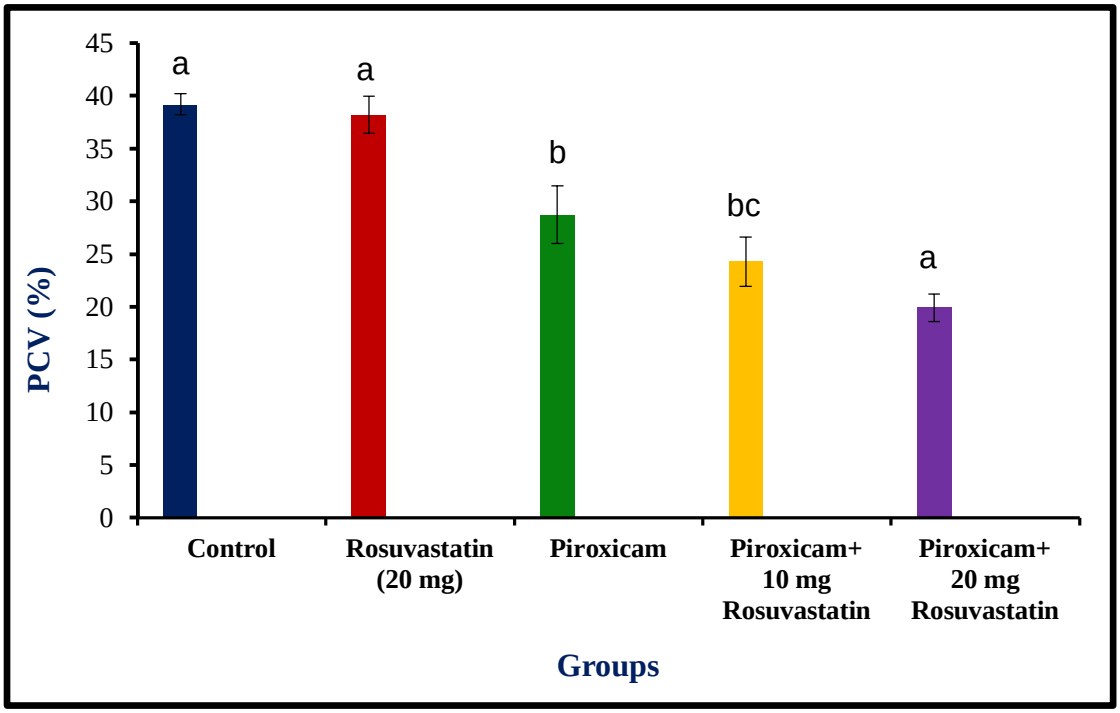


Table (5): Effect of rosuvastatin and/or piroxicam on PCV (%) in blood of rats (n=5).

Groups	PCV (%)
Control Group (Saline, i.p.)	39.20 ± 0.99 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	38.21 ± 1.76 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	28.74 ± 2.73 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	24.28 ± 2.35 [bc]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	19.91 ± 1.29 [a]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (4): Effect of rosuvastatin and/or piroxicam on PCV (%) in blood of rats (n=5).



4.2. Effect of rosuvastatin and/or piroxicam on biochemical parameters:

The concentration of the AST in serum was increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg treated group, AST concentration was significantally increased when compared to control group, while in piroxicam+rosuvastatin 20 mg/kg treated group, AST concentration was non significantally increased when compared to control group and these results were shown in **table(5)** and illustrated in **figure (5).**

The concentration of the ALT in serum was increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, ALT concentration was non significantally

increased when compared to control group, and these results were shown in **table (6)** and illustrated in **figure (6).**

The concentration of the total protein in serum was decreased in piroxicam treated group and this decrease was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg treated group, total protein concentration was significantally decreased when compared to control group, while in piroxicam+rosuvastatin 20 mg/kg treated group, total protein concentration was non significantally decreased when compared to control group and these results were shown in **table (7)** and illustrated in **figure (7).**

The concentration of the albumin in serum was decreased in piroxicam treated group and this decrease was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, albumin concentration was non significantally decreased when compared to control group and these results were shown in **table (8)** and illustrated in **figure (8).**

The concentration of the creatinine in serum was increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, creatinine concentration was non significantally increased when compared to control group and these results were shown in **table (9)** and illustrated in **figure (9).**

The concentrations of the urea in serum were increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, urea concentration was non significantally increased when compared to control group and these results were shown in **table (10)** and illustrated in **figure (10).**

Table (6): Effect of rosuvastatin and/or piroxicam on aspartate aminotransferase (AST) concentration (U/L) in serum of rats (n=5).

Groups	**AST concentrations (U/L)**
Control Group (Saline, i.p.)	114.20 ± 6.64 [b]
Rosuvastatin (20 mg/kg b.wt, p.o.)	106.18 ± 2.31 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	144.35 ± 3.30 [a]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	141.52 ± 4.39 [a]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	122.12 ± 10.03 [b]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (5): Effect of rosuvastatin and/or piroxicam on aspartate aminotransferase (AST) concentration (U/L) in serum of rats (n=5).

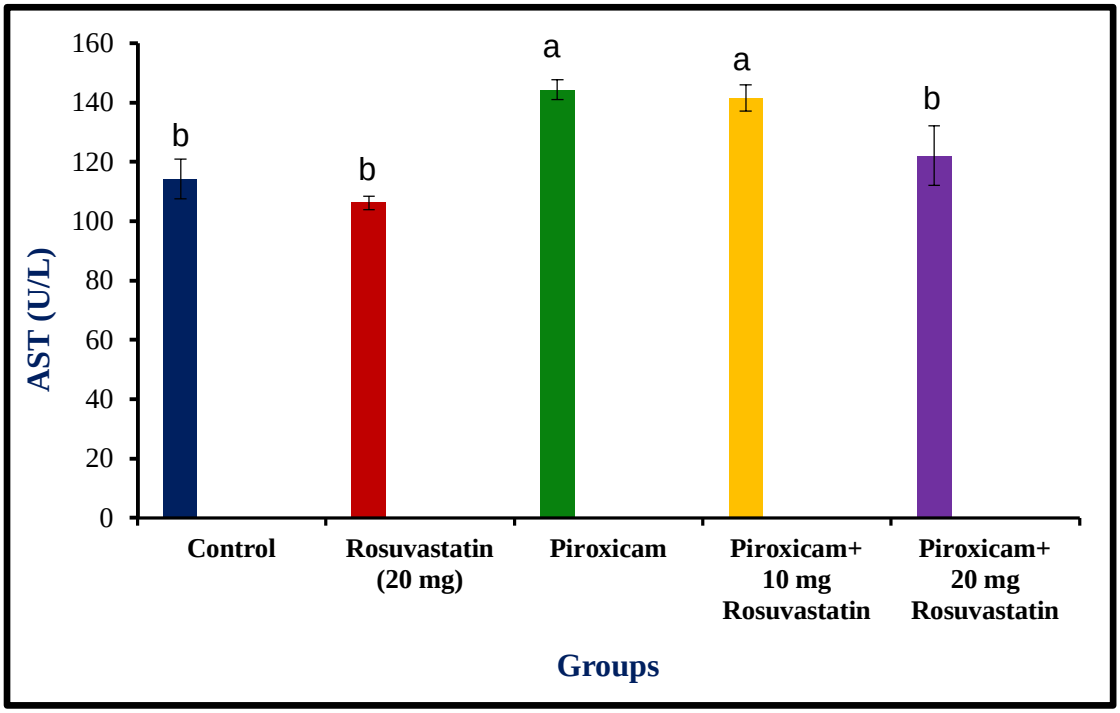


Table (7): Effect of rosuvastatin and/or piroxicam on alanine aminotransferase (ALT) concentration (U/L) in serum of rats (n=5).

Groups	**ALT concentrations (U/L)**
Control Group (Saline, i.p.)	22.66 ± 0.88 [b]
Rosuvastatin (20 mg/kg b.wt, p.o.)	20.80 ± 0.37 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	74.20 ± 10.24 [a]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	32.27 ± 1.65 [b]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	25.62 ± 1.22 [b]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (6): Effect of rosuvastatin and/or piroxicam on alanine aminotransferase (ALT) concentration (U/L) in serum of rats (n=5).

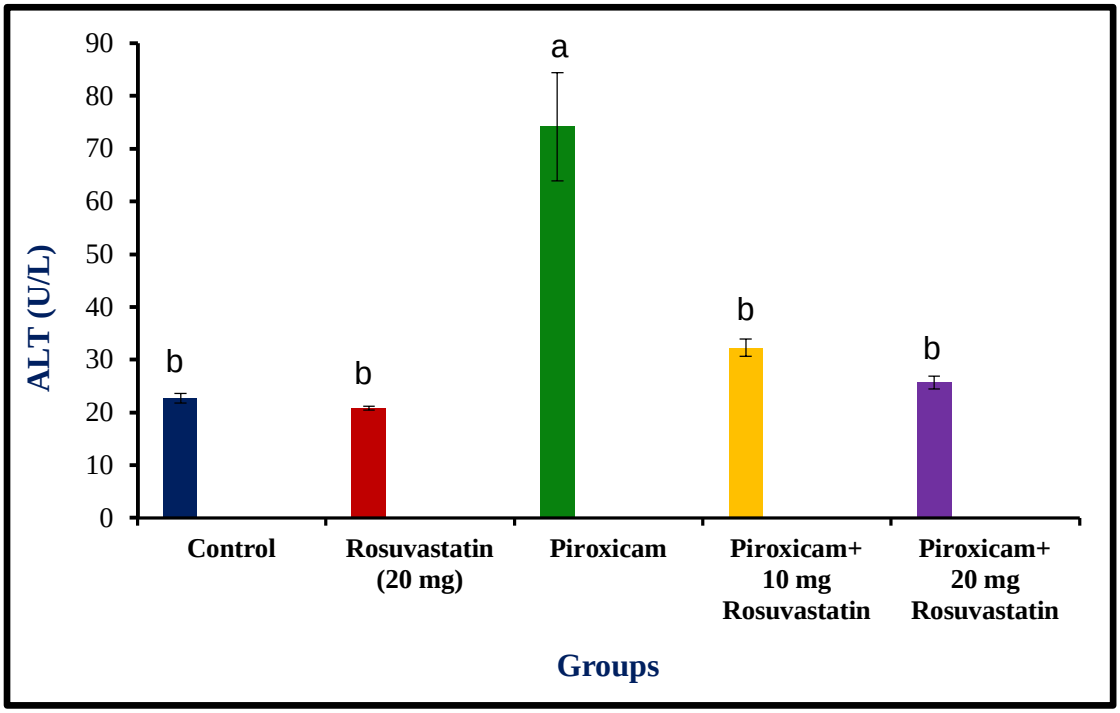


Table (8): Effect of rosuvastatin and/or piroxicam on total protein concentration (g/dl) in serum of rats (n=5).

Groups	T. protein concentrations (g/dl)
Control Group (Saline, i.p.)	7.02 ± 0.27 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	6.98 ± 0.16 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	5.34 ± 0.26 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	5.30± 0.32 [b]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	6.28 ± 0.14 [a]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (7): Effect of rosuvastatin and/or piroxicam on total protein concentration (mg/dl) in serum of rats (n=5).

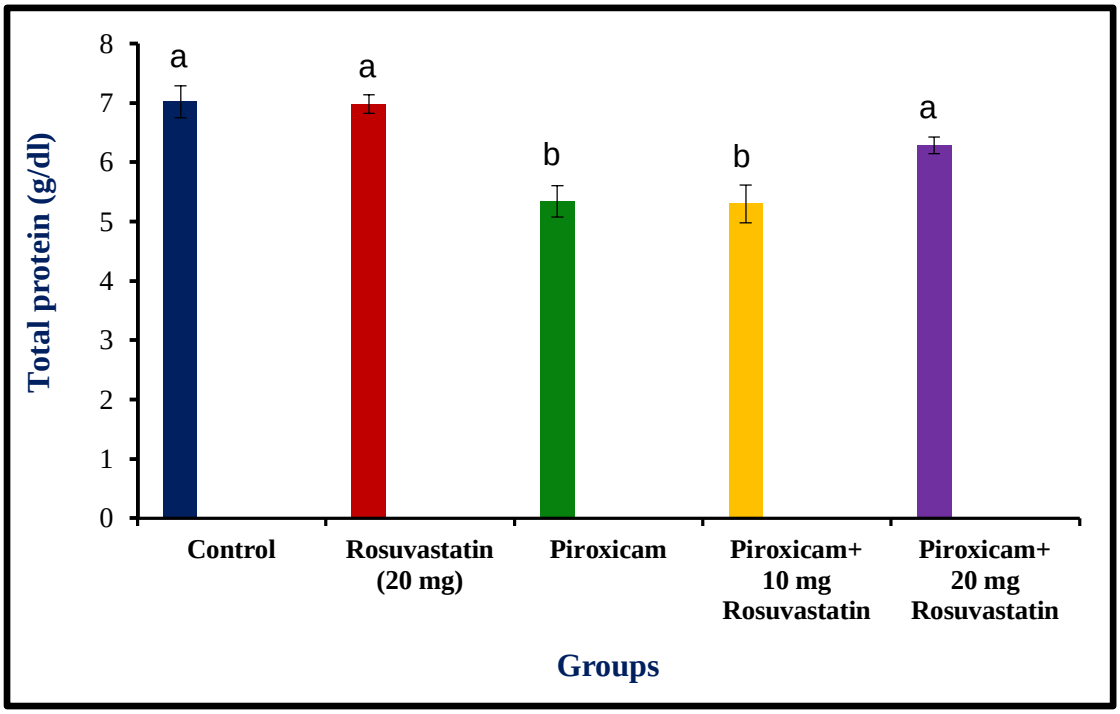


Table (9): Effect of rosuvastatin and/or piroxicam on albumin concentration (g/dl) in serum of rats (n=5).

Groups	Albumin concentrations (g/dl)
Control Group (Saline, i.p.)	3.50 ± 0.10 [ab]
Rosuvastatin (20 mg/kg b.wt, p.o.)	3.69 ± 0.01 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	2.63 ± 0.08 [c]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	3.12 ± 0.15 [b]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	3.34 ± 0.23 [ab]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (8): Effect of rosuvastatin and/or piroxicam on albumin concentration (g/dl) in serum of rats (n=5).

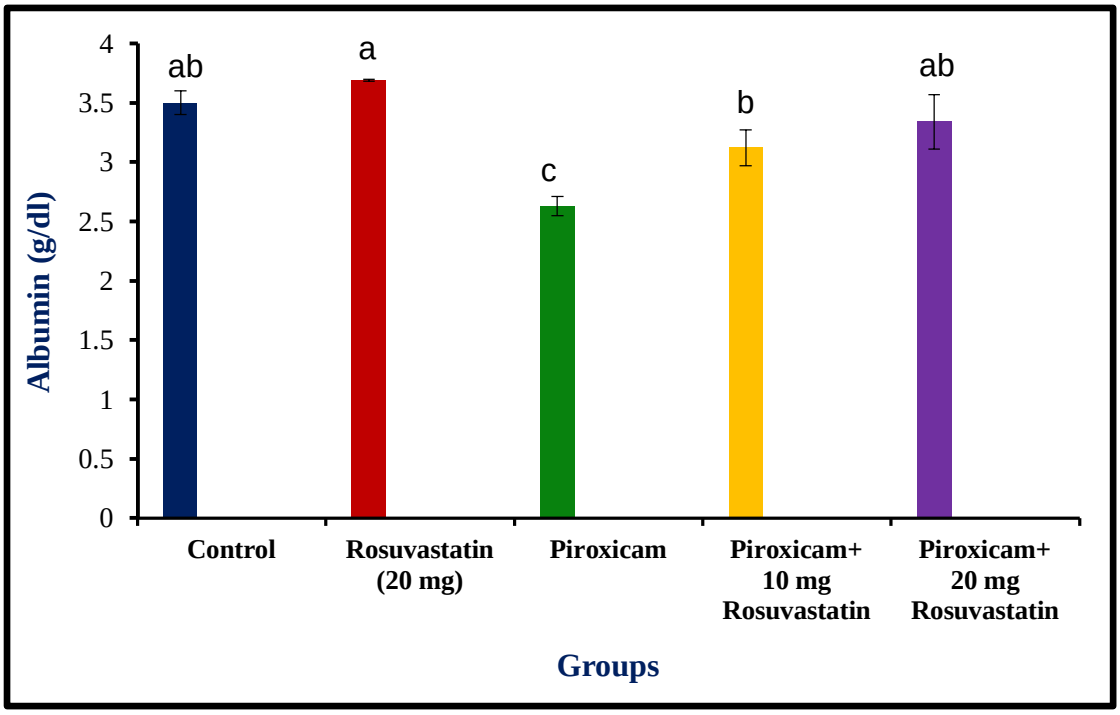


Table (10): Effect of rosuvastatin and/or piroxicam on creatinine concentration (mg/dl) in serum of rats (n=5).

Groups	**Creatinine concentrations (mg/dl)**
Control Group (Saline, i.p.)	0.63 ± 0.02 ^{bc}
Rosuvastatin (20 mg/kg b.wt, p.o.)	0.59 ± 0.03 ^{c}
Piroxicam treated (7 mg/kg b.wt, i.p.)	0.92 ± 0.09 ^{a}
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	0.81 ± 0.06 ^{ab}
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	0.72 ± 0.04 ^{bc}

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (9): Effect of rosuvastatin and/or piroxicam on creatinine concentration (mg/dl) in serum of rats (n=5).

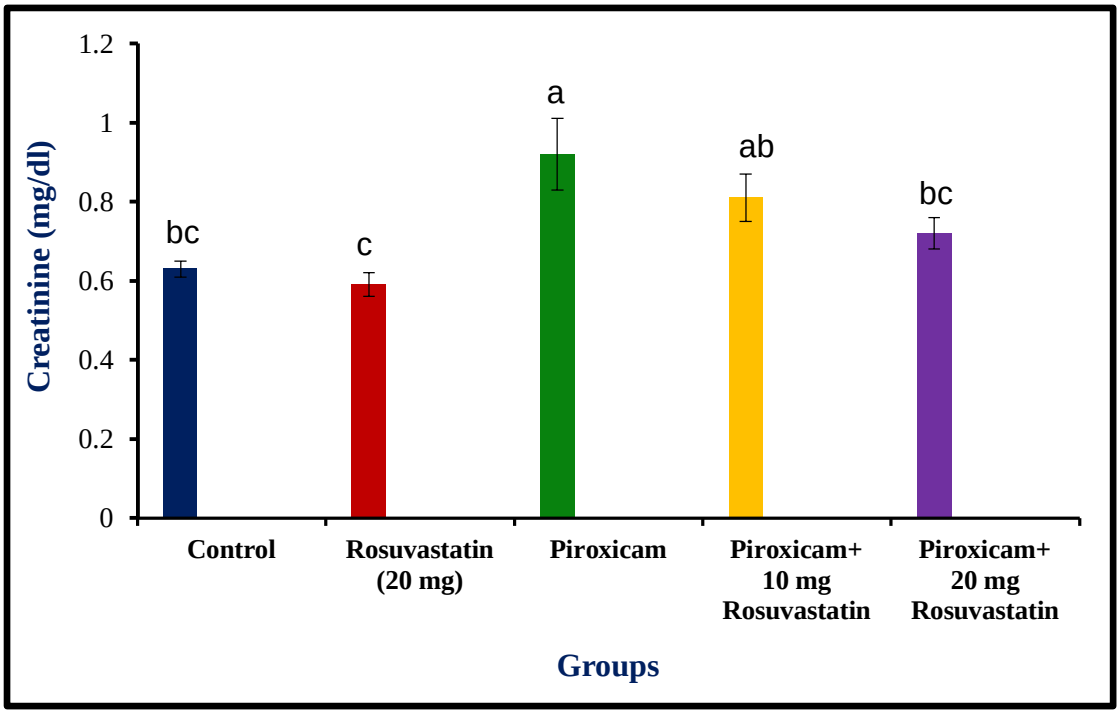
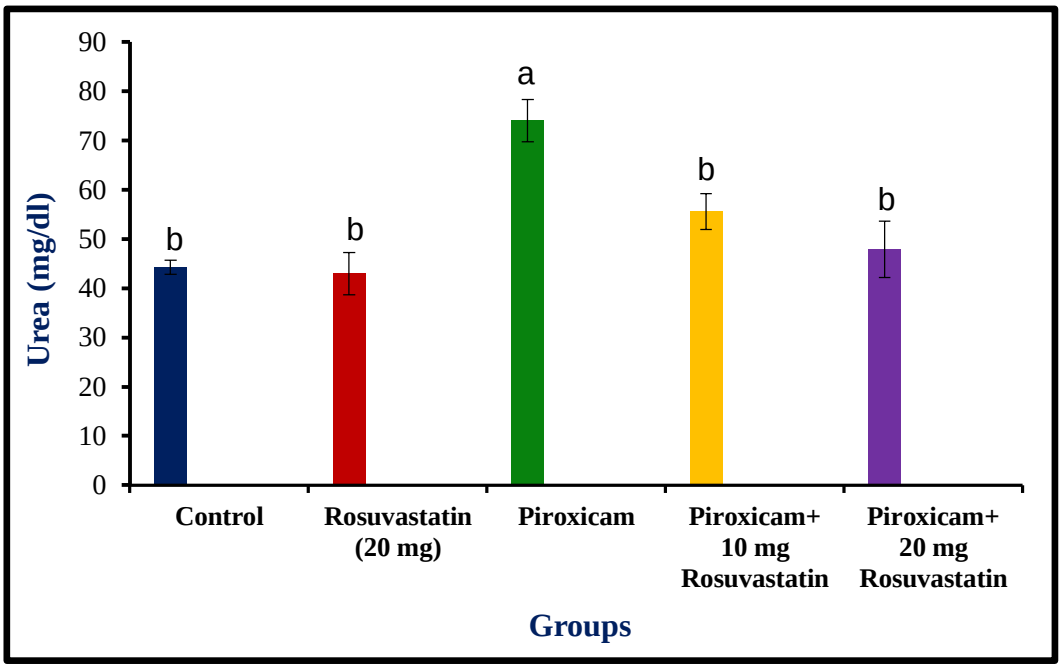


Table (11): Effect of rosuvastatin and/or piroxicam on urea concentration (mg/dl) in serum of rats (n=5).

Groups	**Urea concentrations (mg/dl)**
Control Group (Saline, i.p.)	44.26 ± 1.83 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	43 ± 4.27 [b]
Rosuvastatin (20 mg/kg b.wt, p.o.)	74.02 ± 4.31 [a]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	55.62 ± 3.63 [b]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	47.92 ± 5.70 [b]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure(10): Effect of rosuvastatin and/or piroxicam on urea concentration (mg/dl) in serum of rats (n=5).



4.3. Effect of rosuvastatin and/or piroxicam on lipid profile:

The concentrations of the cholesterol in serum were decreased in piroxicam treated group and this decrease was non significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+ rosuvastatin 20 mg/kg treated groups, cholesterol concentration was significantally decreased when compared to control group. Rosuvastatin treated group showed significant decrease in cholesterol concentration and these results were shown in **table (11)** and illustrated in **figure (11).**

The concentrations of the triglycerides in serum were decreased in piroxicam treated group and this decrease was non significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+ rosuvastatin 20 mg/kg treated groups, triglycerides concentration was significantally decreased when compared to control group. Rosuvastatin treated group showed non significant decrease in triglycerides concentration and these results were shown in **table (12)** and illustrated in **figure (12).**

Table (12): Effect of rosuvastatin and/or piroxicam on cholesterol concentration (mg/dl) in serum of rats (n=5).

Groups	Cholesterol concentrations (mg/dl)
Control Group (Saline, i.p.)	74.42 ± 5.79 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	56.77 ± 3.97 [bc]
Piroxicam treated (7 mg/kg b.wt, i.p.)	67.58 ± 4.46 [ab]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	46.60 ± 2.27 [cd]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	42.95 ± 2.47 [d]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (11): Effect of rosuvastatin and/or piroxicam on cholesterol concentration (mg/dl) in serum of rats (n=5).

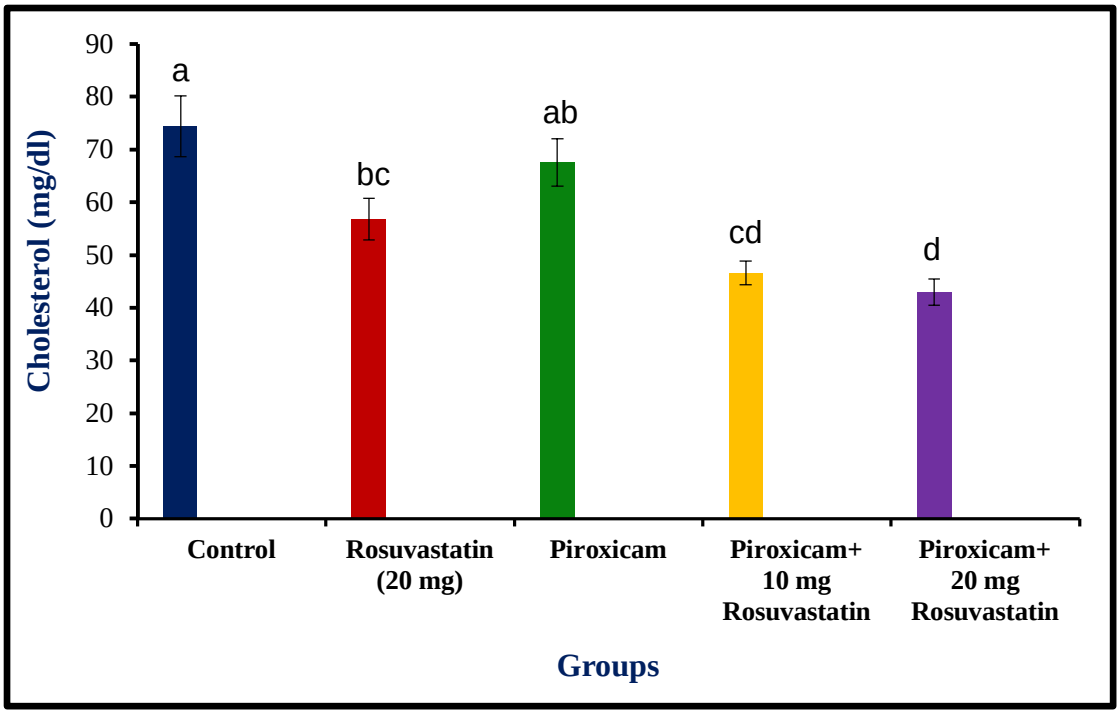
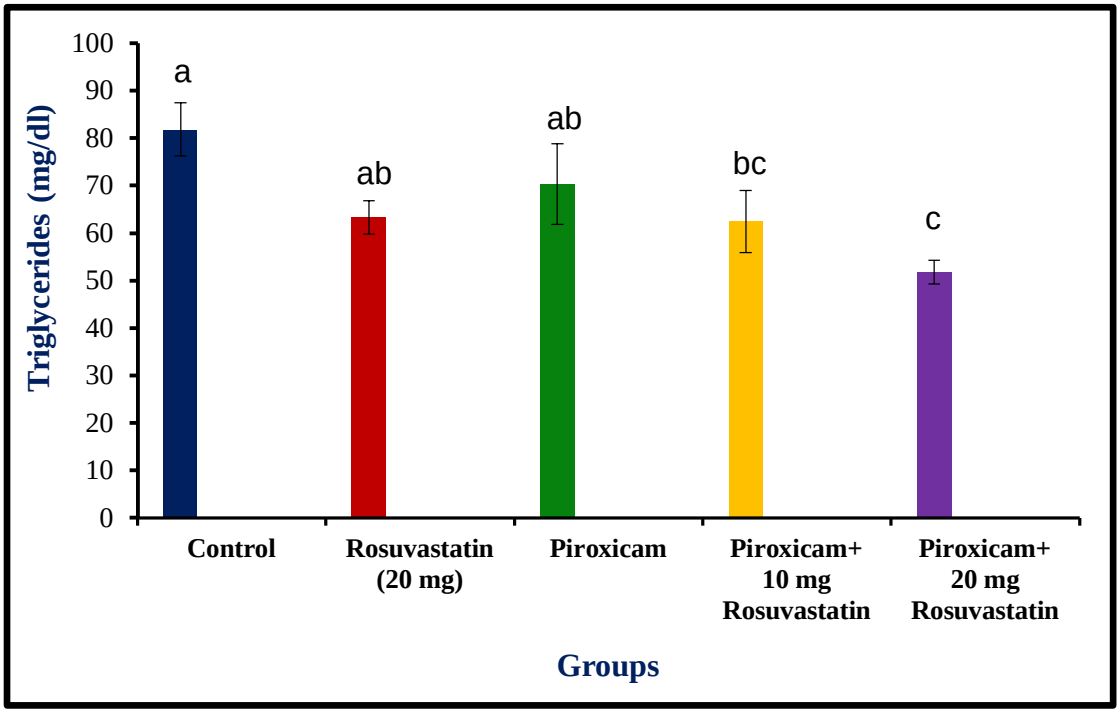


Table (13): Effect of rosuvastatin and/or piroxicam on triglycerides concentration (mg/dl) in serum of rats (n=5).

Groups	Triglycerides concentrations (mg/dl)
Control Group (Saline, i.p.)	81.82 ± 5.58 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	63.35 ± 3.51 [ab]
Piroxicam treated (7 mg/kg b.wt, i.p.)	70.32 ± 8.50 [ab]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	62.48 ± 6.53 [bc]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	51.77 ± 2.49 [c]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (12): Effect of rosuvastatin and/or piroxicam on triglycerides concentration (mg/dl) in serum of rats (n=5).



4.4. Effect of rosuvastatin and/or piroxicam on oxidative stress markers:

In the present study, there were substantial increases in MDA level along with dramatic decreases in GSH, and CAT in the liver and kidney tissues of piroxicam-intoxicated rats, indicating the presence of oxidative stress. These results for GSH, CAT and MDA in liver tissues were shown in **tables (13, 14, 15)** and illustrated in **figures (13, 14, 15)**, respectively. The results for GSH, CAT and MDA in kidney tissues were shown in **tables (16, 17, 18)** and illustrated in figures **(16, 17, 18)**, respectively.

Table (14): Effect of rosuvastatin and/or piroxicam on Glutathione (GSH) concentration (mg/gm) in liver tissue (n=5).

Groups	**GSH concentrations (mg/gm)**
Control Group (Saline, i.p.)	57.26 ± 4.70 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	52.52 ± 1.66 [ab]
Piroxicam treated (7 mg/kg b.wt, i.p.)	38.90 ± 1.32 [d]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	44.72 ± 1.34 [cd]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	47.58 ± 1.81 [bc]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (13): Effect of rosuvastatin and/or piroxicam on Glutathione (GSH) concentration (mg/gm) in liver tissue (n=5).

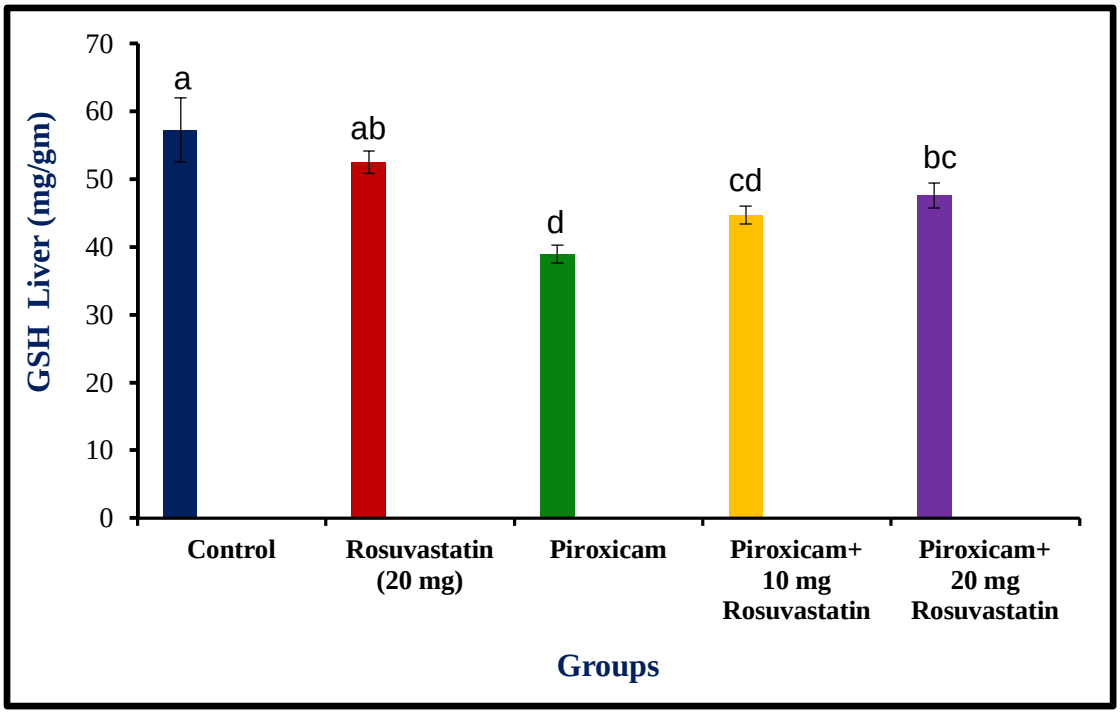


Table (15): Effect of rosuvastatin and/or piroxicam on catalase (CAT) activity (U/gm) in liver tissue (n=5).

Groups	CAT activity (U/gm)
Control Group (Saline, i.p.)	486.29 ± 8.84 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	482.49 ± 6.62 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	437.15 ± 14.93 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	469.32 ± 18.25 [ab]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	476.98 ± 12.44 [a]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (14): Effect of rosuvastatin and/or piroxicam on catalase (CAT) activity (U/gm) in liver tissue (n=5).

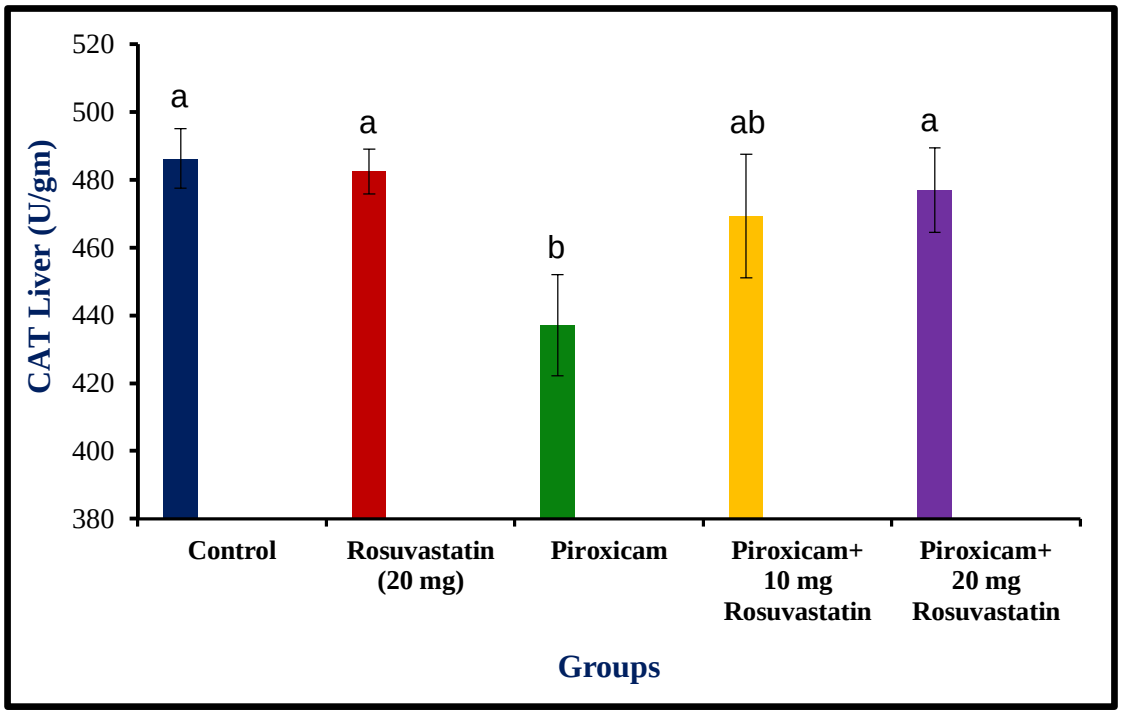


Table (16): Effect of rosuvastatin and/or piroxicam on malonaldehyde (MDA) activity (nmol/gm) in liver tissue (n=5).

Groups	MDA activity (nmol/gm)
Control Group (Saline, i.p.)	274.84 ± 23.46 [b]
Rosuvastatin (20 mg/kg b.wt, p.o.)	272.49 ± 24.69 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	503.68 ± 43.50 [a]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	369.74 ± 34.38 [ab]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	301.09 ± 26.48 [b]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure(15): Effect of rosuvastatin and/or piroxicam on malonaldehyde (MDA) activity (nmol/gm) in liver tissue (n=5).

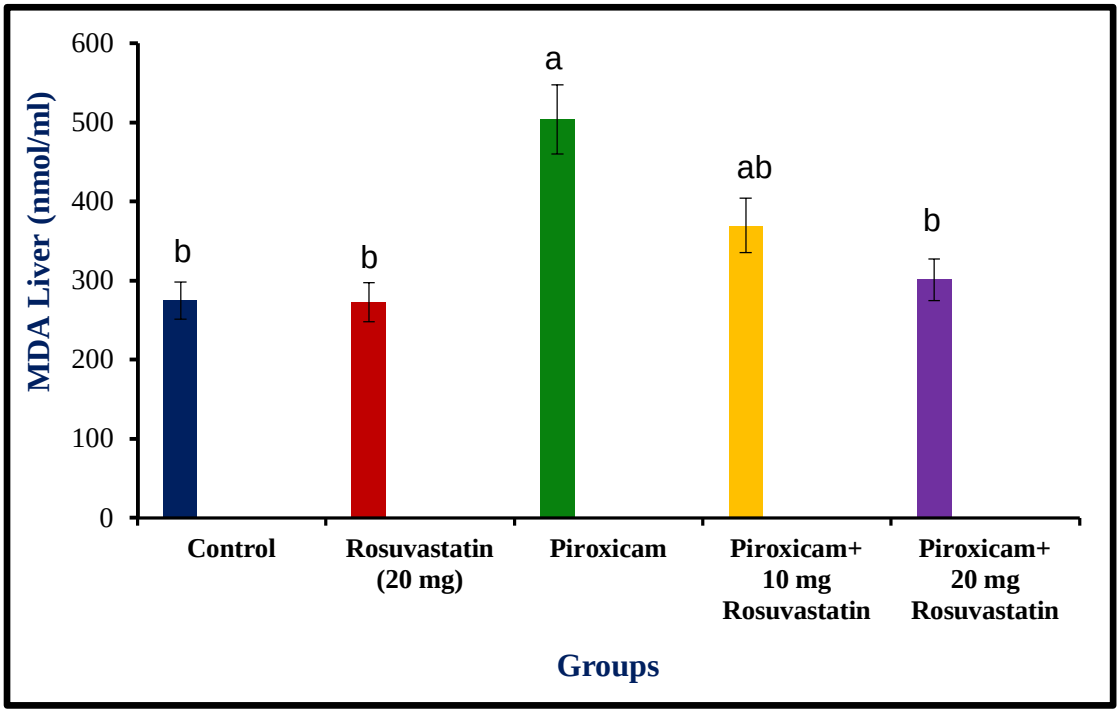


Table (17): Effect of rosuvastatin and/or piroxicam on reduced Glutathione (GSH) concentration (mg/gm) in kidney tissue (n=5).

Groups	**GSH concentrations (mg/gm)**
Control Group (Saline, i.p.)	67.08 ± 2.83 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	66.64 ± 2.52 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	52.17 ± 3.51 [b]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	60.72 ± 1.78 [a]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	61.52 ± 0.36 [a]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (16): Effect of rosuvastatin and/or piroxicam on Glutathione (GSH) concentration (mg/gm) in kidney tissue (n=5).

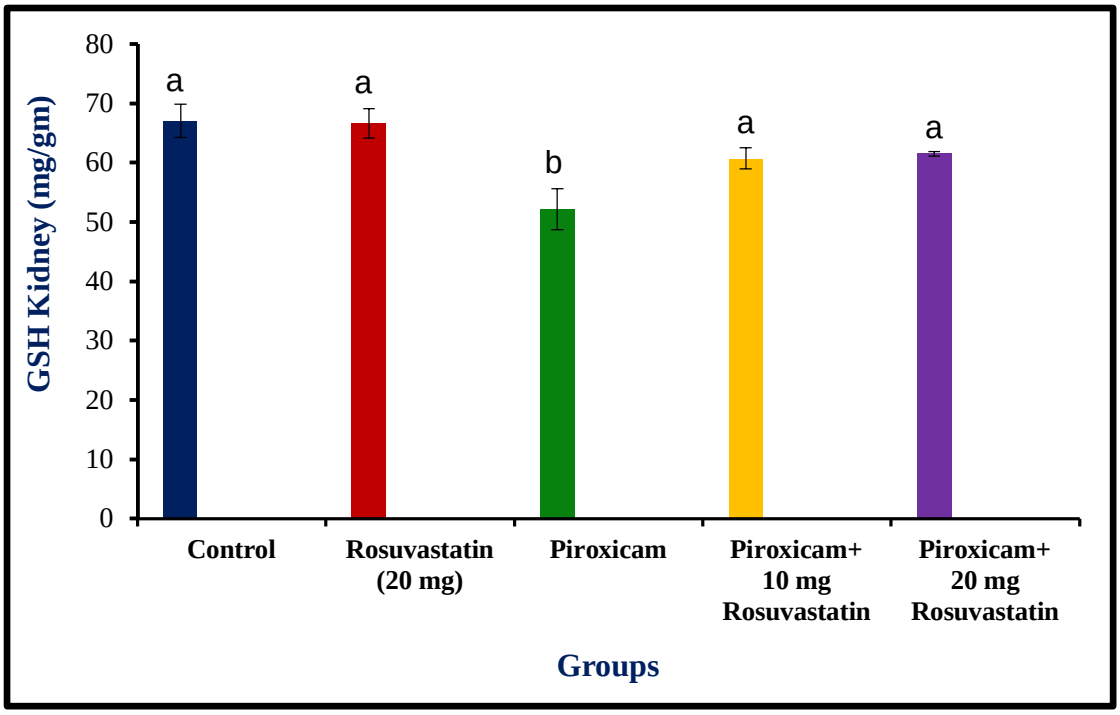


Table (18): Effect of rosuvastatin and/or piroxicam on catalase (CAT) activity (U/gm) in kidney tissue (n=5).

Groups	CAT acticity (U/L)
Control Group (Saline, i.p.)	509.35 ± 7.45 [a]
Rosuvastatin (20 mg/kg b.wt, p.o.)	509.09 ± 8.48 [a]
Piroxicam treated (7 mg/kg b.wt, i.p.)	438.40 ± 18.87 [c]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	461.30 ± 16.89 [bc]
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	480.93 ± 2.96 [ab]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (17): Effect of rosuvastatin and/or piroxicam on catalase (CAT) activity (U/gm) in kidney tissue (n=5).

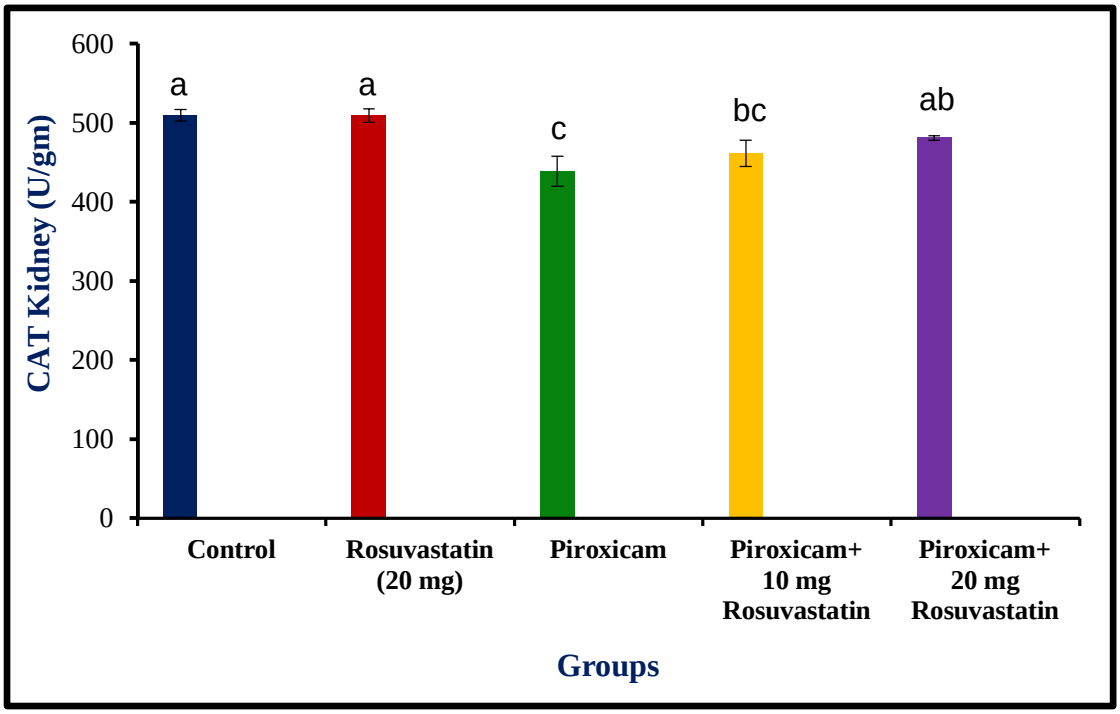
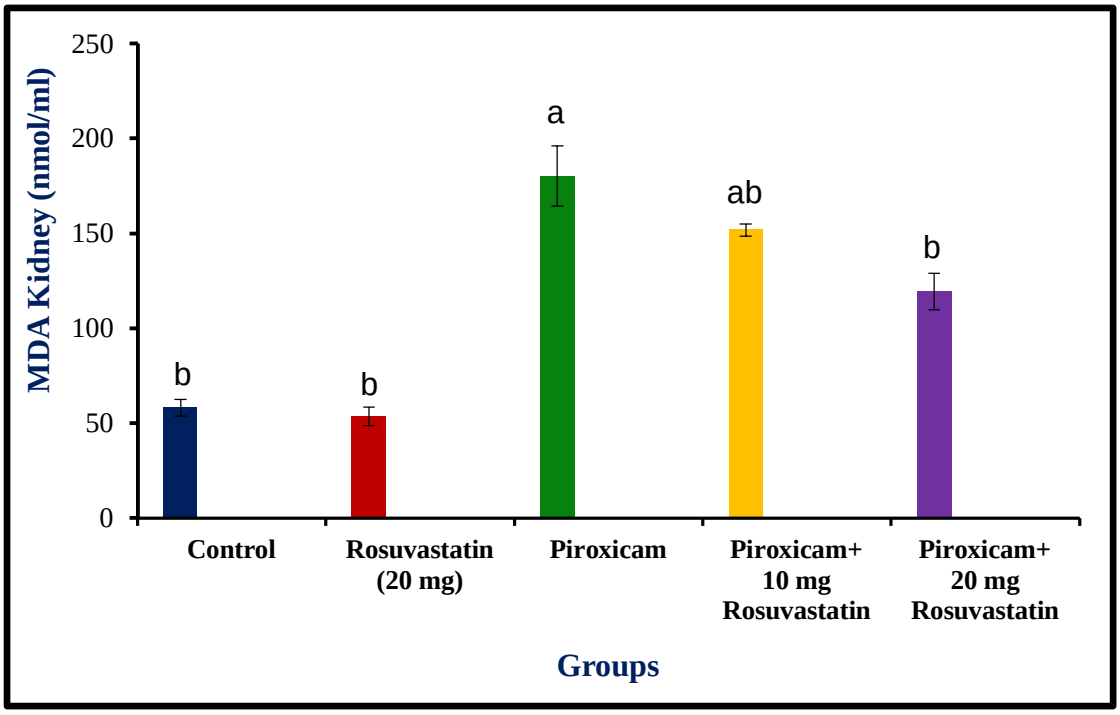


Table (19): Effect of rosuvastatin and/or piroxicam on malonaldehyde (MDA) activity (nmol/gm) in kidney tissue (n=5).

Groups	MDA concentrations (nmol/gm)
Control Group (Saline, i.p.)	58.14 ± 4.47 [b]
Rosuvastatin (20 mg/kg b.wt, p.o.)	53.48 ± 4.92 [b]
Piroxicam treated (7 mg/kg b.wt, i.p.)	180.19 ± 15.83 [a]
Piroxicam (10 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	151.72 ± 3.10 [a]b
Piroxicam (20 mg/kg b.wt, i.p.) and **Rosuvastatin** (7 mg/kg b.wt, p.o.)	119.42 ± 9.69 [b]

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

Figure (18): Effect of rosuvastatin and/or piroxicam on malonaldehyde (MDA) activity (nmol/gm) in kidney tissue (n=5).



4.5. Effect of rosuvastatin and/or piroxicam on histopathological changes in liver and kidney:

In piroxicam intoxicated rat liver tissue showed, sever lymphocytic infiltrations, dilatation of portal vein, and fatty degenerations. Piroxicam and rosuvastatin treated groups showed marked improvement in the hepatic histological appearance indicated by mild fatty degenerations and inflammatory cell infiltrations.

Kidney of piroxicam intoxicated rat showed, sever loss of brush border, tubular vacuolization and dilatation, and mononuclear inflammatory cell infiltration. Piroxicam and rosuvastatin treated groups showed marked improvement in the kidney histological appearance indicated by mild loss of brush border and inflammatory cell infiltration.

5. DISCUSSION

Piroxicam is a commonly prescribed NSAID for its analgesic, antipyretic, and anti-inflammatory properties. However, it has been reported that piroxicam has deleterious effects on liver and kidney tissues induction of oxidative stress and initiation of apoptotic mechanisms. ROSU was described to have antioxidant effect independent to its anti-hyperlipidemic action. Therefore, this study was designed to investigate the potential protective effect of ROSU against piroxicam-induced oxidative damage and apoptosis in liver and kidney tissues.

In the current study, the effect of piroxicam at the dose 7 mg/kg for 28 days on hematology, serum biochemical and histopathological changes have been investigated. RBC_S count, Hb, PCV, and WBC_S were estimated to evaluate the effect of piroxicam on the blood picture. Transaminases were determined to find out effect of piroxicam on hepatic system. ALP was measured to see the cellular damage and non-specific tissue irritation due to piroxicam. Total protein and albumin were measured to determine the effect of piroxicam on protein metabolism. Creatinine and BUN were estimated to estimate the effect of piroxicam on kidney function and excretory system. Histopathology and immunohistochemistry were performed as well to evaluate the degree of piroxicam-induced tissue damage in liver and kidney.

In the present study, there was significant reduction in RBCs, Hb, and PCV suggesting the development of anemia as a result of the effect of piroxicam on DNA synthesis as well as protein synthesis **(Amare, 2009)**.The decreased Hb suggests the hypochromic anaemia due to adverse effect of piroxicam this results are in agreement with **Abatan *et al.*, (2006).** These data are in the same line with that obtained by

Misraulia (2002) who studied the effect of meloxicam at the rate of 0.2, 1.0 and 2.0 mg/kg b.wt. In addition, there was increasing in WBC_s value which might be due to illumination of inflammatory cell infiltrations in response to piroxicam insult. This results are in agreement with the previous studies **(El-Banhawy *et al.*, 1994; McCafferty *et al.*,1995)** who suggested that abundance of leucocytes were prominent in response of body tissues facing any injurious impact.

Liver is well known to have three main functions: storage, metabolism, and biosynthesis. Glucose was converted to glycogen and stored; when needed for energy, it is converted back to glucose. Cholesterol uptake also occurs in the liver. Fat-soluble vitamins, fat and other nutrients are also stored in the liver. Fatty acids were metabolized and converted to lipids, then it conjugated with proteins which synthesized in the liver and released into blood as lipoproteins. Numerous functional proteins such as, enzymes and blood-coagulating factors are also synthesized by the liver. In addition, the liver, which contains numerous xenobiotic metabolizing enzymes, is the main site of xenobiotic metabolism **(Hogson and Levi, 2004)**. The more specific parameter to liver was ALT, and thus is a better parameter for examining the liver injury. AST mainly found in mitochondria of hepatocytes. Thus, to evaluate liver injury, AST and ALT are the most common biochemical markers **(Girish *et al.*, 2009)**.

Aminotransferases (ALT, AST) and ALP are cytoplasmic enzymes whose rise in serum levels are attributed to damaged structural integrity of the liver resulting from their released into the blood circulation after the rupture of the plasma membranes **(David *et al.*, 2014;Velmurugan *et al.*, 2014)**. The aminotransferases (ALT, AST), and ALP are among serum biomarkers of hepatic function which their increases in the serum

indicate hepatic damage **(David *et al.*, 2014)**, whereas decreased levels of total protein and albumin in the serum indicate hepatic damage **(Kanwal *et al.*, 2012)**. Our data revealed that piroxicam caused severe liver damage indicated by increases in serum ALT, AST, and ALP levels along with significant decreases in serum total protein and albumin when compared with the control animals confirming the data obtained by **lapeyer *et al.*, (2006) and Sahu and Ghosal, (2007)**.

Serum proteins are formed by lymphocytic tissues and the liver. A low protein level is observed in blood loss, hepatopathy, and malnutrition. Loss of protein during inflammation or ulceration of the gastrointestinal tract could lead to impaired absorption as well as loss of serum protein due to injured mucosal cells. Damage to the kidney is also responsible for the loss of plasma protein and causes their low concentration **(Jain, 1986)**. The obtained data are in agreement with that reported by **Misraulia (2002)** who observed reduction in serum total protein in the rats treated with various NSAIDs and their combinations for 10 days.

Moreover, **MacAllister *et al.*, (1993)** studied the relative toxicity of phenylbutazone, flunixin meglumine and ketoprofen in healthy adult horses. Phenylbutazone and ketoprofen treated horses had a significant decrease in serum total protein and albumin concentrations. Reduction in total serum protein has also been reported in dogs following prolonged treatment of loxoprofen sodium **(Peter *et al.*, 2003)**. The decrease in plasma total protein in the present study could be due to blood loss and ulceration observed in intestine and stomach during post mortem examination. **Gomes *et al.*, (1999)** reported that the reduction in proteins may be resulted from an hepatocytes impairment and a reduce in amino

acid availability and/or in hepatic protein capability would decrease the total serum concentration.

Kidney has important role in removing wastes like creatinine and urea, regulating the balance of electrolytes and controlling the body's fluid balance. For the kidneys to carry out their normal functions they have to be in good condition both functionally and structurally **(Thomas, 2005)**. The present study revealed a significant increase in serum creatinine and BUN concentrations in piroxicam treated group as compared to control. It has been reported that BUN level was significantly increased when male calves were treated with flunixin meglumine **(Nazifi *et al.*, 2002)**. Creatinine is formed from creatine which stores energy in muscles in the form of phosphocreatine. When physical activity of the body is normal, the creatinine in blood remains within normal range. In agreement with this result, **Ramesh *et al.*, (2001)** reported significant increase in serum creatinine level in dog treated with nimesulide (2 mg/kg). Increased levels of both creatinine and urea levels in serum have been considered as index of assessing nephrotoxicity **(Ali *et al.*, 2001)**. High urea level indicates kidney dysfunction, but its values varies with liver metabolic capacity, protein intake and renal perfusion so it gives a poor indication for measuring the renal function, however, creatinine shows the excretion of waste products through urine **(Khan and Anderson, 2003)**.

Rosuvastatin administration ameliorated both liver and kidney changes confirming the protective role of rosuvastatin against hepatic and renal toxicity induced by piroxicam in rats and that is agreed with results of **İşeri et al., (2007)** who examined the protective effect of simvastatin against cisplatin-induced renal toxicity in rats. Furthermore, **Ozbek *et al.*, (2009)** evaluated the effect of atorvastatin against gentamicin-induced

nephrotoxicity in rats. Co-administration of atorvastatin prevented gentamicin-induced increases in BUN and serum creatinine levels. **Mousah *et al.*, (2016)** assessed the protective effect of atorvastatin, on acetaminophen induced hepatotoxicity in rats. Acetaminophen treated group showed a significant elevation of serum ALT and AST levels. All of these results were ameliorated by administration of atorvastatin, which have protective effects against acetaminophen induced hepatotoxicity. **Selim *et al.*, (2017)** also mentioned that amikacin significantly increased serum levels of urea and creatinine. Meanwhile, serum levels of total protein and albumin were decreased. Conversely, administration of rosuvastatin with amikacin ameliorated such renal damage.

Cholesterol and triglycerides were the important lipids whose increasing was implicated in these disease conditions. High levels of low-density cholesterol, triglycerides, and total cholesterol with decreased HDL-cholesterol will enhance atherosclerosis development and others cerebrovascular disturbances **(Nwanjo, 2004)**. In the present study, piroxicam and rosuvastatin lowered the cholesterol and triglycerides levels. **Kourounakis *et al.*, (2002)** studied the effects of ip administration of NSAIDs in experimentally induced hyperlipidemia in rats The NSAIDs used in this experimental model were selective or non-selective COX-1 inhibitors as well as one non selective COX-2 inhibitor. Most of the drugs significantly reduced the total cholesterol and triglycerides concentrations in the plasma of hyperlipidemic rats. While studies link atheromatosis to inflammation, these results potentially also link anti-inflammatory activity with hypolipidemia. Thus, NSAIDs not only may address the inflammatory aspect of atherosclerosis but also may contribute directly by inducing hypolipidemia.

Ahmed *et al*., (2015) suggested that celecoxib and nimesulide might be used in combination with atorvastatin or other drugs. In this way, both nimesulide and celecoxib will increase levels of HDL-cholesterol as well decrease levels of LDL-cholesterol. This approach of treating dyslipidemia is quite novel and fruitful instead of using high dose of statins with increased probability of adverse effects, especially in the elderly.

ROS are naturally generated in all mammalian cells during normal cellular respiration. Since ROS are cytotoxic molecules even when produced during normal respiration, for cell survival, they are naturally neutralized by the endogenous antioxidant defense system, primarily GSH, SOD, and CAT **(Avery, 2011; Small et al., 2012)**. When there is an imbalance between ROS production and antioxidants, the cell becomes vulnerable to severe oxidative stress-induced damage. ROS can attack cell membranes and other cellular molecules, causing lipid peroxidation, protein oxidation, and DNA damage, which results in cell disruption and loss of function and can lead to diseases such as cancers, atherosclerosis, diabetes, and renal failure **(Abdel-Daim *et al*., 2015)**. In the current study, MDA, a marker of lipid peroxidation, was drastically increased with significant decreases in GSH and CAT levels in piroxicam-intoxicated animals. This finding indicates cell membrane damage in hepatic and renal cells, which is attributed to the increased production of OH.

However, ROSU administration ameliorated both liver and kidney changes confirming the protective role of ROSU against hepatic and renal toxicity induced by piroxicam in rats and that is agreed with results of **İşeri *et al*., (2007)** who mentioned that simvastatin decreased cisplatin-induced increase in myeloperoxidase (MPO) activity in the kidney but did

not improve cisplatin-induced changes in renal MDA and GSH contents. **Ozbek *et al.*, (2009)** evaluated the effect of atorvastatin against gentamicin-induced nephrotoxicity in rats. Co-administration of atorvastatin prevented gentamicin-induced reduction in renal tissue GSH levels and elevation of kidney MDA and NO levels. In addition to **An *et al.*, (2011)** who mentioned that cisplatin significantly increased renal MDA level, decreased kidney GSH level, and inhibited activities of antioxidant enzymes including CAT, SOD and GPx. All these oxidative changes significantly attenuated by pravastatin administration.

Our data concerning oxidative stress biomarkers were consistent with that obtained by **Selim *et al.*, (2017)** who found that amikacin significantly increased the kidney content of MDA, whereas GSH content and CAT activity were decreased. Conversely, administration of rosuvastatin with amikacin ameliorated such damage and reduced DNA fragmentation, apoptosis, and necrosis.

The potential antioxidant and anti-apoptotic capacity of ROSU have been also investigated by **Abdel-Daim and Abdeen (2018)** who proved that administration of ROSU ameliorated the fipronil-induced oxidative damage and apoptosis, possibly through its antioxidant properties.

Our histopathological examination showed that liver of piroxicam intoxicated rat has sever lymphocytic infiltrations, dilatation of portal vein, and fatty degenerations was caused due to increased permeability of blood vessels that occurs when the contraction of the endothelial cells of blood vessels in response to certain chemicals or as a result of loss of particles desmosomes, which lies between the endothelial cells, which allows the passage of blood vessels. When blood vessels expand caused the rush of inflammatory cells from the center to the periphery

endothelial lining the blood of to find its way out of the vessel **(Majeed and Abass, 2017). Abatan *et al.*, (2006)** also reported mild periportal hepatic necrosis and Kupffer cell proliferation in rats treated with indomethacin at 5mg/kg; piroxicam at 15mg/kg; aspirin at 20mg/kg; and phenylbutazone at 10 mg/kg for 14 days. Similarly piroxicam induced severe hepatocellular necrosis was reported by **Ebaid *et al.*, (2007)** who studied piroxicam-induced hepatic and renal histopathological changes in mice.

Piroxicam and rosuvastatin treated groups showed marked improvement in the hepatic histological appearance indicated by mild fatty degenerations and inflammatory cell infiltrations.

Whereas, kidney of piroxicam intoxicated rat showed, sever loss of brush border, tubular vacuolization and dilatation, and mononuclear inflammatory cell infiltration. These effects may be appeared because the drug inhibited both Na^+ transport- dependent and Na^+ independent ATP utilization as well as mitochondrial oxidative phosphorylation in the renal proximal tubules. The results of the present study are in accordance with the previous studies such as that reported by **Mogliner *et al.*, (2006)** who studied the toxic effect of indomethacin, piroxicam, and dicholenac in rats. These changes were also consistent with those induced histopathological changes and increasing occurrence of hepatotoxicity suggests that these toxic metabolites can induce oxidative stress in the liver in rat **(chen *et al.*, 2002)** Also these results are in agreement with **(Ebiad *et al.*, 2007)** observed that the Piroxicam may cause liver toxicity by reduction of poly saccharine, and total protein in the liver tissue, the decrease in carbohydrate content may be due to increased stress in organs.

On the other hand, piroxicam and rosuvastatin treated groups showed marked improvement in the kidney histological appearance indicated by mild loss of brush border and inflammatory cell infiltration.

Bcl-2 (B-cell lymphoma 2) represents a specific functional protein that regulates cell fate **(Czabotar *et al.*, 2014)** especially inhibition of apoptosis. Instead, apoptosis regulator BAX is a protein that functions as pro-apoptotic regulator involved in cell death **(Liu *et al.*, 2016)**. Therefore, our findings exhibited that abnormally altered expressions of Bcl-2, and BAX proteins in liver and kidney cells by piroxicam treatment, indicating that apoptosis-dependent pathway was occurred in piroxicam-damaged liver and kidney as well (figure.21,22,23&24 in B photo).

In contrast, here, co-administration of ROSU could ameliorate piroxicam induced damage through neutralization of ROS and up-regulation of antioxidant enzymes with down-regulation of Bcl-2 and Bax protecting the cell from lipid peroxidation, DNA damage, and from apoptosis as well (figure.21,22,23&24 in C, D photos). Consistently, **Selim et al., (2017)** has examined the protective effects of ROSU against amikacin-induced nephrotoxicity. His findings support ours that administration of ROSU could ameliorate the amikacin-induced tissue damage, apoptosis, and necrosis.

7. CONCLUSIONS

From the present study it was concluded that:

- Statins (rosuvastatin) and nonsteroidal anti-inflammatory drugs (NSAIDs) (piroxicam) are among the most commonly prescribed medications. So, using (piroxicam) for its anti-infalammatory, analgesic and anti-pyretic activities in hyperlipidemic condition (as rosuvastatin was recommended).

- Rosuvastatin bring all the parameters affected by piroxicam near to normal values.

- Rosuvastatin has been shown to be effective against piroxicam induced toxicity as it showed marked improvement in hematological and biochemical alterations, tissue damage and oxidative changes in liver and kidney.

- Thus rosuvastatin has protective effect which minimizes the hepato-renal toxicity induced by piroxicam, thereby suggesting its use as a potent hepatic and nephro protective agent.

7. SUMMARY

This experimental study was conducted to evaluate the hepatorenal protective effect of rosuvastatin and antioxidant activity against experimentally hepatorenal toxicity induced by piroxicam in rats. Twenty five adult male Wistar albino rats (185-210 g) were randomly grouped into five groups (each of 5 rats).

Group 1: Rats which served as the control was injected (i.p) saline (the vehicle) once daily for 28 consecutive days.

Group 2: Rats in this group were received rosuvastatin (20 mg/kg b.wt.), orally once daily for 28 consecutive days.

Group 3: Rats in this group were served as piroxicam toxic control and were injected (i.p) (7 mg/kg b.wt.), once daily for 28 days.

Group 4: Rats in this group were received both piroxicam (7 mg/kg b.wt, i.p) and rosuvastatin (10 mg/kg b.wt, orally) once daily for 28 consecutive days.

Group 5: Rats in this group were received both piroxicam (7 mg/kg b.wt, i.p) and rosuvastatin (20 mg/kg b.wt, orally) once daily for 28 consecutive days

In the present study, there was significant reduction in RBCs, Hb, and PCV and significant increase in WBCs following piroxicam administration at the dose rate of 7 mg/kg body weight once daily orally for 28 days when compared to control group. In groups (piroxicam+rosuvastatin 10 mg/kg & piroxicam+rosuvastatin 20 mg/kg), significant reduction in RBCs, Hb, and PCV and significant increase in WBCs when compared to control group.

The concentrations of the AST in serum were increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg treated group, AST concentrations were significantally increased when compared to control group, while in piroxicam+rosuvastatin 20 mg/kg treated group, AST concentrations were non significantally increased when compared to control group.

The concentrations of the ALT in serum were increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, ALT concentrations were non significantally increased when compared to control group

The concentrations of the total protein in serum were decreased in piroxicam treated group and this decrease was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg treated group, total protein concentration was significantally decreased when compared to control group, while in piroxicam+rosuvastatin 20 mg/kg treated group, total protein concentration was non significantally decreased when compared to control group.

The concentrations of the albumin in serum were decreased in piroxicam treated group and this decrease was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, albumin concentration was non significantally decreased when compared to control group and these results.

The concentrations of the creatinine in serum were increased in piroxicam treated group and this increase was significantly compared to

control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, creatinine concentration was non significantally increased when compared to control group.

The concentrations of the urea in serum were increased in piroxicam treated group and this increase was significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, urea concentration was non significantally increased when compared to control group.

The concentrations of the cholesterol in serum were decreased in piroxicam treated group and this decrease was non significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, cholesterol concentration was significantally decreased when compared to control group. Rosuvastatin treated group showed significant decrease in cholesterol concentration.

The concentrations of the triglycerides in serum were decreased in piroxicam treated group and this decrease was non significantly compared to control group. In piroxicam+rosuvastatin 10 mg/kg and piroxicam+rosuvastatin 20 mg/kg treated groups, triglycerides concentration was significantally decreased when compared to control group. Rosuvastatin treated group showed non significant decrease in triglycerides concentration.

In the present study, there were substantial increases in MDA level along with dramatic decreases in GSH, and CAT in the liver and kidney tissues of piroxicam-intoxicated rats, indicating the presence of oxidative stress.

Histopathologically, liver of piroxicam intoxicated rat showed, sever lymphocytic infiltrations, dilatation of portal vein, and fatty degenerations. Piroxicam and rosuvastatin treated groups showed marked improvement in the hepatic histological appearance indicated by mild fatty degenerations and inflammatory cell infiltrations.

Histopathologically, kidney of piroxicam intoxicated rat showed, sever loss of brush border, tubular vacuolization and dilatation, and mononuclear inflammatory cell infiltration. Piroxicam and rosuvastatin treated groups showed marked improvement in the kidney histological appearance indicated by mild loss of brush border and inflammatory cell infiltration.

Histopathological effect of piroxicam on liver and kidney tissues was also markedly improved by co-administration of rosuvastatin and piroxicam.

From the present study it could be concluded that, rosuvastatin with its antioxidant properties reduces liver and kidney damage caused by piroxicam in rats.

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السيرة الذاتية للبـــاحثه

- الإسم: دينا عبدالناصر جودة الجزار.
- ولدت الباحثه عام ١٩٨٤ ببنها – محافظة القليوبية.
- حصلت الباحثه علي شهادة إتمام الدراسة الإبتدائية عام١٩٩٥.
- حصلت الباحثه علي شهادة إتمام الدراسة الإعداديه عام١٩٩٨.
- حصلت الباحثه علي شهادة إتمام الدراسة الثانويه عام٢٠٠١.
- إلتحقت الباحثه بكلية الصيدلة – جامعة مصر للعلوم والتكنولجيا وحصلت علي شهادة البكالوريوس في العلوم الصيدلية٢٠٠٦.
- سجلت الباحثه للحصول علي درجة الماجستير في العلوم الطبيه البيطريه (مادة الفارماكولوجيا) عام ٢٠١٦.
- تعمل الباحثه حاليا صيدلانية بمستشفى بنها الجامعي.

الملخص العربى

استهدفت هذه الدراسه تقييم التأثير الوقائي الكبدي والكلوي للروسوفاستاتين في الجرزان المُحدَث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام.

فى هذه الدراسه تم اجراء التجربه على خمس وعشرون ذكرا من الجرزان البيضاء من النوع الألبينو ويتراوح متوسط اوزنها ما بين (١٨٥ - ٢١٠ جم) وقد تم تقسيم الجرزان الى خمس مجموعات و كل مجموعه تتكون من خمسة جرزان :

- **المجموعه الأولى (الضابطة):** تم حقن الجرزان في هذه المجموعه محلول ملح عن طريق الحقن البروتوني مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية.
- **المجموعه الثانيه:** تم تجريع الجرزان في هذه المجموعه بالروسوفاستاتين(٢٠ ملليجرام/كجم وزن) عن طريق الفم مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية.
- **المجموعه الثالثه:** تم حقن الجرزان في هذه المجموعه بالبيروكسيكام (٧ ملليجرام/كجم وزن) عن طريق الحقن البروتوني مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية.
- **المجموعه الرابعه:** تم حقن الجرزان في هذه المجموعه بالبيروكسيكام (٧ ملليجرام/كجم وزن) عن طريق الحقن البروتوني مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية + تجريع الجرزان في هذه المجموعه بالروسوفاستاتين (١٠ ملليجرام/كجم وزن)عن طريق الفم مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية.
- **المجموعه الخامسه:** تم حقن الجرزان في هذه المجموعه بالبيروكسيكام (٧ ملليجرام /كجم وزن) عن طريق الحقن البروتوني مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية + تجريع الجرزان في هذه المجموعه بالروسوفاستاتين (٢٠ ملليجرام/كجم وزن).عن طريق الفم مره واحده يوميا لمده ثمانيه وعشرون يوم متتالية .

كما إشتملت الدراسه أيضا على التأثير علي صوره الدم (عدد كرات الدم الحمراء (RBCs) وكرات الدم البيضاء (WBCs) وحجم الخلايا المعبأه (PCV) وعلى تركيزالهيموجلوبين Hb). والتأثيرات البيوكيميائية للبيروكسيكام والروسوفاستاتين فى الجرزان و التى تشمل التأثير على تركيز الإنزيمات الكبدية، البروتين، الألبومين، الكرياتينين، اليوريا، الكوليستيرول والدهون الثلاثيه (ثلاثي الجلسريد) فى مصل الدم. وتأثير الروسوفاستاتين كمضاد

للأكسده. كما تم أيضا إستبيان تأثير الروسوفاستاتين على التغيرات الهستوباثولوجية للكلي والكبد فى الجرزانالمُحدَث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام.

وكانت النتائج كالتالى :-

البيروكسيكام يسبب انخفاض في عدد كرات الدم الحمراء (RBCs) وحجم الخلايا المعبأه (PCV) وعلى تركيزالهيموجلوبين (Hb) وزياده في عدد كرات الدم البيضاء (WBCs). إعطاء الروسوفاستاتين١٠ملليجرام /كجم اوالروسوفاستاتين٢٠ملليجرام/كجم مع البيروكسيكام٧ملليجرام/كجم يؤدي الي عودة تركيزات هذه القياسات نحو المعدلات الطبيعيه بعد ٢٨ يوم.

الروسوفاستاتين ١٠ ملليجرامم/كجم او٢٠ملليجرام/كجم يقلل تركيز الإنزيمات الكبدية (اسبرتات امينو ترانسفيراز و انزيم الالنين امينو ترانسفيراز) والكرياتينين والبولينا والكوليستيرول والدهون الثلاثيه فى مصل الدم في الجرزان المحدث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام والذي يؤدي الي ارتفاع تركيزات هذه القياسات بعد ٢٨ يوم.

أيضا الروسوفاستاتين ١٠ ملليجرام/كجم او ٢٠ملليجرام/كجم يزيد تركيزات البروتين الكلي والألبومين فى مصل الدم في الجرزان المحدث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام والذي يؤدي الي إنخفاض تركيزات هذه القياسات بعد ٢٨ يوم.

الروسوفاستاتين ١٠ ملليجرام/كجم او٢٠ملليجرام/كجم يقلل تركيز الكوليستيرول والدهون الثلاثيه فى مصل الدم في الجرزان المحدث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام ٧ملجم/كجم والذي يؤدي الي إنخفاض تركيزات هذه القياسات بعد ٢٨يوم.

أيضا تمت دراسة التاثيرالمضاد للاكسده للروسوفاستاتين١٠ ملليجرام/كجم او ٢٠ملليجرام/كجمفي الجرزان باستخدام نموذج التليف الكبدي والكلوي المُحدَث بالبيروكسيكام ٧ملليجرام/كجم ونتج عن إعطاء البيروكسيكام انخفاض في مستوي الكاتاليز (CAT) والجلوتاثيون (GSH) بينما ارتفع مستوي المالونالدهيد (MDA). نجد ان الروسوفاستاتين أدي الي رجوع هذه القياسات نحو معدلاتها الطبيعيه بعد ٢٨ يوم.

أما بالنسبة للتغيرات الهستوباثولوجية للكبد والكلي نجد أن الروسوفاستاتين له تأثير واقي للكبد والكلي ويتمتع بدرجه جيده من الخصائص المضاده للاكسده في الجرزان المُحدَث لها تسمم كبدي وكلوي تجريبيا بالبيروكسيكام والمعروف بتدميره لأنسجه الكبد والكلي.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿قَالُوا سُبْحَانَكَ لَا عِلْمَ
لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العظيم

(البقرة: ٣٢)