Study on the possible alleviative effect of ticagrelor against diclofenac induced hepato-renal toxicity in adult male albino rats: Sight on mTOR-NLRP3-ASC-Caspase 1 pathway

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Abstract

Background: DIC is one of the most used nonsteroidal anti-inflammatory drugs (NSAIDs) and despite its extensive therapeutic utility, diclofenac may cause multiple severe side effects, including renal injury and hepatotoxicity. Ticagrelor beside its potent antiplatelet activity has anti-inflammatory, anti-oxidative and anti-apoptotic effect, herein, we will study its possible protective effect against DIC induced hepatorenal toxicity.

Aim: To assess the possible protective effects of ticagrelor against hepatic toxicity and renal toxicity induced by diclofenac

Methods: Fifty four male albino rats, weighing 200-250 g, were used randomly allocated to nine groups each one contain 6 rats as follow: Group 1(Control negative): given saline orally for 28 days. Group 2 (Ticagrelor): given ticagrelor alone (20 mg/kg/day, orally) for 7 days. Group 3 (Diclofenac): given diclofenac (10 mg/kg/day, i.p.) for 7 days. Group 4 (s Small dose prophylactic group (SP)): given ticagrelor (10 mg/kg/day, orally) concomitantly with diclofenac (10 mg/kg/day, i.p.) for 7 days. Group 5 (Large dose prophylactic group (LP)): given ticagrelor (20 mg/kg/day, orally) concomitantly with diclofenac (10 mg/kg/day, i.p.) for 7 days. Group 6 (Short term small dose treatment group (SST)): given diclofenac as in Group 3, followed by ticagrelor (10 mg/kg/day, orally) for another 7 days. Group 7 (Short term large dose treatment group (SLT)): v diclofenac as in Group 3, followed by ticagrelor (20 mg/kg/day, orally) for another 7 days. Group 8 (Long term small dose treatment group (LST)): given diclofenac as in Group 3, followed by ticagrelor (10 mg/kg/day, orally) for another 28 days. Group 9 (Long term large dose treatment group (LLT)): given diclofenac as in Group 3, followed by ticagrelor (20 mg/kg/day, orally) for another 28 days. *At the end of the experiment, the following parameters were measured:* Portal blood pressure (cm/H2O), liver and kidney functions, Serum ASC, mTOR, NLRP3, IL1, Serum GSDMD, caspase1, superoxide dismutase levels(SOD), Hepatic and renal MDA and histopathological examination.

Results: The results of this work showed that diclofenac (10 mg/kg/day, i.p.) for 7 days produced hepatotoxicity and nephrotoxicity by decreasing serum SOD level and by increasing serum AST, ALT, hepatic MDA, portal blood pressure ,serum urea , creatinine ,renal MDA ,serum NLRP3 ,ASC, mTOR ,GSDMD ,IL1 ,caspase1 levels and microscopic scoring of renal and hepatic tissues. While ticagrelor (10 and 20mg/kg/day, orally) produced a prophylactic effect by increasing serum SOD level and by decreasing serum AST, ALT, hepatic MDA ,portal blood pressure ,serum urea, creatinine ,renal MDA ,serum NLRP3 ,ASC, mTOR ,GSDMD ,IL1 ,caspase1 levels and microscopic scoring of renal and hepatic tissues in a dose dependent manner. The results of this work also showed that ticagrelor (10 and 20mg/kg/day, orally) for 7 and 28 days produced a curative effect by increasing serum SOD level and by decreasing serum AST, ALT, hepatic MDA ,portal blood pressure ,serum urea , creatinine ,renal MDA ,serum NLRP3 ,ASC, mTOR ,GSDMD ,IL1 ,caspase1 levels and microscopic scoring of renal and hepatic tissues .The effect of ticagrelor (20mg/kg/day, orally) for 28 days produced the most significant results.

Conclusion: In light of the results of this study we concluded that ticagrelor in a dose of 10 and 20 mg/kg was proved to have prophylactic and curative effect against diclofenac induced hepatotoxicity and nephrotoxicity by its antioxidant, anti-inflammatory and anti-apoptotic effect but further experimental studies are required to confirm our results

Keywords: alleviative effect, ticagrelor, diclofenac induced hepato-renal toxicity

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Introduction

Diclofenac (DIC) is one of the most used nonsteroidal anti-inflammatory drugs (NSAIDs) and despite its extensive therapeutic utility, DIC may cause multiple severe side effects, including renal injury, hepatotoxicity, gastrointestinal injury, and cardiovascular risks **(1)**

DIC causes generalized inhibition of prostaglandins (PG)through inhibition of cyclo-oxygenase 1, which has a protective role in the kidney and other organs. It has been reported that DIC reduced GFR and other kidney functions in a dose-deponent manner by inhibiting PG biosynthesis **(1)**

In contrast to other classical NSAIDs, diclofenac is known to inhibit the COX-2 enzyme with greater efficiency than the COX-1 enzyme **(2)**

DIC triggers mitochondrial permeability transition pores liberating cytochrome c which produces excessive free radicals and, in this manner, induces caspase cascade and lipid peroxidation culminating at cellular apoptosis. It also hinders the mitochondrial electron transport chain from ATP formation in the renal cells. It is bio-transformed to benzoquinineimines which are involved in shifting redox homeostasis towards the pro-oxidant side leading to severe renal damage **(3)**

Accumulating evidence that arises from laboratory animal studies emphasizes its nephrotoxicity manifested by induction of DNA fragmentation and oxidative stress and appearance of several histopathological lesions, along with an increase in renal damage markers **(3)**

The mechanism of DIC induced liver toxicity in humans is idiosyncratic. Diclofenac is metabolized in hepatocyte by multiple cytochrome P-450 enzymes resulting in formation of drug-protein adducts, glutathione (GSH) conjugation, and mitochondrial dysfunction and organ damage. However, it has been documented that diclofenac rather than its metabolite is responsible for its toxicity **(2)**

nucleotide-binding oligomerization domain-like receptors domains–containing protein 3 inflammasome (NLRP3), an important component of innate immunity, also could be activated by reactive oxygen species (ROS) generation. NLRP3 inflammasome signal pathway can regulate the caspase-1 activity, which contributes to the activation of cytokine precursor pro-IL-1β and pro-IL-18.

Powerful inflammatory factors TNF-α and IL-1β further aggravate inflammation response, resulting in tissue damage as previously studied in a rat model of lithium induced nephrotoxicity (3)

Ticagrelor, a platelet P2Y12 receptor inhibitor with a strong anti-platelet aggregation effect, can abate inflammation and oxidative stress by suppressing the nitric oxide/ reactive oxygen species/ nuclear factor kappa B (NOX4/ROS/NF-κB) signaling pathway in a rat model of myocardial ischemia-reperfusion injury (4)

In vitro and in vivo data demonstrate that ticagrelor inhibits activation of the NLRP3 inflammasome independent of the $P2Y_{12}$ receptor. ticagrelor exerts dual functions to regulate chloride efflux in the inhibition of the NLRP3 inflammasome. First, ticagrelor induces intracellular chloride ions (CLIC) degradation in an autophagy-dependent manner, and then it suppresses the localization of CLICs to the plasma membrane, even if CLICs are not degraded. Thus, the high levels of intracellular chloride ions induced by ticagrelor block the adaptor molecule apoptosis-associated speck-like protein containing a CARD(ASC) oligomerization. Notably, ASC is critical for the maturation of IL-1β, which is released by the NLRP3 (4)

Aim of this study was to assess the possible protective effects of ticagrelor against hepatic toxicity and renal toxicity induced by diclofenac.

Materials and Methods:

This experimental study was carried out in Animal unit, Faculty of Medicine, Zagazig University assuming the mean ALT was 40.19 _+ 2 vs 42.89 _+ 2.4 is in control vs intervention group At 80% test power and 95% confidence level ,the estimated sample is 54 rats , 6 rats in each group, measured by open epi program .**Criteria of inclusion:** only male rats weighing 200-250gm**. Criteria of exclusion:** only female rats and rats weighing less than 200 gm or above 250gm**.**

Fifty-four male albino rats, weighing 200-250 g, were purchased from the Faculty of Veterinary Medicine-animal unit, Zagazig University, Egypt. The procedures in this experimental study was performed by the National Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC) with approval number) ZU-IACUC/3/F/398/2022), Duration of approval (29-12-2022) to (29-12-2025).

Drugs and chemicals used: **Diclofenac sodium (Voltaren):** Each 3ml ampoule contains 75mg diclofenac sodium, Novartis , Egypt**, Ticagrelor (Brilique tablets**): each tablet contains 90mg , AstraZeneca UK). **Normal Saline 0.9% solution:** E.I.P.I. Co. A.R.E. **Pentobarbital**: (Sigma Aldrich, Egypt branch). **Formalin solution 10%:** EL Nasr Pharmaceutical Company, Egypt. **Hematoxylin and Eosin (H&E):** sigma Aldrich (st . Louis , MO,USA) . **Liquid Nitrogen:** (Al Gomhuria Company, Egypt). **Kits for estimation of the following parameters Caspase 1(CASP1):** Rat CASP1ELISA Kit, CLOUD-CLONE CORP. (CCC, USA) catalogue NO. SEB592Ra. **Interleukin 1 Beta (IL1b):** Rat IL1b ELISA Kit CLOUD-CLONE CORP. (CCC, USA) catalogue NO. SEA563Ra. **Gasdermin D (GSDMD):** Rat GSDMD ELISA Kit MyBiosource, Inc. Southern California, San Diego (USA) catalogue NO. MBS2032003. **NLR Family Pyrin Domain Containing Protein 3 (NLRP3):** Rat NLRP3 ELISA Kit MyBiosource, Inc. Southern California, San Diego (USA) Catalogue NO. MBS7255410. **Creatinine:** by Colorimetric method biodiagnostic 29 El-Tahrer St. - Dokki- Giza - Egypt catalogue No. CR 12 50. **Urea:** by Colorimetric method Abcam Cambridge, UK. catalogue No. ab83362 **Lipid Peroxide (Malondialdehyde):** by Colorimetric method biodiagnostic 29 El-Tahrer St. - Dokki- Giza - Egypt catalogue No. MD 25 29. **Superoxide Dismutase (SOD):** by Colorimetric method bio diagnostic 29 El-Tahrer St. - Dokki- Giza - Egypt catalogue No. SD 25 21.**Alanine aminotransferase (ALT):** by Colorimetric method Egyptian Co for Biotechnology – Spectrum Diagnostics. Obour city industrial area, Block 20008, Piece 19 A, PO Box 30 Cairo, Egypt.catalogue No. 264 001. **Aspartate aminotransferase(AST):** by Colorimetric method Egyptian Co for Biotechnology – Spectrum Diagnostics. Obour city industrial area, Block 20008, Piece 19 A, PO Box 30 Cairo, Egypt.catalogue No. 260 001. **Mammalian target of rapamycin (mtor):** by Western blot analysis of (mTOR), Santa Cruz Biotechnology, Inc. 10410 Finnell Street Dallas, Texas 75220 U.S.A. catalogue No.sc-517464. **Apoptosis-associated speck-like protein containing a CARD(ASC) :** by Western blot analysis of (ASC), Santa Cruz Biotechnology, Inc. 10410 Finnell Street Dallas, Texas 75220 U.S.A. catalogue No. sc-514414. **β-Actin:** by Western blot analysis of β-Actin, Santa Cruz Biotechnology, Inc. 10410 Finnell Street Dallas, Texas 75220 U.S.A. catalogue No. sc-47778.

Rats were allowed to accommodate for 2 weeks in individual stainless-steel wire mesh cages and kept in a regulated environment (25±1°C, 50±2% humidity), with 12 h light/dark cycles, with free access to food and water ad-labium.

The 54 rats were randomly assigned to nine groups each one contain 6 rats as follow:

Group 1(Control negative): given saline orally for 28 days.

Group 2 (Ticagrelor): given ticagrelor alone (20 mg/kg/day, orally). for 7 days.

Group 3 (Diclofenac): given diclofenac (10 mg/kg/day, i.p.) for 7 days.

Prophylactic groups

Group 4 (Small dose prophylactic group (SP)): given ticagrelor(10 mg/kg/day, orally) concomitantly with diclofenac (10 mg/kg/day, i.p.) for 7 days.

Group 5 (large dose prophylactic group): given ticagrelor (20 mg/kg/day, orally) concomitantly with diclofenac (10 mg/kg/day, i.p.) for 7 days.

Treatment groups

Short term therapyfor 7 days (Owumi and Dim, 2019).

Group 6 (Short term small dose treatment group (SST)): given diclofenac as in Group 3, followed by ticagrelor (10 mg/kg/day, orally) for another 7 days.
Group 7 (Short term large dose treatment group (SLT)): given diclofenac as in Group 3, followed by ticagrelor (20 mg/kg/day, orally) for another 7 days.

Long term therapy for 28 days (Alabi and Akomolafe, 2020).
Group 8 (Long term small dose treatment group (LST)): given diclofenac as in Group 3, followed by ticagrelor (10 mg/kg/day, orally) for another 28 days.
Group 9 (Long term large dose treatment group (LLT)): given diclofenac as in Group 3, followed by ticagrelor (20 mg/kg/day, orally) for another 28 days.
Blood samples were collected from the retro-orbital plexus under light anesthesia by pentobarbital 75mg/kg/i.p,1 ml blood samples were collected in EDTA containing tubes, These Blood samples were centrifugated at 3000 rpm for 10 minutes to get clear serum and kept at - 20°c for bioassay of liver and kidney functions, NLRP3, ASC, IL1, GSDMD , mTOR, caspase1,superoxide dismutase.
Rats were euthanized, and one kidney and a portion of hepatic tissue were fixed in 10% formalin for 24 h for histopathological studies, and the other kidney and aportion of hepatic tissue were taken and be kept at - 80°c for measuring MDA

METHODS

Induction of nephrotoxicity & hepatotoxicity by using diclofenac :
Rats received diclofenac (10 mg/kg/day, i.p.) for 7 days in all groups except group1&2.

Oral administration of ticagrelor :
Tica was dissolved in saline and administered once daily by oral gavage in a volume not exceed 0.3 ml/100 gm for 7days in groups 2 ,4,5,6,7 and for 28 days in groups 8,9.

Portal blood pressure measuring
Rats were anesthetized by pentobarbital 75mg/kg/i.p . A midline abdominal incision was made and the portal pressure was measured by inserting a normal saline filled 20-gauge needle into the portal vein.The needle was joined to a PE-50 tube which was fixed to a metered recording scale fixed to make the zero reading in mid-axilla.The pressure reading(cm/water) was considered satisfactory when a stable recording was produced (4)

Biochemical assay:

Estimation of caspase 1:
The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of CASP1 in rat tissue homogenates, cell lysates and other biological fluids.

[CALCULATION OF RESULTS]
Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve with CASP1 concentration on the y-axis and absorbance on the x-axis.
Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

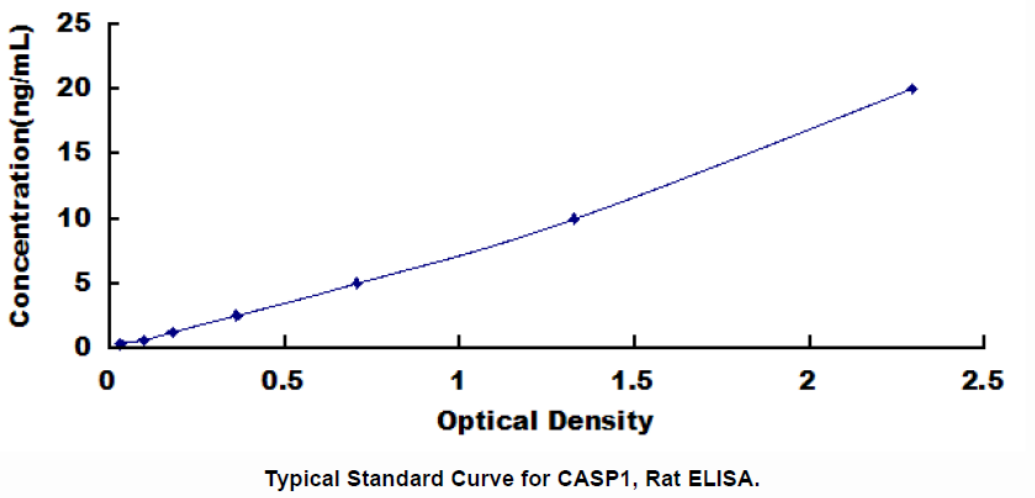

Figure (1):typical standard curve for CASP1,Rat ELISA.

Estimation of IL1b:

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IL1b in rat serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

- **[CALCULATION OF RESULTS]:**

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with IL1b concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

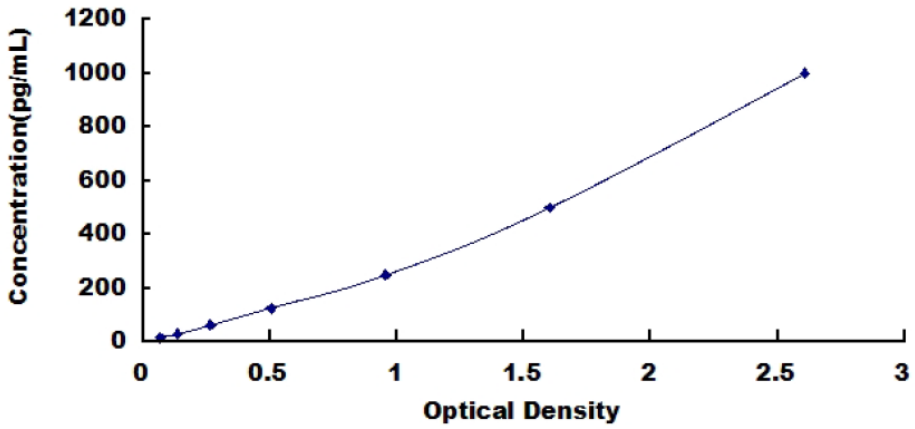

Figure (8): Typical Standard Curve for IL1b, Rat ELISA.

Estimation of GSDMD:

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of GSDMD in rat tissue homogenates, cell lysates and other biological fluids.

[CALCULATION OF RESULTS]

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve with GSDMD concentration on the y-axis and absorbance on the x-axis.Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

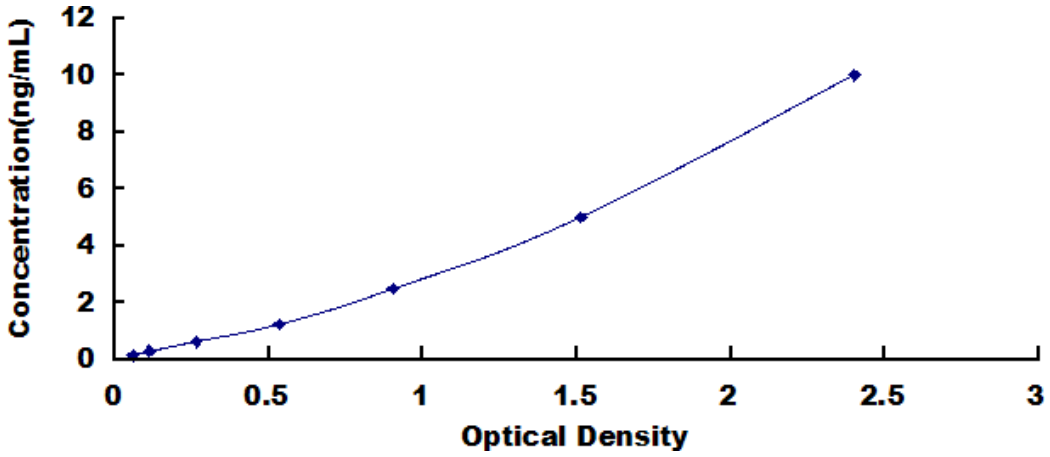

Figure (2): Typical Standard Curve for **GSDMD** , Rat ELISA.

Estimation of NLRP3:

This NLRP3 ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Rat NLRP3.

[CALCULATION OF RESULTS]

The standard curve is used to determine the amount of samples.

First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation. DO NOT subtract the O.D of standard zero.Construct a standard curve by plotting the concentration on the horizontal (X) axis against the average O.D. for each standard on the vertical (Y) axis, and draw a best fit curve e using graph paper or statistical software to generate a four paramater logistic (4-PL) curve-fit or logit-log linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.Calculate the concentration of samples corresponding to the mean absorbance from the standard curve. Standard curve for demonstration only.

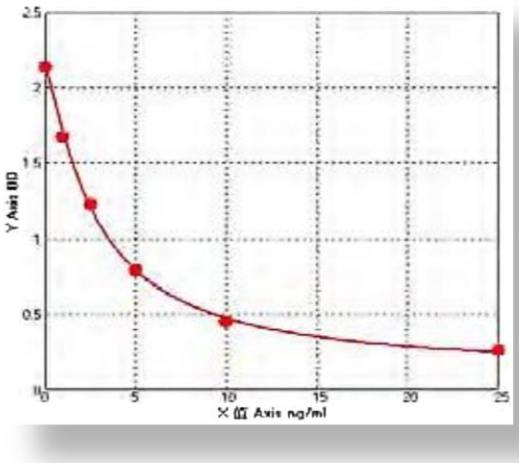

Figure (3): Typical Standard Curve for NLRP3, Rat ELISA.

Estimation of Creatinine:

Colorimetric Method

[TEST PRINCIPLE]
Creatinine forms a colored complex with picrate in an alkaline medium (Jaffé, 1886) .

Estimation of Urea:

[TEST PRINCIPLE](Fawcett and Scott, 1960). Urea Assay Kit (Colorimetric) (ab83362) provides a rapid, simple, sensitive, and reliable for measurement of Urea level in a variety of samples such as serum, plasma, and urine, etc. In the assay, Urea reacts as substrate with compounds in the presence of enzymes to form a product that reacts with the probe to generate color (ODmax=570nm). The optical density of produced color has a direct relationship with Urea concentration in the solution. The kit can detect as low as 0.5 nmol per well or 10 μM of Urea. The assay is also suitable for high throughput studies.

[CALCULATION OF RESULTS]
Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates). Average the duplicate reading for each standard and sample.
If the sample background control is significant, then subtract the sample background from sample reading.
Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
Plot the corrected absorbance values for each standard as a function of the final concentration of Urea.
Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
Extrapolate sample readings from the standard curve plotted using the following equation:

$$Sa = \frac{\text{Corrected absorbance} - (\text{y intercept})}{\text{Slope}}$$

Concentration of urea in the test samples is calculated as:

$$Urea\ Concentration = \frac{\text{Sa}}{\text{Sv}} * D$$

Where:
Sa = Sample amount (nmol) from standard curve.
Sv = Sample volume (μL) added into the wells.
D = Dilution factor of sample.
Urea Molecular Weight is 60.07 g/mol

TYPICAL STANDARD CURVE

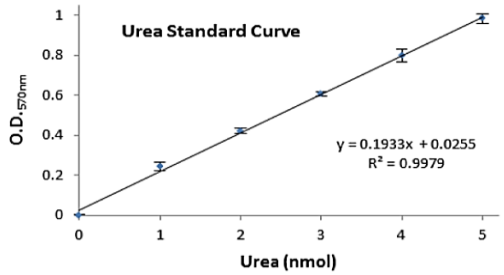

Figure (4): Typical Standard Curve for urea.

Estimation of MDA:

Colorimetric Method

[TEST PRINCIPLE]
Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm

Estimation of SOD:

Colorimetric Method
[TEST PRINCIPLE]
This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye
[CALCULATION OF RESULTS]

$$\text{Percent inhibition} = \frac{(\Delta \text{Acontrol}) - (\Delta \text{Asample})}{(\Delta \text{Acontrol})}$$

Where
ΔAcontrol =
The change in absorbance at 560 nm over 5 min. following the addition of PMS to the reaction mixture in the absence of sample .
ΔAsample =
The change in absorbance at 560 nm over 5 min. following the addition of PMS to the reaction mixture in the presence of sample .
Purified SOD was shown to inhibit the initial rate of photo activated phenazine methosulfate mediated reduction of O2˙ to O2 which then reduced nitroblue tetrazolium. 1.5 U/assay of the purified enzyme produced 80% inhibition.
SOD activity can be expressed as a function of any other relevant parameter such as protein or hemoglobin content which has been measured separately.

SOD Activity:
U/ml = % inhibition x 3.75

$$\text{U/gm tissue} = \%\ \text{inhibition} \times 3.75 \times \frac{1}{gm\ tissue\ used}$$

$$\text{U/gm Hb} = \%\ \text{inhibition} \times 3.75 \times \frac{1}{gm\ Hb\ used}$$

Estimation of ALT:

TEST PRINCIPLE] ALT – (colorimetric method).

Assay Principle (Reitman and Frankel, 1957) The reaction involved in the assay system is as follows:
The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.
[CALCULATION OF RESULTS]
Calculate the number of units / ml of ALT of sample using the standard curve.
Estimation of AST:

[TEST PRINCIPLE] AST – (Colorimetric method). **Assay Principle (Reitman and Frankel, 1957).**
The reaction involved in the assay system is as follows: The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding

oxaloacetate and L-glutamate.

- **[CALCULATION OF RESULTS]**

Calculate the number of units / ml of AST of sample using the standard curve.

10. Western blot analysis of ASC & mTOR:

After extracting protein solutions from the liver and kidney tissues, equal amounts of protein (20–30 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (10% acrylamide gel) using a Bio-Rad Mini-Protein II system. The protein was transferred to polyvinylidene difluoride membranes (Pierce) with a Bio-Rad Trans-Blot system. Membranes were then washed with PBS and blocked for 1 h at room temperature with 5% (w/v) skim milk powder in PBS. The manufacturer's instructions were followed for primary antibody reactions. After blocking, the blots were developed using antibodies against ASC, mTOR and β-actin (dilution 1:1000) (Santa Cruz Biotechnology, Inc,USA), washed and incubated with peroxidase-labeled secondary antibodies at 37°C for 1 h. Band intensity was assessed with a ChemiDoc™ imaging system with Image LabTM software version 5.1 (Bio-Rad Laboratories Inc.). Results are presented in arbitrary units after normalization to β-actin protein expression.

Histopathological work up :

Tissue specimens from liver and kidney were fixed in neutral buffered formalin 10% for 48 hours, dehydrated in ascending grades of ethanol (70%-100%), cleared in xylene, embedded in paraffin wax. 5μm thickness of paraffin sections were obtained by using automated microtome then stained with routine Hematoxylin and Eosin (H & E)**.** All section photos were photographed using a Leica® microscope combined with AmScope® microscope digital camera. Lesions score system was evaluated as the following: (0 = no detectable histopathological alterations, 1 = rarely minimal or focal, 2 = multifocal, 3 = patchy or diffuse) with a semiquantitative method.

STATISTICAL ANALYSIS:

The obtained results were tabulated as means ± standard error of mean (SE). Comparisons between different groups were made using one-way analysis of variances (one-way ANOVA) followed by Post-Hoc Tukey test as described. The differences were considered to be significant when $p < 0.05$. Statistical Package of Social Sciences (SPSS) computer software (version 26) was used to carry out the statistical analysis while non parametric data were analyzed by Kruskal-Wallis followed by Duncan test.

Results

Effect of administration of Ticagrelor either prophylactic or curative on serum AST (IU/ml), and ALT (IU/ml) on diclofenac induced nephrotoxicity and hepatotoxicity (table 1)

In control negative group(1) serum AST was 41.27±0.66, and ALT was 25.62±0.61, while Diclofenac treated group(2) in a dose of (10 mg/kg/day, i.p.) showed a significant (P <0.0001) increase in the levels of AST to 86.75±0.89 (↑110.2%), and ALT to 77.74±0.47 (↑202%) as compared to the control negative group.
In Ticagrelor group(3) given (20 mg/kg/day, orally) serum AST and ALT were non-significantly reduced to 40.82±0.92 and 25.47±0.59 respectively as compared to the control negative group.
In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days serum AST was significantly (P <0.0001) reduced to 65.75±1.54 (↓24.2%), and ALT was significantly (P <0.0001) reduced to 54.02±3.1 (↓30.5%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.
In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days serum AST was significantly (P <0.0001) reduced to 51.98±1.65 (↓40%), and ALT was significantly (P <0.0001) reduced to 38.43±1.77 (↓50.56%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.
In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days serum AST was significantly (P <0.0001) reduced to 56.02±1.27 (↓35.4%), and ALT was significantly (P <0.0001) reduced

to 53.69±1.94 (↓30.9%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.
In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days serum AST was significantly (P <0.0001) reduced to 49.99±1.52 (↓42.3%), and ALT was significantly (P <0.0001) reduced to 46.17±1.72 (↓40.6%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.
In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days serum AST was significantly (P <0.0001) reduced to 50.68±1.76 (↓41.6%), and ALT was significantly (P <0.0001) reduced to 45.41±1.31 (↓41.58%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.
In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days serum AST was significantly (P <0.0001) reduced to 48.74±1.05 (↓43.8%), and ALT was significantly (P <0.0001) reduced to 28.31±1.61 (↓63.58%) as compared to the Diclofenac treated group, meanwhile AST value was significantly (P <0.0001) higher than control negative group, but ALT value was non-significant with control negative group.

Table 1: Effect of administration of Ticagrelor either prophylactic or curative on serum ALT and AST levels

	G(1) **Control negative**	G(2) **Ticagrelor**	G(3) **Diclofenac (Diseased)**	G(4) **Small dose prophylactic group (SP)**	G(5) **Large dose prophylactic group (LP)**	G(6) **Short term small dose treatment group (SST)**	G(7) **Short term large dose treatment group (SLT)**	G(8) **Long term small dose treatment group (LST)**	G(9) **Long term large dose treatment group (LLT)**
AST (IU/ml)	41.27^{e} ±0.66	40.82^{e} ±0.92	86.75^{a} ±0.89	65.75^{b} ±1.54	51.98^{c} ±1.65	56.02^{c} ±1.27	49.99^{d} ±1.52	50.68^{cd} ±1.76	48.74^{d} ±1.05
% of change in relation to control negative	-----	↓1.09%	↑110.2%	↑59%	↑25.9%	↑35.75%	↑21.1%	↑22.8%	↑18.1%
% of change in relation to control diseased	-----	↓52.9%	-----	↓24.2%	↓40%	↓35.4%	↓42.3%	↓41.6%	↓43.8%
ALT (IU/ml)	25.62^{e} ±0.61	25.47^{e} ±0.59	77.74^{a} ±0.47	54.02^{b} ±3.1	38.43^{d} ±1.77	53.69^{b} ±1.94	46.17^{c} ±1.72	45.41^{cd} ±1.31	28.31^{e} ±1.61
% of change in relation to control Negative	-----	↓0.78%	↑203.4%	↑110.8%	↑50%	↑109.5%	↑80.2%	↑77.2%	↑10.5%
% of change	-----	↓ 67.23%	-----	↓30.5%	↓50.56%	↓30.9%	↓40.6%	↓41.58%	↓63.58%

in relation to control diseased									

Results are presented as Mean ± Std Error (n= 6 rats).
Values in the same row with different superscript letters are significantly (P <0.0001) different. Where a represent highest value and e represent lowest value.
Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method.
n: number of rats in each group.
AST: Aspartate aminotransferase ALT: alanine aminotransferase

Effect of administration of Ticagrelor either prophylactic or curative on serum Urea (mg/dL), Creatinine (mg/dL) and Portal blood pressure (mm Hg) on diclofenac induced nephrotoxicity and hepatotoxicity (table 2**)**

In control negative group (1) serum Urea was 21.94±0.50, Creatinine was 0.22±0.03, and Portal blood pressure was 3.5±0.16, while Diclofenac treated group(2) in a dose of (10 mg/kg/day, i.p.) showed a significant (P <0.0001) increase in the levels of Urea to 67.74±0.74 (↑208.7%), Creatinine to 1.867±0.11 (↑748.6%) and Portal blood pressure to 16.23±0.39 (↑363.7%) as compared to the control negative group.

In Ticagrelor group(3) given (20 mg/kg/day, orally) serum Urea was insignificantly increased to 23.47±0.62 (↑7%), Creatinine was was insignificantly increased to 0.24±0.03 (↑9%) and Portal blood pressure was insignificantly decreased to 3.45±0.17 (↓1.4%) as compared to the control negative group.

In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days serum Urea was significantly (P <0.0001) reduced to 47.19±1.17 (↓30.3%), Creatinine was significantly (P <0.0001) reduced to 0.88±0.02 (↓52.9%) and Portal blood pressure was significantly (P <0.0001) reduced to 9.66±0.35 (↓40.5%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.

In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days serum Urea was significantly (P <0.0001) reduced to 37.27±1.86 (↓45%), Creatinine was significantly (P <0.0001) reduced to 37.27±1.86 (↓61.9%) and Portal blood pressure was significantly (P <0.0001) reduced to 6.30±0.33 (↓61.9%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.

In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days serum Urea was significantly (P <0.0001) reduced to 50.86±0.52 (↓24.9%), Creatinine was significantly (P <0.0001) reduced to 0.61±0.01 (↓67.3%) and Portal blood pressure was significantly (P <0.0001) reduced to 6.51±0.19 (↓59.9%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.

In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days serum Urea was significantly (P <0.0001) reduced to 43.84±1.39 (↓35.3%), Creatinine was significantly (P <0.0001) reduced to 0.49±0.03 (↓73.8%) and Portal blood pressure was significantly (P <0.0001) reduced to 4.90±0.36 (↓69.8%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.

In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days serum Urea was significantly (P <0.0001) reduced to 43.91±1.25 (↓35.2%), Creatinine was significantly (P <0.0001) reduced to 0.49±0.02 (↓73.8%) and Portal blood pressure was significantly (P <0.0001) reduced to 5.83±0.49 (↓64.1%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group.

In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days serum Urea was significantly (P <0.0001) reduced to 31.65±0.77 (↓53.3%), Creatinine was significantly (P <0.0001) reduced to 0.29±0.02 (↓84.5%) and Portal blood pressure was significantly (P <0.0001) reduced to 3.90±0.43 (↓75.9%) as compared to the Diclofenac treated group, meanwhile these values were significantly (P <0.0001) higher than control negative group, but Creatinine, and Portal blood pressure were non-significant with control negative group.

Table 2. Effect of administration of Ticagrelor either prophylactic or curative on serum urea and creatinine level.

Results are presented as Mean ± Std Error (n= 6 rats).

Values in the same row with different superscript letters are significantly (P <0.0001) different. Where a represent highest value and e,f,d represent lowest value. Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method.

parameters	G(1) **Control negative**	G(2) **Ticagrelor**	G(3) **Diclofenac (Diseased)**	G(4) **Small dose prophylactic group (SP)**	G(5) **Large dose prophylactic group (LP)**	G(6) **Short term small dose treatment group (SST)**	G(7) **Short term large dose treatment group (SLT)**	G(8) **Long term small dose treatment group (LST)**	G(9) **Long term large dose treatment group (LLT)**
urea (mg/dL)	21.94 ±0.50^{f}	23.47 ±0.62^{f}	67.74 ±0.74^{a}	47.19 ±1.17^{bc}	37.27 ±1.86^{d}	50.86 ±0.52^{b}	43.84 ±1.39^{c}	43.91 ±1.25^{c}	31.65 ±0.77^{e}
% of change in relation to control negative	-----	↑7%	↑208.7%	↑115.1%	↑69.8%	↑131.8%	↑99.8%	↑100.1%	↑43.8%
% of change in relation to control diseased	-----	-----	-----	↓30.3%	↓45%	↓24.9%	↓35.3%	↓35.2%	↓53.3%
Creatinine (mg/dL)	0.22 ±0.03^{e}	0.24 ±0.03^{e}	1.867 ±0.11^{a}	0.88 ±0.02^{b}	0.71 ±0.03^{bc}	0.61 ±0.01^{c}	0.49 ±0.03^{cde}	0.49 ±0.02^{de}	0.29 ±0.02^{e}
% of change in relation to control negative	-----	↑9%	↑748.6%	↑300%	↑222.7	↑177.2%	↑122.7%	↑122.7%	↑31.8%
% of change in relation to control diseased	-----	-----	-----	↓52.9%	↓61.9%	↓67.3%	↓73.8%	↓73.8%	↓84.5%
Portal blood pressure (mm Hg)	3.5±0.16^{d}	3.45±0.17^{d}	16.23±0.39^{a}	9.66±0.35^{b}	6.30±0.33^{c}	6.51±0.19^{c}	4.90±0.36^{c}	5.83±0.49^{c}	3.90±0.43^{d}
% of change **in relation to control negative**	-----	↓1.4%	↑363.7 %	↑176%	↑80%	↑86%	↑40%	↑66.5%	↑11.4%
% of change in relation to control diseased	-----	-----	-----	↓40.5%	↓61.9%	↓59.9%	↓69.8%	↓64.1%	↓75.9%

n: number of rats in each group

Effect of administration of Ticagrelor either prophylactic or curative on liver and kidney MDA (ng/mL) and SOD (u/g) on diclofenac induced nephrotoxicity and hepatotoxicity (table 3)

In control negative group(1) levels of kidney and liver MDA levels were 0.80±0.01, 31.77±0.63 and SOD was 10.2±0.50, while Diclofenac treated group(2) in a dose of (10 mg/kg/day, i.p.) showed a significant (P <0.0001) increase in the levels of liver and kidney MDA were 5.05±0.09 (↑531.3%), 57.73±0.76 (↑81.7%) and SOD decreased significantly to 2.30±0.25 (↓77.2%) as compared to the control negative group.

In Ticagrelor group(3) given (20 mg/kg/day, orally) kidney and liver MDA levels were 0.810±0.02(↑0.8%) , 31.10±0.33(↓2.1%) . and SOD was 10.2±0.50 (↓1.2%) as compared to the control negative group.

In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 2.91±0.07 (↓42.3%), 47.10±0.98 (↓18.5%), and SOD was

increased 2.89±0.16 (↑25.6%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were significantly (P <0.0001) higher than control negative group, but SOD level was significantly lower than control negative group.

In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 1.32±0.11 (↓73.9%), 45.47±0.98 (↓21.2%), and SOD was increased 4.87±0.27 (↑111.7%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were significantly (P <0.0001) higher than control negative group, but SOD level was significantly lower than control negative group.

In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 3.10±0.10 (↓38.6%), 48.75±0.94 (↓15.6%), and SOD was increased 3.81±0.08 (↑65.7%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were significantly (P <0.0001) higher than control negative group, but SOD level was significantly lower than control negative group.

In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 1.58±0.18 (↓68.7%), 42.32±0.91 (↓26.7%), and SOD was increased 5.26±0.14 (↑128.7%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were significantly (P <0.0001) higher than control negative group, but SOD level was significantly lower than control negative group.

In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 2.25±0.07 (↓55.4%), 42.08±0.85 (↓27.1%), and SOD was increased 6.138±0.12 (↑166.9%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were significantly (P <0.0001) higher than control negative group but SOD level was significantly lower than control negative group.

In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days kidney and liver MDA levels were significantly (P <0.0001) reduced to 1.00 ±0.04 (↓81.2%), 34.48±1.44 (↓40.3%), and SOD was increased 8.00±0.34 (↑247.8%) as compared to the Diclofenac treated group, meanwhile liver and kidney MDA values were non-significantly different than control negative group, but SOD level was significantly lower than control negative group.

Table 3. Effect of administration of Ticagrelor either prophylactic or curative on kidney and liver MDA and SOD levels.

	G(1) **Control negative**	G(2) **Ticagrelor**	G(3) **Diclofenac (Diseased)**	G(4) **Small dose prophylactic group (SP)**	G(5) **Large dose prophylactic group (LP)**	G(6) **Short term small dose treatment group (SST)**	G(7) **Short term large dose treatment group (SLT)**	G(8) **Long term small dose treatment group (LST)**	G(9) **Long term large dose treatment group (LLT)**
Kidney MDA (ng/mL)	0.80 ±0.01^{e}	0.810 ±0.02^{e}	5.05 ±0.09^{a}	2.91 ±0.07^{b}	1.32 ±0.11^{de}	3.10 ±0.10^{b}	1.58 ±0.18^{d}	2.25 ±0.07^{c}	1.00 ±0.04^{e}
% of change in relation to control negative	-----	↑.8%	↑531.3%	↑263%	↑65%	↑287.5%	↑97.5%	↑181.2%	↑25%
% of change in relation to control diseased	-----	-----	-----	↓42.3%	↓73.9%	↓38.6%	↓68.7%	↓55.4%	↓81.2%
SOD (u/g)	10.08 ±0.59^{a}	10.2 ±0.50^{a}	2.30 ±0.25^{f}	2.89 ±0.16^{de}	4.87 ±0.27^{ef}	3.81 ±0.08^{de}	5.26 ±0.14^{cd}	6.138 ±0.12^{cd}	8.00 ±0.34^{b}
% of change	-----	↓1.2%	↓77.2%	↓71.3%	↓51.7%	↓62.2%	↓47.8%	↓39.1%	↓20.6%

in relation to control negative									
% of change in relation to control diseased	-----	-----	-----	↑25.6%	↑111.7%	↑65.7%	↑128.7%	↑166.9%	↑247.8%
Liver MDA (ng/mL)	31.77±0.63^{d}	31.10±0.33^{d}	57.73±0.76^{a}	47.10±0.98^{b}	45.47±0.98^{bc}	48.75±0.94^{b}	42.32±0.91^{c}	42.08±0.85^{c}	34.48±1.44^{d}
% of change in relation to control negative	-----	↓2.1%	↑81.7%	↑48.25%	↑43.1%	↑53.4%	↑33.2%	↑32.4%	↑8.5%
% of change in relation to control diseased	-----	-----	-----	↓18.5%	↓21.2%	↓15.6%	↓26.7%	↓27.1%	↓40.3%

Results are presented as Mean ± Std Error (n= 6 rats).
Values in the same row with different superscript letters are significantly (P <0.0001) different. Where a represent highest value and e,d, represent lowest value.
Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method.
n: number of rats in each group.
MDA: Malondialdehyde, SOD: Superoxide Dismutase

Effect of administration of Ticagrelor either prophylactic or curative on mRNA gene expression of IL-1, CASPASE-1, Gasdermin on diclofenac induced nephrotoxicity and hepatotoxicity (table 4)

In control negative group(1) mRNA gene expression of Gasdermin was 0.82±0.01, CASPASE-1 was 40.16±1.12 and IL-1 was 23.47±1.01, while Diclofenac treated group (2) in a dose of (10 mg/kg/day, i.p.) showed a significant (P <0.0001) increase in the levels of Gasdermin was 5.82±0.17 (↑ 609.7%), CASPASE-1 was 324.6±5.83 (↑ 708.2%) and IL-1 was 229.9±10.43 (↑ 879.5%), as compared to the control negative group.

In Ticagrelor group(3) given (20 mg/kg/day, orally) mRNA gene expression of Gasdermin was 0.810±0.02 (↑0%), CASPASE-1 was 41.08±1.10 (↑2.3%), and IL-1 was 23.64±0.82 (↑0.7%), as compared to the control negative group.

In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 2.97±0.08 (↓48.7%), CASPASE-1 was significantly (P <0.0001) reduced to 169.1±11.08 (↓47.9%), and IL-1 was significantly (P <0.0001) reduced to 97.15±2.02 (↓57.7%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.

In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 1.44±0.06 (↓75.2%), CASPASE-1 was significantly (P <0.0001) reduced to 84.10±3.08 (↓74.1%), and IL-1 was significantly (P <0.0001) reduced to 73.73±2.63 (↓67.9%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.

In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 4.18±0.09 (↓28.2%), CASPASE-1 was significantly (P <0.0001) reduced to 229.5±8.46 (↓29.3%), and IL-1 was significantly (P <0.0001) reduced to 148.3±2.95 (↓35.5%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control

negative group.
In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 3.78±0.06 (↓35.1%), CASPASE-1 was significantly (P <0.0001) reduced to 194.6±3.83 (↓40.04%), and IL-1 was significantly (P <0.0001) reduced to 124.7±3.14 (↓45.8%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.
In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 3.89±0.06 (↓33.2%), CASPASE-1 was significantly (P <0.0001) reduced to 170.6±4.49 (↓47.4%), and IL-1 was significantly (P <0.0001) reduced to 110.5±4.52 (↓51.9%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.
In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 2.20±0.20 (↓62.2%), CASPASE-1 was significantly (P <0.0001) reduced to 99.40±2.74 (↓69.4%), and IL-1 was significantly (P <0.0001) reduced to 86.93±2.79 (↓62.2%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.

Table 4. Effect of administration of Ticagrelor either prophylactic or curative on mRNA gene expression of IL-1, CASPASE-1, Gasdermin.

	G(1) **Control negative**	G(2) **Ticagrelor**	G(3) **Diclofenac (Diseased)**	G(4) **Small dose prophylactic group (SP)**	G(5) **Large dose prophylactic group (LP)**	G(6) **Short term small dose treatment group (SST)**	G(7) **Short term large dose treatment group (SLT)**	G(8) **Long term small dose treatment group (LST)**	G(9) **Long term large dose treatment group (LLT)**
Gasdermin	0.82±0.01^{e}	0.82±0.02^{e}	5.82±0.17^{a}	2.97±0.08^{c}	1.44±0.06^{d}	4.18±0.09^{b}	3.78±0.06^{b}	3.89±0.06^{b}	2.20±0.20^{c}
% of change in relation to control negative	-----	0%	↑609.7%	↑262.1%	↑75.6%	↑409.7%	↑360.9%	↑374.3%	↑168.2 %
% of change in relation to control diseased	-----	-----	-----	↓48.7%	↓75.2%	↓28.2%	↓35.1%	↓33.2%	↓62.2%
Caspase 1	40.16±1.12^{e}	41.08±1.10^{e}	324.6±5.83^{a}	169.1±11.08^{c}	84.10±3.08^{d}	229.5±8.46^{b}	194.6±3.83^{c}	170.6±4.49^{c}	99.40±2.74^{d}
% of change in relation to control negative	-----	↑2.3%	↑708.2%	↑321.5%	↑109.4%	↑471.5%	↑384.5%	↑324.8%	↑147.5 %
% of change in relation to control diseased	-----	-----	-----	↓47.9%	↓74.1%	↓29.3%	↓40.04%	↓47.4%	↓69.4%
IL-1	23.47 ±1.01^{f}	23.64 ±0.82^{f}	229.9 ±10.43^{a}	97.15 ±2.02^{d}	73.73 ±2.63^{e}	148.3 ±2.95^{b}	124.7 ±3.14^{c}	110.5 ±4.52^{cd}	86.93 ±2.79^{de}
% of change in relation to control negative	-----	↑0.7%	↑879.5%	↑313.9%	↑214.2%	↑531.9%	↑431.3%	↑370.8%	↑277.8 %
% of change in relation to control diseased	-----	-----	-----	↓57.7%	↓67.9%	↓35.5%	↓45.8%	↓51.9%	↓62.2%

Results are presented as Mean ± Std Error (n= 6 rats).
Values in the same row with different superscript letters are significantly (P <0.0001) different. Where a represent highest value and e,f, represent lowest value.

Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method.
n: number of rats in each group.
IL-1:Interleukin

Effect of administration of Ticagrelor either prophylactic or curative on mRNA gene expression of mTOR, NLRP3, and ASC on diclofenac induced nephrotoxicity and hepatotoxicity (table 5)

In control negative group(1) mRNA gene expression of mTOR was 0.85±0.01, NLRP3 was 0.958±0.009 and ASC was 1.597±0.05, while Diclofenac treated group(2) in a dose of (10 mg/kg/day, i.p.) showed a significant (P <0.0001) increase in the levels of mTOR was 3.263±0.06 (↑ 283.8%), ASC was 3.860±0.04 (↑ 302.9%) and NLRP3 was 8.759±0.28 (↑ 448.5%), as compared to the control negative group.
In Ticagrelor group(3) given (20 mg/kg/day, orally) mRNA gene expression of mTOR was 0.8633±0.02 (↑1.1%), ASC was 0.941±0.01 (↓1.7%), and NLRP3 was 1.550±0.05 (↓2.9%), as compared to the the control negative group.
In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days mRNA gene expression of mTOR was significantly (P <0.0001) reduced to 1.402±0.09 (↓57.3%), ASC was significantly (P <0.0001) reduced to 1.260±0.02 (↓67.3%), and NLRP3 was significantly (P <0.0001) reduced to 5.832±0.13 (↓33.4%), as compared to the Diclofenac treated group, meanwhile these values were higher than the control negative group.
In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days mRNA gene expression of mTOR was significantly (P <0.0001) reduced to 1.035±0.01 (↓68.2%), ASC was significantly (P <0.0001) reduced to 1.023±0.01 (↓73.5%), and NLRP3 was significantly (P <0.0001) reduced to 4.825±0.11 (↓44.9%), as compared to the Diclofenac treated group, meanwhile mTOR and ASC values were nonsignificantly higher than the control negative group but NLRP3 value was significantly higher.
In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days mRNA gene expression of mTOR was significantly (P <0.0001) reduced to 2.612±0.04 (↓19.9%), ASC was significantly (P <0.0001) reduced to 2.240±0.03 (↓41.9%), and NLRP3 was significantly (P <0.0001) reduced to 5.051±0.11 (↓42.3%), as compared to the Diclofenac treated group, meanwhile these values were significantly higher than the control negative group.
In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days mRNA gene expression of mTOR was significantly (P <0.0001) reduced to 2.038±0.01 (↓37.5%), ASC was significantly (P <0.0001) reduced to 1.982±0.01 (↓48.7%), and NLRP3 was significantly (P <0.0001) reduced to 4.071±0.05 (↓53.5%), as compared to the Diclofenac treated group, meanwhile these values were significantly higher than the control negative group.
In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 1.903±0.07 (↓41.6%), ASC was significantly (P <0.0001) reduced to 1.753±0.03 (↓54.6%), and NLRP3 was significantly (P <0.0001) reduced to 3.208±0.17 (↓63.4%), as compared to the Diclofenac treated group, meanwhile these values were significantly higher than the control negative group.
In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days mRNA gene expression of Gasdermin was significantly (P <0.0001) reduced to 1.327±0.08 (↓59.3%), ASC was significantly (P <0.0001) reduced to 1.302±0.06 (↓66.2%), and NLRP3 was significantly (P <0.0001) reduced to 2.243±0.14 (↓74%), as compared to the Diclofenac treated group, meanwhile these values were significantly higher than the control negative group.

Table 5. Effect of administration of Ticagrelor either prophylactic or curative on mRNA gene expression of mTOR, NLRP3, and ASC on diclofenac induced nephrotoxicity and hepatotoxicity.

	G(1) **Control negative**	G(2) **Ticagrelor**	G(3) **Diclofenac (Diseased)**	G(4) **Small dose prophylactic group (SP)**	G(5) **Large dose prophylactic group (LP)**	G(6) **Short term small dose treatment group (SST)**	G(7) **Short term large dose treatment group (SLT)**	G(8) **Long term small dose treatment group (LST)**	G(9) **Long term large dose treatment group (LLT)**
mTOR	0.85 ±0.01^{e}	0.86 ±0.02^{e}	3.263 ±0.06^{a}	1.402 ±0.09^{d}	1.035 ±0.01^{e}	2.612 ±0.04^{b}	2.038 ±0.01^{c}	1.903 ±0.07^{c}	1.327 ±0.08^{d}
% of change in relation to control negative	-----	↑1.1%	↑283.8%	↑64.9%	↑21.7%	↑207.2%	↑139.7%	↑123.9%	↑56.1%
% of change in relation to control diseased	-----	-----	-----	↓57.03%	↓68.2%	↓19.9%	↓37.5%	↓41.6%	↓59.3%
ASC	0.958 ±0.009^{f}	0.941 ±0.01^{f}	3.860 ±0.04^{a}	1.260 ±0.02^{e}	1.023 ±0.01^{f}	2.240 ±0.03^{b}	1.982 ±0.01^{c}	1.753 ±0.03^{d}	1.302 ±0.06^{e}
% of change in relation to control negative	-----	↓1.7%	↑302.9%	↑31.5%	↑6.8%	↑133.8%	↑106.8%	↑82.9%	↑35.9%
% of change in relation to control diseased	-----	-----	-----	↓67.3%	↓73.5%	↓41.9%	↓48.7%	↓54.6%	↓66.2%
NLRP3	1.597 ±0.05^{g}	1.550 ±0.05^{g}	8.759 ±0.28^{a}	5.832 ±0.13^{b}	4.825 ±0.11^{c}	5.051 ±0.11^{c}	4.071 ±0.05^{d}	3.208 ±0.17^{e}	2.243 ±0.14^{f}
% of change in relation to control negative	-----	↓2.9%	↑448.5%	↑265.1%	↑202.1%	↑216.3%	↑154.9%	↑100.9%	↑40.4%
% of change in relation to control diseased	-----	-----	-----	↓33.4%	↓44.9%	↓42.3%	↓53.5%	↓63.4%	↓74.%

Results are presented as Mean ± Std Error (n= 6 rats).
Values in the same row with different superscript letters are significantly (P <0.0001) different. Where a represent highest value and e,f,g represent lowest value.
Statistical comparisons were carried out using one-way ANOVA followed by post-hoc tests using LSD method.
n: number of rats in each group.
NLRP3: NACHT, LRR and PYD domains-containing protein 3, mTOR: Mammalian target of rapamycin, ASC: Apoptosis-associated speck-like protein

Western blot analysis of ASC and mTOR

In control negative group(1) expression of mTOR and ASC were reduced as compared to the Diclofenac treated group(2) in a dose of (10 mg/kg/day, i.p.).
In Ticagrelor group(3) given (20 mg/kg/day, orally) expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were same level of the control negative group.
In Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were higher than the control negative group.
In Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were higher than the control negative group.

In Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were higher than the control negative group.
In Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were same level of the control negative group.
In Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were same level of the control negative group.
In Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days expression of mTOR and ASC were reduced as compared to the Diclofenac treated group, meanwhile these were same level of the control negative group.

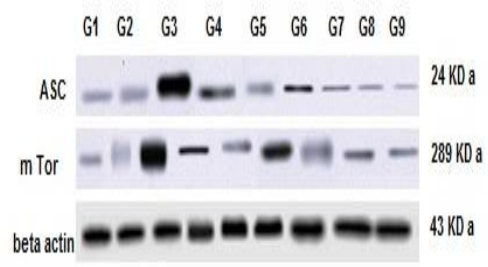

Figure 5. Effect of administration of Ticagrelor either prophylactic or curative on ASC and mTOR
G1:control ,G2:ticagrelor , G3:diclofenac,G4:small dose prophylaxis
G5:large dose prophylaxis , G6: small dose short term ttt ,
G7: large dose short term ttt , G8: small dose long term ttt,
G9: large dose long term ttt

Histopathological findings:

Microscopic examination of livre tissue

Sections from liver "control negative group(1) (**fig. 6, 7)** revealed normal histological structures of hepatic lobules, hepatic cords, sinusoids, portal triads, and central veins.
Ticagrelor group(3) of liver given (20 mg/kg/day, orally) **(fig.8, 9)** showed normal histological architectures of hepatic cords, portal triads, sinusoids and central veins.
But, Diclofenac group (2) given (10 mg/kg/day, i.p.) exhibited marked steatosis which commonly seen within periportal areas**(fig.10)**. Some examined sections revealed centrilobular degenerated and necrotic hepatic cells adjacent to mononuclear inflammatory cells aggregates mainly lymphocytes **(fig.11)**. Also, ruptured of some fatty degenerated cells were detected which coalesced to form fatty cysts. Some necrotic cells may be leaving empty spaces filled with minute number of leukocytes and cellular debris **(fig.12)**. In addition, congested hepatic blood vessels and sinusoids were seen **(fig.13)**.
Small dose prophylactic group (4) given (10 mg/kg/day, orally) for 7 days (SP): showed few number of fatty degenerated cells particularly within periportal areas **(fig.14)**. Some sections revealed moderate number of randomly distributed signet ring appeared cells **(fig.15)**. Focal perivascular necrotic areas were also encountered **(fig.16)**.
Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days (LP) **(fig.17,18,19)** exhibited interstitial and perivascular minute areas of round cells infiltrates. Hyperplastic and hypertrophied kupffer cells with minute sinusoidal lymphocytic aggregation could be noticed beside mildly dilated central vein.
Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days (SST) demonstrated presence of many fatty degenerated cells within periportal areas **(fig.20)**. The latter were infiltrated with round cells infiltrations in some examined sections **(fig.21)**. Accidental and programmed cell deaths could be detected at centro lobular areas in many examined sections **(fig.22)**. The necrotic cells appeared as swollen

cells with pyknotic or karyolysis nuclei and cytplasmlysis. While, apoptotic cells manifested by shrinked cells with clumped chromatin and homogenous eosinophilic cytoplasm.

Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days (SLT) showed few number of hydropic degenerated cells alternated with unicellular hepatic necrosis with pyknotic nuclei at some centrolobular areas **(fig.23)**. Unicellular fatty change beside interstitial inflammatory cells aggregtions were noticed **(fig.24)**. Congested hepatic blood vessels and sinusoids were also detected **(fig.25)**.

Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days (LST) showed still presence of periportal fatty change **(fig.26)**. Various types of acute cell swelling were observed in some examined sections primarily hydropic degeneration. Also, dilated central veins were also seen. Moreover, Necrotic hepatic cells in some fields were replaced by mononuclear inflammatory cells **(fig.27)**. The latter were also demonstrated at perivascular areas **(fig.28)**.

Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days (LLT) revealed preserved hepatocytes arrangement, sinusoids, central veins and portal areas in the majority of examined sections **(fig.29)**. However, some sections exhibited presence of minute area of hydropic degeneration **(fig.30)** and unicellular fatty degenerated cells **(fig. 31)**.

Microscopic examination of renal tissue

Kidney of control negative group(1) showed normal histology of glomerular tufts, renal tubules and stromal structures **(fig.32,33)**.

Ticagrelor group(3) of kidney given (20 mg/kg/day, orally) revealed normal structures of glomerular corpuscles and renal tubular epithelium **(fig.34,35)**.

Sections from Diclofenac group(2) given (10 mg/kg/day, i.p.) revealed necrosed some glomerular corpuscles **(fig.36)** and atrophied of few number of glomerular tufts **(fig.37)**. The latter were dilated in some examined section. Some renal tubular epithelium suffered from degenerative changes with co-agulative necrosis in other tubules **(fig.38)**. Intraluminal hyaline casts were seen within some renal tubules. Congested peritubular capillaries were also seen **(fig.39)**.

Small dose prophylactic group(4) given (10 mg/kg/day, orally) for 7 days (SP): showed hydropic degeneration in some renal tubular epithelium **(fig.40,41)**. Congestion of renal blood vessels and hemorrhages between tubules were also seen **(fig.42)**.

Large dose prophylactic group(5) given (20 mg/kg/day, orally) for 7 days (LP) displayed cloudy swelling in few number of renal epithelium **(fig.43,44)**. Most renal corpuscles and renal tubules showed maintenance their histological structures **(fig.45)**.

Short term small dose treatment group(6) given (10 mg/kg/day, orally) for 7 days (SST) exhibited focal degeneration in some tubular epithelium with intracytoplasmic hyaline droplets **(fig.46)**. Necrotic and destructed few tubules with perivascular edema and peritubular round cells infiltration were noticed **(fig.47)**. Congested blood vessels and glomerular capillaries were also seen **(fig.48)**.

Short term large dose treatment group(7) given (20 mg/kg/day, orally) for 7 days (SLT) showed degenerative changes in a moderate number of renal tubules beside intraluminal hyaline casts **(fig.49)**. Shrined glomerular tufts **(fig.50)** and dilated peritubular capillaries were also observed **(fig.51)**.

Long term small dose treatment group(8) given (10 mg/kg/day, orally) for 28 days (LST) showed ballooning degeneration in few tubules **(fig.52)**. Perivascular lymphocytic aggregation **(fig.53)** and dilated renal vasculatures was also seen **(fig.54)**.

Long term large dose treatment group(9) given (20 mg/kg/day, orally) for 28 days (LLT) showed regeneration of renal tubules **(fig.55)**, apparent normal histological configurations of renal tubules and glomerular corpuscles **(fig.56,57)**.

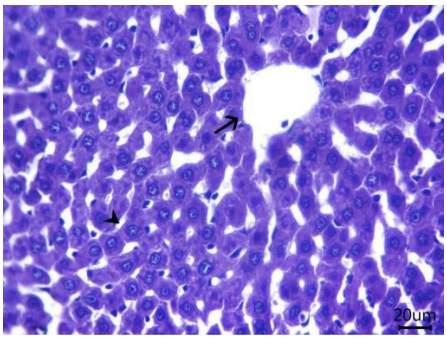

Fig. 6: Photomicrograph of H&E stained sections from control negative group of liver G(1) (**Scale bar 20µm)** showing normal histological structures of hepatic cords (arrowhead), sinusoids, and central vein (arrow).

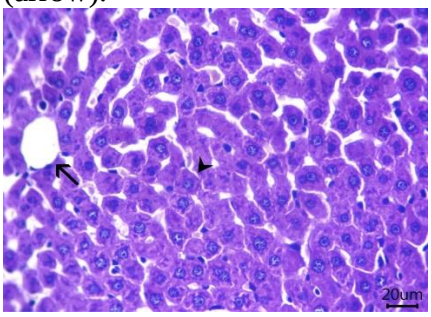

Fig.7: Photomicrograph of H&E stained sections from control negative group of liver G(1)(**Scale bar 20µm)** showing normal histological structures of hepatic cords (arrowhead), sinusoids, and central vein (arrow).

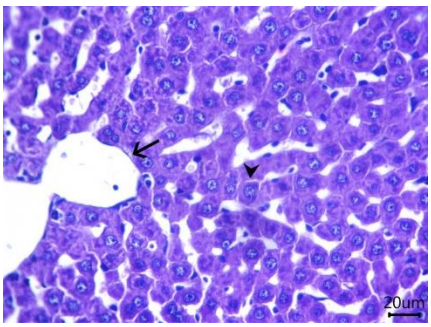

Fig.8: Photomicrograph of H&E stained sections from Ticagrelor group of liver G(2) **(Scale bar 20µm)** showing normal histological architectures of hepatic cells (arrowhead), sinusoids and central vein (arrow).

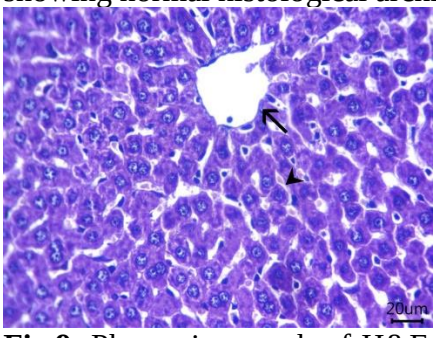

Fig.9: Photomicrograph of H&E stained sections from Ticagrelor group of liver G(2) **(Scale bar 20µm)** showing normal histological architectures of hepatic cells (arrowhead), sinusoids and central vein (arrow).

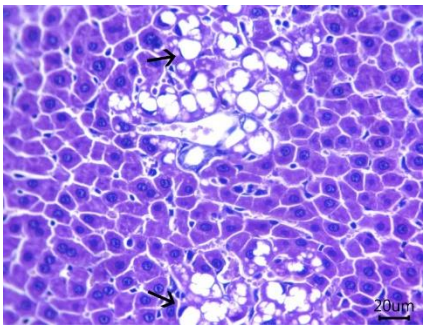

Fig.10: Photomicrograph of H&E stained sections from Diclofenac group of liver G(3) **(Scale bar 20µm)** showing marked periportal steatosis (arrows)

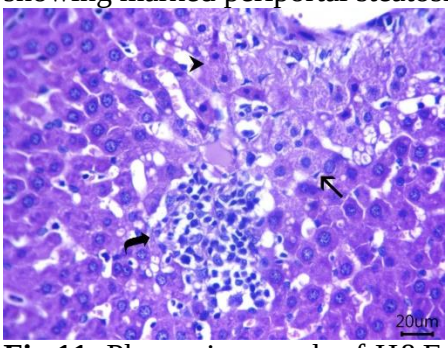

Fig.11: Photomicrograph of H&E stained sections from Diclofenac group of liver G(3) **(Scale bar 20µm)** showing centrolobular degenerated (arrow) and necrotic (arrowhead) hepatic cells adjacent to mononuclear inflammatory cells aggregates mainly lymphocytes (curved arrow).

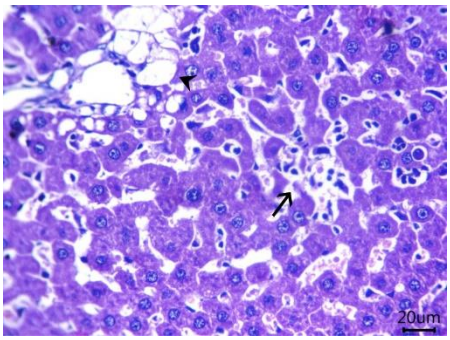

Fig.12: Photomicrograph of H&E stained sections from Diclofenac group of liver G(3) **(Scale bar 20µm)** showing fatty cysts (arrowhead) and empty spaces filled with minute number of leukocytes and cellular debris (arrow).

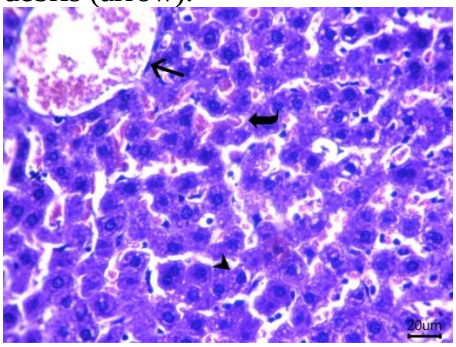

Fig.13: Photomicrograph of H&E stained sections from Diclofenac group of liver G(3) **(Scale bar 20µm)** showing vacuolated hepatic cells (arrowhead), congested central vein (arrow) and sinusoids (curved arrow).

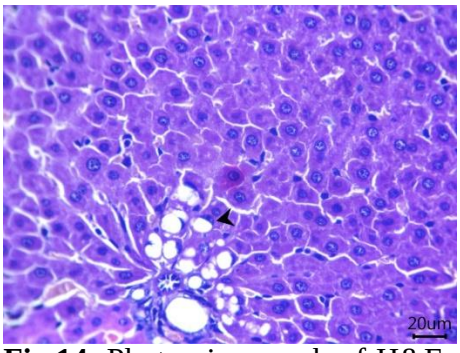

Fig.14: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of liver G(4) **(Scale bar 20µm)** showing few number of fatty degenerated cells particularly within periportal areas **(arrowhead)**.

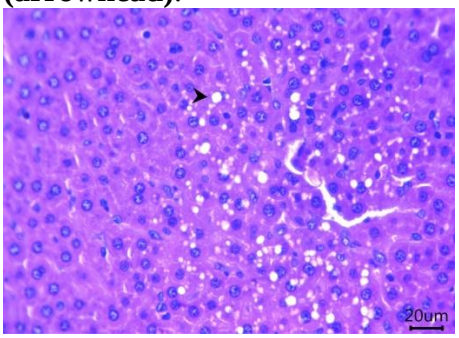

Fig.15: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of liver G(4) **(Scale bar 20µm)** showing moderate number of randomly distributed signet ring appeared cells **(arrowhead)**.

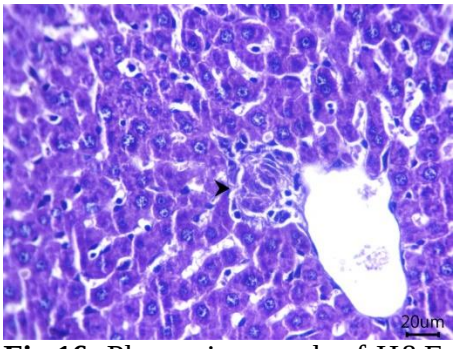

Fig.16: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of liver G(4) **(Scale bar 20µm)** showing focal perivascular necrotic areas (arrowhead)

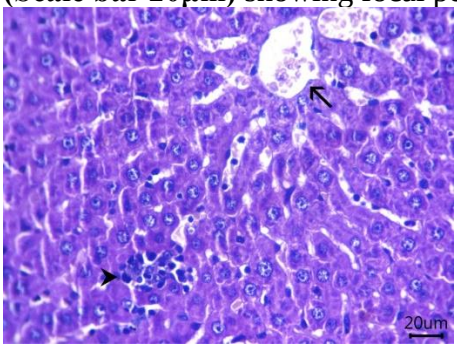

Fig.17: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of liver G(5) **(Scale bar 20µm)** showing minute areas of interstitial round cells infiltrates (arrowhead), sinusoidal lymphocytic aggregation and mildly dilated central vein (arrow)

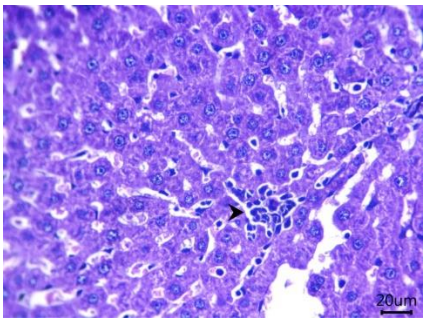

Fig.18: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of liver G(5) **(Scale bar 20µm)** showing perivascular minute areas of round cells infiltrates **(arrowhead)**.

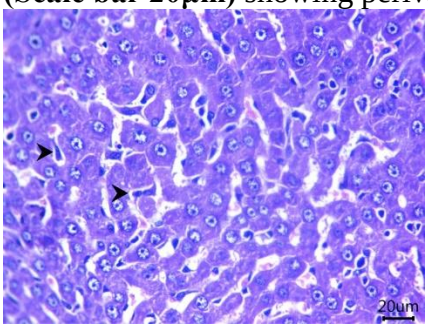

Fig.19: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of liver G(5) **(Scale bar 20µm)** showing hyperplastic and hypertrophied kupffer cells **(arowheads)**

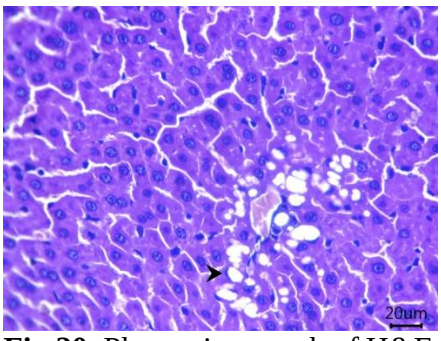

Fig.20: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of liver G(6) **(Scale bar 20µm)** showing presence of many fatty degenerated cells within periportal areas **(arrowhead)**.

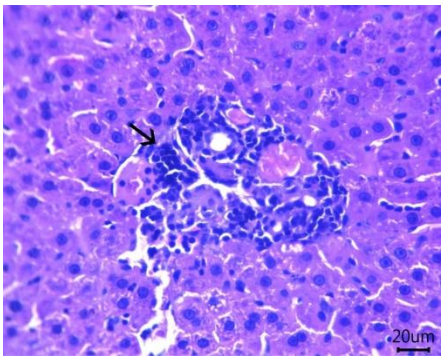

Fig.21: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of liver G(6) **(Scale bar 20µm)** showing round cells infiltrations within some portal area **(arrow)**.

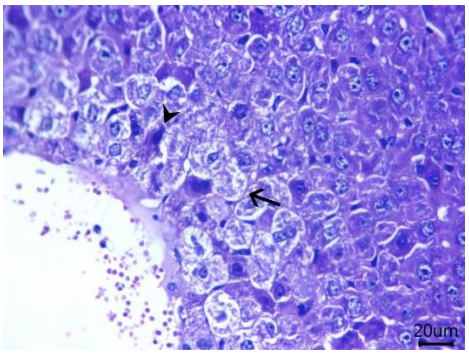

Fig.22: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of liver G(6) **(Scale bar 20µm)** showing accidental cell death (arrow) and programmed cell death (arrowhead) at centro lobular areas.

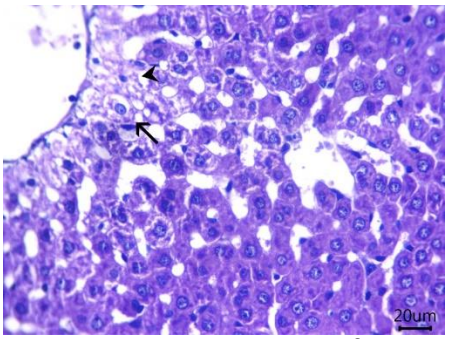

Fig.23: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of liver G(7) **(Scale bar 20µm)** showing few number of hydropic degenerated cells (arrow) alternated with unicellular hepatic necrosis (arrowhead) with pyknotic nuclei at some centrolobular areas **.**

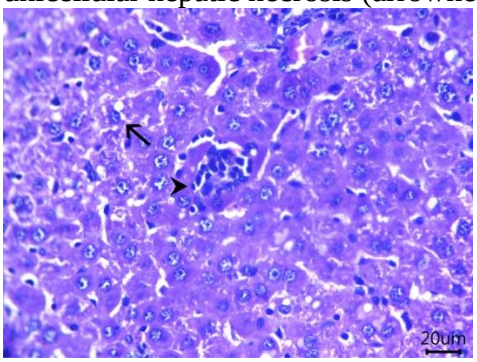

Fig.24: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of liver G(7) **(Scale bar 20µm)** showing unicellular fatty change (arrowhead) beside interstitial inflammatory cells aggregtions (arrow).

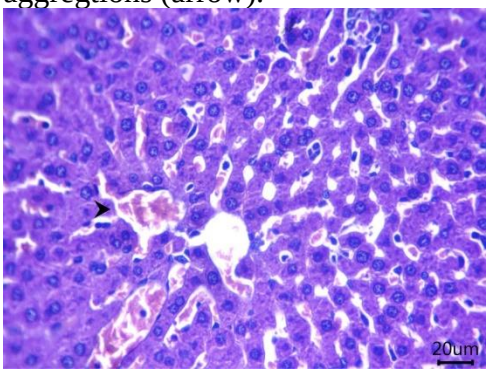

Fig.25: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of liver G(7) **(Scale bar 20µm)** showing congested central vein (arrowhead).

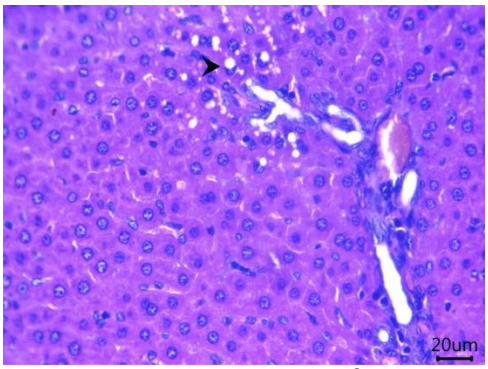

Fig.26: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of liver G(8) **(Scale bar 20µm)** showing still presence of periportal fatty change (arrowhead).

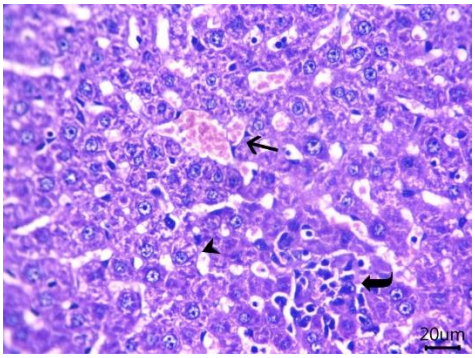

Fig.27: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of liver G(8) **(Scale bar 20µm)** showing various types of acute cell swelling primarily hydropic degeneration (arrowhead), dilated central vein (arrow) and necrotic hepatic cells replaced by mononuclear inflammatory cells (curved arrow).

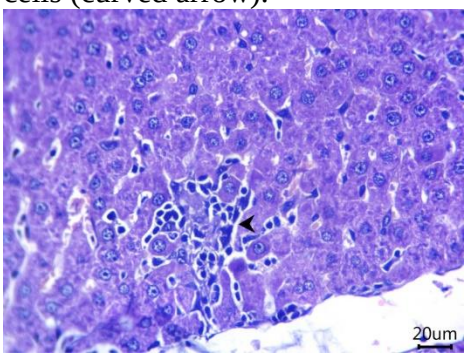

Fig.28: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of liver G(8) **(Scale bar 20µm)** showing mononuclear inflammatory cells at perivascular areas **(arrowhead).**

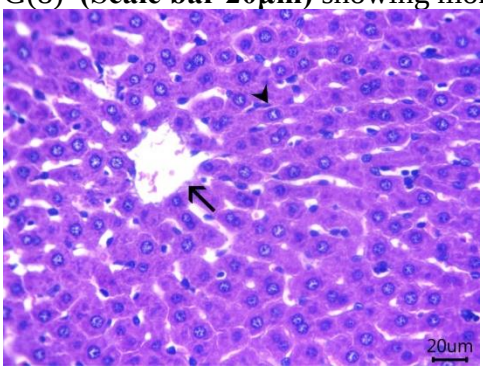

Fig.29: Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of liver **(Scale bar 20µm)** showing preserved hepatocytes arrangement **(arrowhead)**, sinusoids, central vein **(arrow).**

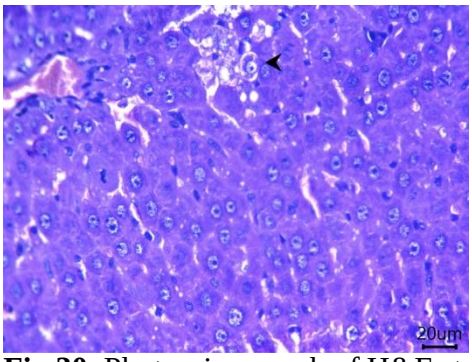

Fig.30: Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of liver G(9) **(Scale bar 20µm)** showing minute area of hydropic degeneration **(arrowhead).**

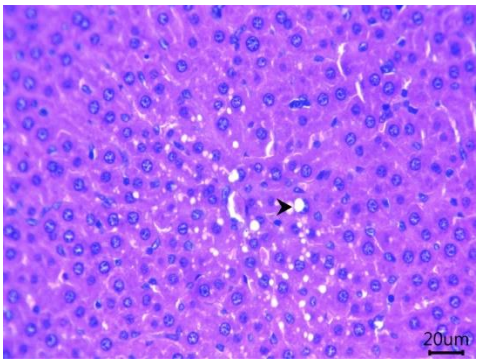

Fig.31: Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of liver G(9) **(Scale bar 20µm)** showing unicellular fatty degenerated cells **(arrowhead).**

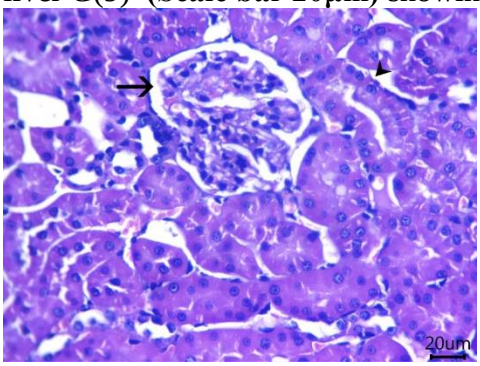

Fig.32: Photomicrograph of H&E stained sections from control negative group of kidney G(1) (**Scale bar 20µm)** showing normal histology of glomerular tufts (arrow) and renal tubules **(arrowhead).**

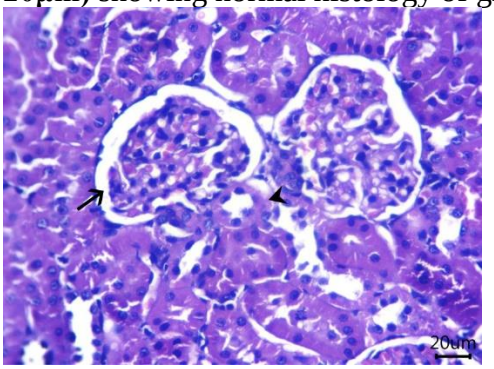

Fig.33: Photomicrograph of H&E stained sections from control negative group of kidney G(1) (**Scale bar 20µm)** showing normal histology of glomerular tufts (arrow) and renal tubules **(arrowhead).**

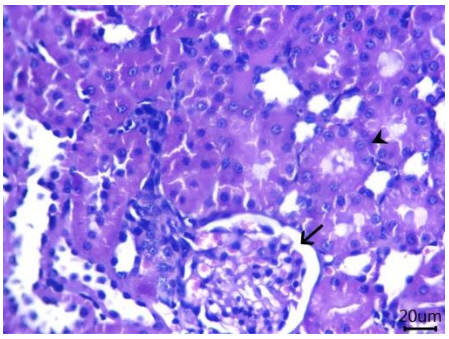

Fig.34: Photomicrograph of H&E stained sections from Ticagrelor group of kidney G(2) (**Scale bar 20µm)** showing normal structures of glomerular corpuscle (arrow) and renal tubular epithelium **(arrowhead).**

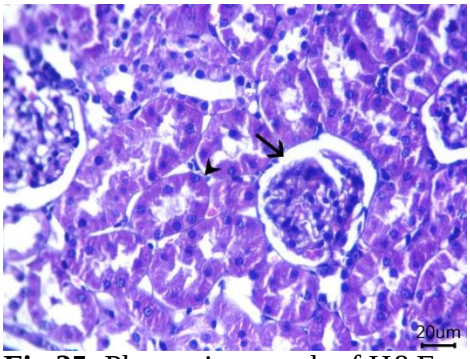

Fig.35: Photomicrograph of H&E stained sections from Ticagrelor group of kidney G(2) (**Scale bar 20µm)** showing normal structures of glomerular corpuscle (arrow) and renal tubular epithelium **(arrowhead).**

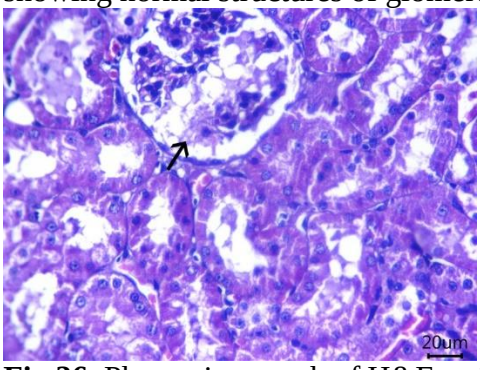

Fig.36: Photomicrograph of H&E stained sections from Diclofenac group of kidney G(3) (**Scale bar 20µm)** showing necrosed some glomerular corpuscles **(arrow).**

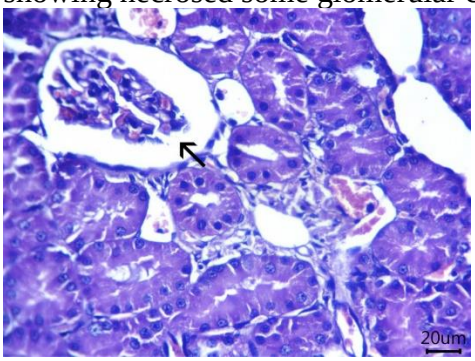

Fig.37: Photomicrograph of H&E stained sections from Diclofenac group of kidney G(3) (**Scale bar 20µm)** showing atrophied few number of glomerular tufts **(arrow).**

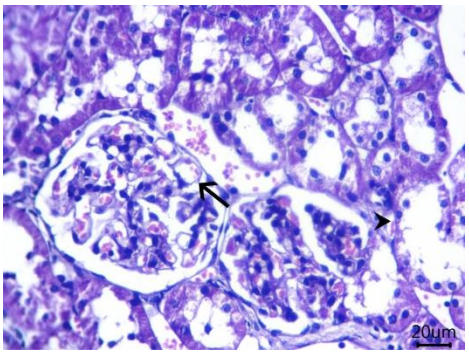

Fig.38: Photomicrograph of H&E stained sections from Diclofenac group of kidney G(3) (**Scale bar 20µm)** showing dilated glomerular capillaries (arrow), degenerative changes and co-agulative necrosis **(arrowhead)** in some renal tubular epithelium**.**

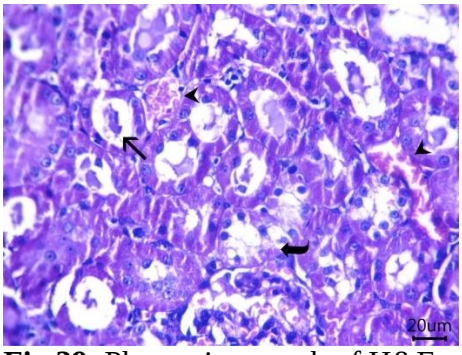

Fig.39: Photomicrograph of H&E stained sections from Diclofenac group of kidney G(3) (**Scale bar 20µm)** showing intraluminal hyaline casts (arrow), Congested peritubular capillaries (arrowhead) and unicellular epithelial necrosis (curved arrow).

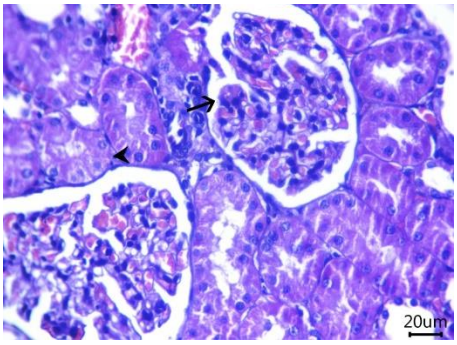

Fig.40: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of kidney G(4) (**Scale bar 20µm)** showing normal morphology of glomerular tufts (arrow) and hydropic degeneration in some renal tubular epithelium (arrowhead).

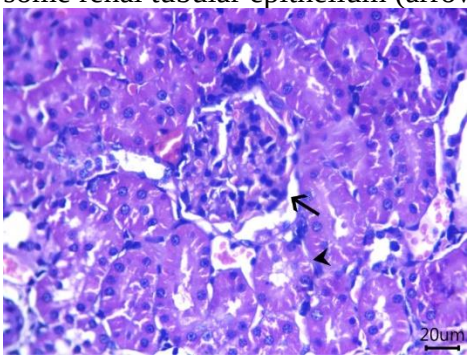

Fig.41: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of kidney G(4) (**Scale bar 20µm)** showing normal morphology of glomerular tufts (arrow) and hydropic degeneration in some renal tubular epithelium (arrowhead).

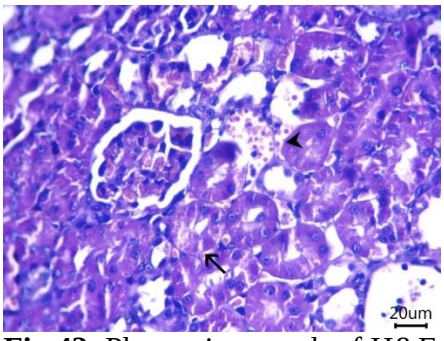

Fig.42: Photomicrograph of H&E stained sections from Small dose prophylactic group (SP) of kidney G(4) (**Scale bar 20µm)** showing congestion of renal blood vessels, hemorrhages between tubules (arrowhead) and degenerated renal tubules (**arrow)**.

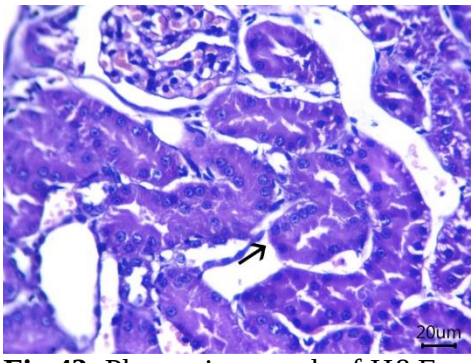

Fig.43: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of kidney G(5) (**Scale bar 20µm)** showing cloudy swelling in few number of renal epithelium **(arrow)**.

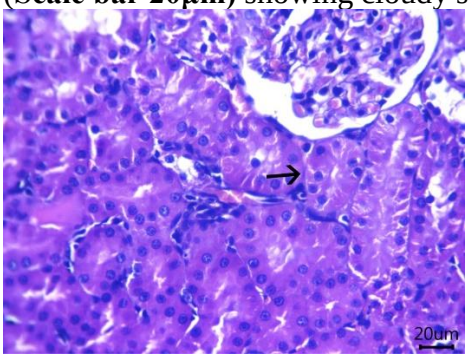

Fig.44: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of kidney G(5) (**Scale bar 20µm)** showing cloudy swelling in few number of renal epithelium **(arrow)**

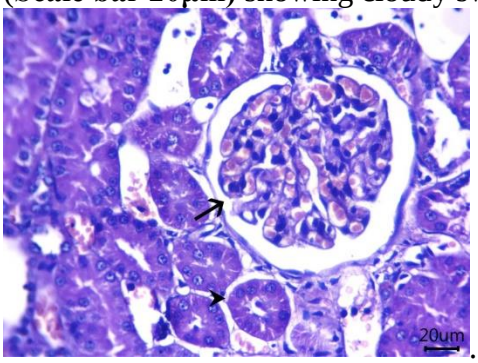

Fig.45: Photomicrograph of H&E stained sections from Large dose prophylactic group (LP) of kidney G(5) (**Scale bar 20µm)** showing maintenance of histological structures of most renal corpuscle (arrow) and renal tubule (arrowhead).

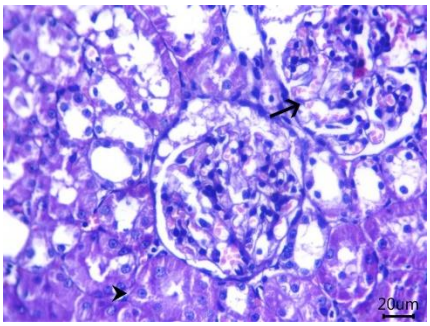

Fig.46: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of kidney G(6) (**Scale bar 20µm)** showing focal degeneration in some tubular epithelium with intracytoplasmic hyaline droplets (arrowhead), dilated glomerular capillaries (arrow).

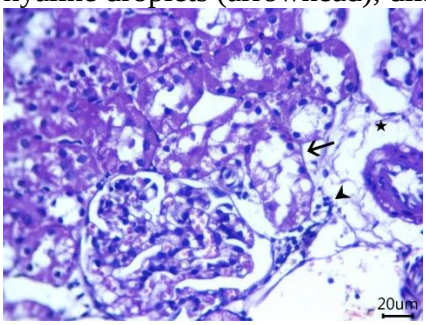

Fig.47: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of kidney G(6) (**Scale bar 20µm)** showing Necrotic and destructed few tubules (arrow) with perivascular edema (star) and peritubular round cells infiltration (arrowhead).

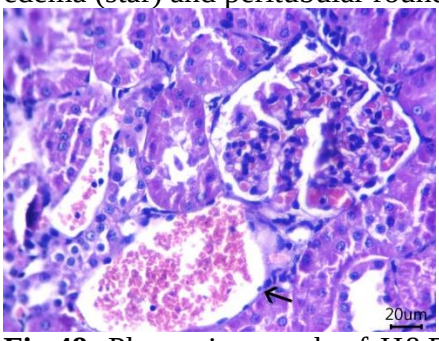

Fig.48: Photomicrograph of H&E stained sections from Short term small dose treatment group (SST) of kidney G(6) (**Scale bar 20µm)** showing congested blood vessels (arrow) and glomerular capillaries.

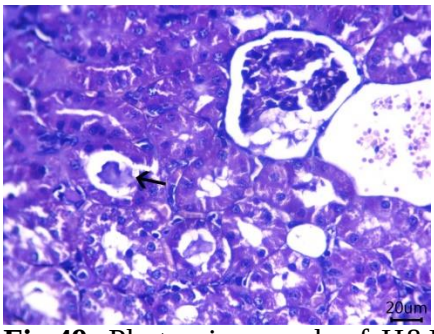

Fig.49: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of kidney G(7) (**Scale bar 20µm)** showing degenerative changes in a moderate number of renal tubules beside intraluminal hyaline casts (arrow).

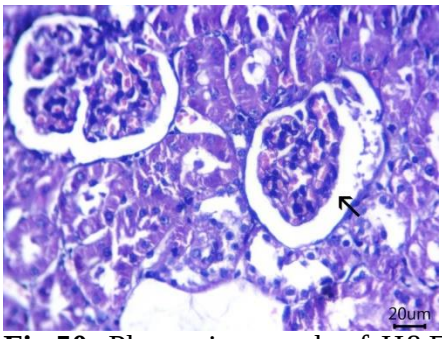

Fig.50: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of kidney G(7) (**Scale bar 20µm)** showing Shrinked glomerular tufts (arrow).

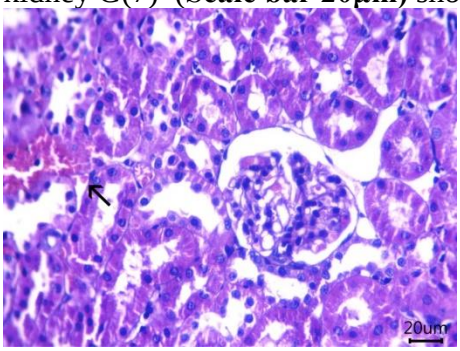

Fig.51: Photomicrograph of H&E stained sections from Short term large dose treatment group (SLT) of kidney G(7) (**Scale bar 20µm)** showing dilated peritubular capillaries (arrow).

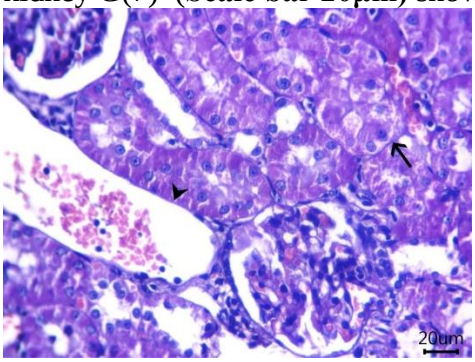

Fig.52: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of kidney G(8) (**Scale bar 20µm)** showing ballooning degeneration in few tubules (arrow) and dilated vasculature (arrowhead).

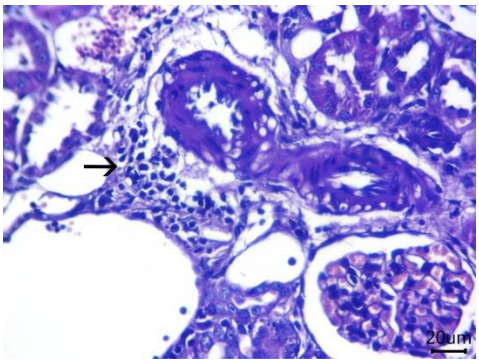

Fig.53: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of kidney G(8) (**Scale bar 20µm)** showing Perivascular lymphocytic aggregation (arrow)

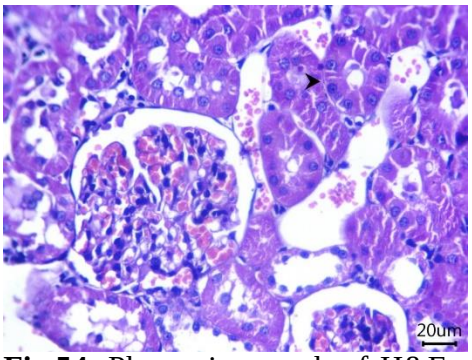

Fig.54: Photomicrograph of H&E stained sections from Long term small dose treatment group (LST) of kidney G(8) (**Scale bar 20µm)** showing dilated renal vasculatures and apparent normal tubular epithelium (arrowhead)

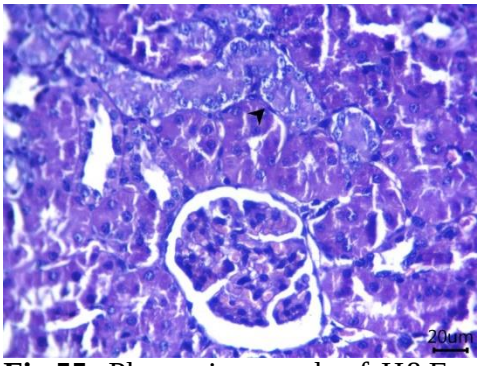

Fig.55: Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of kidney G(9) (**Scale bar 20µm)** showing regeneration of renal tubules (arrowhead)

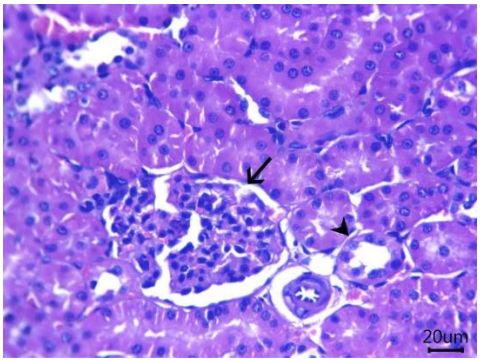

Fig.56; Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of kidney G(9) (**Scale bar 20µm)** showing apparent normal histological configurations of renal tubule (arrowhead) and glomerular corpuscle (arrow).

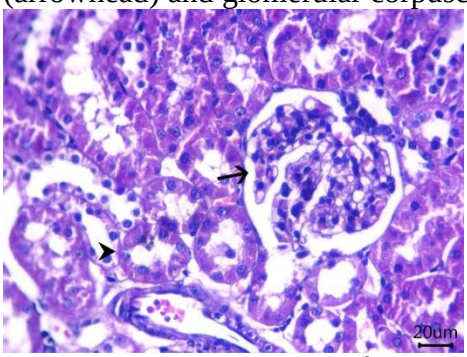

Fig.57: Photomicrograph of H&E stained sections from Long term large dose treatment group (LLT) of kidney G(9) (**Scale bar 20µm)** showing apparent normal histological configurations of renal tubule (arrowhead) and glomerular corpuscle (arrow).

Table 6. Lesions score of the severity extent in Hepatic and renal tissues among different experimental groups:

Organ	Lesions	Control negative	Ticagrelor	Diclofenac (Diseased)	Small dose prophylactic group (SP)	Large dose prophylactic group (LP)	Short term dose small treatment group (SST)	Short term dose large treatment group (SLT)	Long term dose small treatment group (LST)	Long term dose large treatment group (LLT)
Liver	**Fatty change**	0	0	3	1	0	2	1	1	0
	Hydropic degeneration	0	0	3	1	1	2	1	1	1
	Hepatic cells necrosis	0	0	1	1	0	1	1	1	1
	Hepatic cells apoptosis	0	0	1	0	0	1	0	0	0
	Congested hepatic vasculatures	0	0	2	0	1	0	1	1	0
	Mononuclear inflammatory cells aggregation	0	0	2	1	1	2	1	1	0
Kidney	**Atrophied glomeruli**	0	0	2	0	0	0	1	0	0
	Necrotic glomeruli	0	0	2	0	0	0	0	0	0
	Congested glomerular tufts	0	0	1	0	0	1	0	0	0
	Cloudy swelling of renal Epithelium	0	0	3	1	1	2	1	1	1
	Ballooning degeneration	0	0	2	2	0	1	1	1	0
	Hyaline droplets	0	0	2	0	1	2	0	0	0
	Hyaline casts	0	0	2	0	0	0	1	0	0
	Co-agulative necrosis	0	0	1	0	0	1	0	0	0
	Congested peritubuar capillaries	0	0	2	1	0	1	1	1	0
	Hemorrhage	0	0	1	1	0	0	0	0	0
	Lymphocytic aggregation	0	0	2	0	0	1	0	1	0

Lesions score system was as follows: (0 = no detectable histopathological lesion, 1 = rarely minimal or focal, 2 = multifocal, 3 = patchy or diffuse) as a semiquantitative method

Discussion

Drug-induced liver injury (DILI) is considered as a serious public health issue furthermore hepatotoxicity has been regarded as the main clinical problem caused by it **(5).**The liver plays a key role in the metabolism of a variety of drugs and toxins and thus is especially susceptible to damage induced by drugs (Mudd and Guddati, 2021) and most latest evidence, suggested that the incidence of hepatotoxicity and prevalence is 15–20 cases per 100,000 individuals around the globe**(6).**

Acute kidney injury (AKI) is a global health challenge of vast proportions, as approximately13.3% of people worldwide are affected annually. The pathophysiology of AKI is very complex, but its main causes are sepsis, ischemia, and nephrotoxicity(7). Medication-induced nephrotoxicity remains one of the most common causes of acute kidney injury (AKI) among hospitalized patients(Morales-Alvarez, 2020) and accounts for 19–26% of all hospitalized cases**(8).**

Owing to the efficacy in reducing pain and inflammation, non-steroidal anti-inflammatory drugs (NSAIDs) are among the most popularly used medicines confirming their position in the WHO's Model List of Essential Medicines. With escalating musculoskeletal complications, as evident from 2016 Global Burden of Disease data, NSAID usage is evidently unavoidable**(9).**

Diclofenic (DIC), a nonsteroidal anti-inflammatory (NSAID), analgesic and antipyretic drug, is widely used globally to relieve pain and fever**(10).**It is extensively consumed for the management of many chronic conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis**(11).**About 940 tons of (DIC)

are consumed yearly**(12).** The presence of DIC as an over-the counter drug may lead to its abuse resulting in threatening deleterious effects on the liver, kidney, and gastrointestinal tract**(13).** Oxidative stress and mitochondrial damage are the main elements collaborating in DIC toxicity**(14).**

Ticagrelor is one of the most recent antiplatelet agents used to inhibit platelet aggregation via blocking the ADP receptors of the subtype $P2Y_{12}$,it was first discovered by Astra Zeneca and approved for use in 2011 by the FDA . Ticagrelor is usually used for the prevention and treatment of thromboembolism in adult patients with acute coronary syndrome **(15).**

Ticagrelor inhibits activation of the NLRP3 inflammasome independent of the P2Y12 receptor(16). The NLRP3 inflammasome is composed of NLRP3 protein, ASC and pro-caspase-1. The NLRP3 activation triggers the cleavage of caspase-1, which promotes the activation IL-1β and cytokines release(17).

The current study was designed to evaluate the possible ameliorative effect of ticagrelor against diclofenac induced hepatotoxicity and nephrotoxicity .

In the present work, hepatotoxicity was evaluated by measuring portal blood pressure, Liver enzymes [serum alanine transferase (ALT) and serum aspartate transferase (AST)],hepatic tissue malondialdehyde (MDA) and histopathological examination for liver pathology.

Nephrotoxicity was evaluated by measuring serum urea and creatinine, renal tissue level of malondialdehyde (MDA) ,in addition to histopathological examination of renal pathology.

Inflammation and oxidative stress were evaluated by measuring NLRP3, mamillian target of rapamycin (mTOR) ,Apoptosis- associated speck-like protein containing a CARD (ASC) ,caspase 1,Interleukin (IL1B),Gasdermin (GSDMD), superoxide dismutase (SOD).

Diclofenac induced hepatorenal toxicity model used in this work was described by **Elbaz et al., (51)** who reported that diclofenac (10 mg/kg/day, intraperitoneal [ip]) in rats for 7 days produced hepatorenal toxicity.

The current study showed that diclofenac (10 mg/kg/day, intraperitoneal [ip]) in rats for 7 days resulted in a significant increase in serum urea , creatinine levels , renal and hepatic tissue MDA,ALT,AST,IL1B with a significant decrease in SOD level in relation to control group .

These results are in agreement with**Alabi et al., (18)** who found a significant increase in serum urea , creatinine level and renal tissue MDA in diclofenac treated rats (10 mg/kg/day) i.m for 7 days in relation to control group.

Also, these results are in consistence with **Ahmed et al., (19)** who showed that the administration of diclofenac sodium (100 mg/kg/day/i.m., for 3 days) induced a significant increase in urea , creatinine concentrations and renal tissue MDA compared with control group.

Also, these results are in consistence with **Alorabi et al., (1)** who showed that SCr level was elevated significantly in DIC -treated rats (150 mg/kg I.P, for 6 days) compared with the control group with percent changes of 117.14 % ($P < 0.05$).

Also ,these results in agreement with **Alkuraishy et al., (20)** who showed that Diclofenac 15 mg/kg led to significant AKI through elevation of serum urea and creatinine levels.

Nephrotoxic effect of diclofenac probably causes a decrease in the rate of excretion of urea and creatinine inducing an increase in serum urea and creatinine concentrations. The rate of excretion is influenced by glomerular filtration rate, and any nephron abnormalities decrease glomerular filtration rate resulted in an increase in serum urea and creatinine **(19).** Previous studies have shown that minor increase in serum creatinine can reflect a marked fall in glomerular filteration rate **(21).** Elevated creatinine and urea levels are associated with the compromised integrity of the glomerular filtration rate barrier, leading to renal failure **(22)**

These results also are in consistence with **Aycan et al., (23)** who showed that intramuscular injection of diclofenac at a dose 9 mg/kg body weight twice daily for 5 successive days caused liver abnormalities manifested as significant increase in ALT and AST in diclofenac treated rats compared to sham group .

furthermore **Moradi et al., (24)** indicated that the levels of the ALT, AST, urea, creatinine, MDA and serum IL-1β were remarkably increased in DIC treated animals (50 mg/kg bw, i.p.) for 7 consecutive days compared to control group.

In the same context **Peter S et al., (25)** proved that Rats treated with DIC showed elevated levels of serum liver function markers such as ALT, AST and significant elevation in the levels of urea and creatinine with significant reduction in the activities of the antioxidant enzyme SOD.

Additionally **Darbar et al., (26)** showed that the administration of Diclofenac at the single dose of 150 mg /kg-1 body weight intraperitonially once daily for 28 days caused a dramatic elevation in serum AST and

ALT, indicating sub-chronic hepatotoxicity with severe damage to hepatic tissue membranes during DIC intoxication and the release of these enzymes into circulation. In cases of hepatotoxicity, the cellular transport of liver cells is modified, altering the plasmatic membrane, which results in the release of these liver enzymes and a subsequent increase in their levels (27).

Also, This is in agreement with **Elshopakey and Elazab, (14**) who indicated that serum ALT and AST activities , Hepatic and renal MDA levels were significantly elevated in DIC treated rats compared to the control group also the hepatic and renal activities of SOD was significantly reduced in DIC- treated groups unlike that of the control rats. The elevation of serum IL-1β level reflect the hepato-renal injury as showed in DIC groups.

Also, **Alabi et al., (27)** showed that the administration of diclofenac caused a significant decrease in superoxide dismutase (SOD) activity when compared with those of the control group, but also resulted in apronounced elevation of malondialdehyde (MDA) levels. These could result from the formation of excess free radicals generated by DIC metabolites that overwhelm the antioxidant status system and cause peroxidation of lipids.

In line with our results **Nouri and Heidarian, (28)** showed that superoxide dismutase (SOD) remarkably reduced in diclofenac treated group relative to the control group and **Prince, (29)** showed an elevation in the level of IL1β on DIC -induced rats which indicated oxidative stress and inflammation.

Our study showed that ticagrelor given in a dose of 10 and 20 mg/kg as a prophylactic agent and in a dose of 10 and 20mg/kg as a curative agent for 7days or 28 days resulted in a significant decrease in serum urea , creatinine level , renal and hepatic tissue MDA,ALT,AST,IL1B with a significant increase in SOD level in relation to diclofenac group.

SOD can protect the human body from peroxidation with the consequent damage to the tissues by inactivating the oxygen-free radicals. MDA is a poisonous end-product of lipid peroxidation. The level of MDA represents the rate and extent of lipid peroxidation directly and shows the capability of eliminating free radicals indirectly **(30)**

These results are in consistence with **Findik et al., (31)** who found that the rats that received 25 mg/kg dose of ticagrelor had lower levels of MDA than the control group.

Also in consistence with **Chen, (32)** who found that the IL-1β content in serum and MDA content in brain tissue of ticagrelor group were significantly lower than those of model group while SOD content was significantly higher than those of model group of cerebral ischemia reperfusion injury in rats This means that ticagrelor can reduce the oxidative stress response.

In a study by **BAĞCIOĞLU et al., (33),** the animals that received ticagrelor 5 mg/kg, 10 mg/kg and 20 mg/kg had significantly less MDA level in renal tissue than the control group.There was less lipid peroxidation product in the animals that received ticagrelor than the control group, and this decrease was statistically significant .

post induction of renal ischemia reperfusion(I/R) ,administration of ticagrelor (20 mg/kg; p.o) for 3 days dwindled creatinine parameter markedly by 71%, as compared to the I/R group it also lessened renal content of MDA by 64% compared to I/R group **(34).**

Lv et al., (35) showed that after treating the mice with ticagrelor, the cecal ligation and puncture CLP-induced increases of creatinine and urea were significantly lower compared to the sepsis model group ,This indicates that ticagrelor can protect renal function in sepsis. Also MDA levels decreased significantly in the ticagrelor treated group with significant increase in SOD level

Also ,**Zhang et al., (12)** showed that Ticagrelor reduced serum levels of IL-1B,ALT,AST and MDA levels in rats treated with a dose of 20 mg/kg/d orally once a day for 12 weeks in relation to model group of rats.

ALSO,Ticagrelor reduced serum levels of IL-1B and SCR levels in a dose dependant manner (10,30,100 mg/kg body weight) in relation to mice in cecal ligation and puncture group **(36).** Ticagrelor treatment reduced SCr and IL-1β expression levels in a model of acute kidney injury in septic rats **(37).**

The current study shows that diclofenac (10 mg/kg/day, intraperitoneal [ip]) in rats for 7 days resulted in a significant increase in serum levels of NLRP3,ASC, caspase-1,gasdermin and mTOR.

This is in agreement with **El-Maadawy et al., (38)** who showed that In DIC -treated group , in a dose of (50 mg/kg, I.M.), DIC upregulated mTOR gene expression when compared to normal group ,significantly increased the NLRP3, ASC and caspase-1 mRNA expressions, which was associated with a marked increase in IL-1β levels. The active caspase-1 mediates the cleavage and activation of gasdermin D (GSDMD) the hallmark protein of pyroptosis **(39).** GSDMD form pores in the plasma membrane leading to cell swelling,

membrane lysis and further release of the pro-inflammatory cytokines **(17).**

The current study shows that ticagrelor given in a dose of 10 and 20 mg/kg as a prophylactic agent and in a dose of 10 and 20mg/kg as a curative agent for 7days or 28 days resulted in a significant decrease in serum levels of NLRP3,ASC, caspase-1,gasdermin and mTOR.

This is in agreement with Wa**ng et al., (4)** who showed that Ticagrelor treatment produce significant decrease in the number of ASC sparks , expression of NLRP3 inflammasome and excessive secretion of IL-1β in rat model of doxorubicin-induced toxicity. Also, ticagrelor treatment inhibited the expression of mTOR in rats challenged with Bleomycin.

Also, our results is in agreement with **Chen et al., (40)** who showed that ticagrelor reduced serum and myocardial IL-1β, mRNA levels of ASC and NLRP3, Caspase-1 and GSDMD-N levels in a model of diabetic cardiomyopathy . Caspase-1 cleaves GSDMD and the N-terminal domain of GSDMD (GSDMD-N) attaches to the cell membrane to form a pore that allows IL-1β and IL-18 release from the cells .

Furthermore, **Dai et al., (41)** showed that Pre-treatment with the high-dose of ticagrelor 100 mg /kg suppressed Myocardial ischemia-reperfusion injury (MIRI) induced upregulation of NLRP3 (0.46-fold), ASC (0.64-fold), and Cleaved caspase-1 (0.80-fold).

Also, **Birnbaum et al., (42)** showed that High-dose of ticagrelor 100 mg /kg reduced serum creatinine and the activation of the NLRP3-inflammasome in mice with Diabetic Nephropathy .

In our study hepatic microscopic examination of diclofenac group exhibited marked steatosis which commonly seen within periportal areas, centrilobular degenerated and necrotic hepatic cells adjacent to mononuclear inflammatory cells aggregates mainly lymphocytes , Also, ruptured of some fatty degenerated cells were detected which coalesced to form fatty cysts. Some necrotic cells may be leaving empty spaces filled with minute number of leukocytes and cellular debris **,** In addition, congested hepatic blood vessels and sinusoids were seen.

This is in agreement with **Elshopakey and Elazab, (14)** who showed that hepatic sections from DIC treated rats displayed necrosis of the hepatocytes with dilatation of the hepatic sinusoids.

Also ,other group researchers from **Alabi et al., (18)** showed dystrophic changes in the structure of the liver like sinusoidal congestion, perivenular zonal necrosis, ballooning degeneration, and mononuclear leukocytes and lymphocytes infiltrated by a microscopic examination in a study using male Wistar rats injected DIC 10 mg/kg I.M for 7 days.

Also, **Elkhishin and Amer, (43)** found that Histological examination of the liver of the rats, received diclofenac 100 mg/ kg IM , revealed enlarged portal area, congested portal vein and central vein, bile duct proliferation and peribiliary cellular infiltration and fibrosis.

These results also are in consistence with **Hassan et al., (44)** who showed that the liver of diclofenac-injected rats at a dose of 3 mg/kilogram body weight (kg b. wt)/day exhibited marked deleterious histological alterations including hydropic and vacuolar degeneration of hepatocytes, inflammatory cells' infiltration, focal hepatic necrosis, apoptosis, congestion of blood vessels and sinusoids, fatty changes, Kupffer cells' proliferation, and hyperplasia of the epithelial lining the bile canaliculi.

The previous histological lesions in diclofenac-injected rats may be attributed to the increase in the oxidative stress and excessive release of free radicals and ROS. This exacerbated production of ROS administration activates apoptosis through intrinsic pathway, aggravates cell necrosis through peroxidation of membrane lipids, and stimulates DNA breakages and mutations by oxidative damage **(44).**

The current study showed that Small dose prophylactic group (SP) showed few number of fatty degenerated cells particularly within periportal areas **,**Some sections revealed moderate number of randomly distributed signet ring appeared cells and Focal perivascular necrotic areas were also encountered.

Large dose prophylactic group (LP) exhibited interstitial and perivascular minute areas of round cells infiltrates. Hyperplastic and hypertrophied kupffer cells with minute sinusoidal lymphocytic aggregation could be noticed beside mildly dilated central vein.

Short term small dose treatment group (SST) demonstrated presence of many fatty degenerated cells within periportal areas**,** the latter were infiltrated with round cells infiltrations in some examined sections **,**accidental and programmed cell deaths could be detected at centro lobular areas in many examined sections **,**the necrotic cells appeared as swollen cells with pyknotic or karyolysis nuclei and cytplasmlysis. While, apoptotic cells manifested by shrinked cells with clumped chromatin and homogenous eosinophilic cytoplasm.

Short term large dose treatment group (SLT) showed few number of hydropic degenerated cells alternated with unicellular hepatic necrosis with pyknotic nuclei at some centrolobular areas ,Unicellular fatty change beside interstitial inflammatory cells aggregation were noticed and Congested hepatic blood vessels and

sinusoids were also detected **.**

Long term small dose treatment group (LST) showed still presence of periportal fatty change, Various types of acute cell swelling were observed in some examined sections primarily hydropic degeneration. Also, dilated central veins were also seen. Moreover, Necrotic hepatic cells in some fields were replaced by mononuclear inflammatory cells and the latter were also demonstrated at perivascular areas.

Long term large dose treatment group (LLT) revealed preserved hepatocytes arrangement, sinusoids, central veins and portal areas in the majority of examined sections, however, some sections exhibited presence of minute area of hydropic degeneration and unicellular fatty degenerated cells.

The hepatoprotective effect of ticagrelor can be explained by its anti-inflammatory effect proved by suppression of hepatic MDA and upregulation of SOD level, also by its inhibitory effect on NLRP3 inflammasome pathway.

In line with our results **Lee, (45)** who revealed that Ticagrelor improved non-alcoholic fatty liver disease by reducing inflammation and fatty acid biosynthesis and attenuating steatosis.

Also, Results revealed that ticagrelor in a dose of 20 mg/kg oral daily can alleviate lung fibrosis by antagonizing PI3K/AKT/mTOR pathway signaling **Wanas et al., (46)** indicating the antifibrotic effect of ticagrelor.

In our study renal microscopic examination of diclofenac group revealed necrosed some glomerular corpuscles and atrophy of few number of glomerular tufts The latter were dilated in some examined section. Some renal tubular epithelium suffered from degenerative changes with co-agulative necrosis in other tubules and Intraluminal hyaline casts were seen within some renal tubules. Congested peritubular capillaries were also seen.

These results are in consistence with **Aycan et al., (23)** who found that intramuscular injection of DIC at a dose 9 mg/kg body weight twice daily for 5 successive days caused degeneration of the kidney tubules, tubular atrophy and dilatation ,desquamation in some tubule epithelia and vacuolar degeneration. Interstitial cell infiltration was prominent in some sections.

In the same context **Wadie et al., (47)** found that administration of diclofenac sodium in a dose of 15 mg/kg/day induced a significant renal injury as evidenced by marked necrosis of epithelial lining renal tubules, congestion and vacuolation of glomerular tufts, atrophy of glomerular tuft and marked inflammatory cells infiltration.

Furthermore **Qasim et al., (48)** found that there was significant detrimental effects of diclofenac on renal tissue architecture, with retracted glomeruli, tubular cast, tubule-interstitial inflammation, and tubular necrosis.

The current study showed that Small dose prophylactic group (SP) showed hydropic degeneration in some renal tubular epithelium, Congestion of renal blood vessels and hemorrhages between tubules were also seen .

Large dose prophylactic group (LP) displayed cloudy swelling in few number of renal epithelium **,** Most renal corpuscles and renal tubules showed maintenance their histological structures

Short term small dose treatment group (SST) exhibited focal degeneration in some tubular epithelium with intracytoplasmic hyaline droplets **,**Necrotic and destructed few tubules with perivascular edema and peritubular round cells infiltration were noticed, Congested blood vessels and glomerular capillaries were also seen

Short term large dose treatment group (SLT) showed degenerative changes in a moderate number of renal tubules beside intraluminal hyaline casts, Shrined glomerular tufts and dilated peritubular capillaries were also observed .

Long term small dose treatment group (LST) showed ballooning degeneration in few tubules, Perivascular lymphocytic aggregation and dilated renal vasculatures was also seen.

Long term large dose treatment group (LLT) showed regeneration of renal tubules and apparent normal histological configurations of renal tubules and glomerular corpuscles.

These results are in agreement with **Yu et al., (37)**who showed that In ticagrelor treated group, the structure of the renal tubules was less damaged, with no luminal occlusion, less inflammatory cell infiltration, slight damage to the renal tubular epithelial cells with a slight edema of vacuolated changes compared with the cecal ligation and puncture (CLP) group, the injury score was decreased .

Also these results are in agreement with **Uil et al., (49)** who showed that Ticagrelor treated diabetic mice in a dose of (300 mg/kg) developed less albuminuria, less glomerular injury, less endothelial cell activation and injury, less tubulointerstitial fibrosis, less inflammation, and less tubular apoptosis in relation to diabetic

group of mice without treatment .

The current study shows that diclofenac (10 mg/kg/day, intraperitoneal [ip]) in rats for 7 days resulted in a significant increase in portal blood pressure this may be explained by the congestion of portal vein and sinusoids which occurred after diclofenac treatment . DILI can lead to cirrhosis with portal hypertension and end-stage liver disease **(50).**

Our study showed that ticagrelor given in a dose of 10 and 20 mg/kg as a prophylactic agent and in a dose of 10 and 20mg/kg as a curative agent for 7days or 28 days resulted in a significant decrease in portal blood pressure as it relieved the congestion of portal vein and sinusoids .

Conclusion

In light of the results of this study we concluded that ticagrelor in a dose of 10 and 20 mg/kg was proved to have prophylactic and curative effect against diclofenac induced hepatotoxicity and nephrotoxicity by its antioxidant, anti-inflammatory and anti-apoptotic effect but further experimental studies are required to confirm our results

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