

EVALUATION AND STUDY OF PATHO-PHYSIOLOGICAL ROLE OF GLUCOCORTICOID RECEPTOR (GR) LIGANDS IN ACETAMINOPHEN-INDUCED LIVER INJURY IN MICE

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Abstract: Stress elicited by various drugs and psychosocial components is extensively prevalent globally. Glucocorticoid receptors control different features of lipid metabolism and lipoproteins in the liver. Acetaminophen or paracetamol is a broadly used antipyretic and analgesic drug. Overdose of acetaminophen causes centrilobular necrosis and fibrosis by generating oxidative stress. This experimental study was conducted to evaluate alone as well as combined systemic effects of glucocorticoid receptor (GR) ligands, Mifepristone (GR antagonist), and Dexamethasone (GR agonist) on oxidative damage and liver injury induced by acetaminophen. Evaluation of parameters of liver functions showed a substantial increase in AST ALT levels accompanied by elevation in TOS and reduction in TAC in the APAP-induced hepatotoxic group. While the groups that received dexamethasone, RU-486, and Dex+RU-486 as pretreatment significantly decreased the ALT, AST, and TOS levels compared to the toxic group (APAP). The protective effects of glucocorticoid receptor ligands in APAP-induced liver injury were further verified by histopathological examination. However, additional studies are necessary to illustrate the hepato-protective efficacy, safety profile, basic underlying process of protection, and therapeutic applications of glucocorticoid receptor ligands.

Keywords: Mice, Glucocorticoid, Acetaminophen, AST, TAC, APAP

Introduction

Psychosocial or psychological stress is a highly challenging and demanding societal issue. The psychological approach mainly emphasizes one's perception regarding stress given by life incidents on the derivation of their own conscious effort, to solve different problems and estimate the threats imposed on an individual and the credibility of available resources (Cohen et al., 2016). Different life-fluctuating or menacing events are known as "stressors." Psychological stress possesses an intricate association with liver inflammation and fibrosis. Stress influences gastrointestinal tract physiological functions such as gut motility, mucosal blood flow, mucosal permeability, visceral sensitivity, and gastric secretion (Konturek et al., 2011). When someone is under the influence of stressors, exclusive pathways that cause stimulation of the HPA (hypothalamic pituitary adrenal) axis within the brain are activated. This is designated as a stress response, which further causes the release of peripheral mediators (Vere et al., 2009). Glucocorticoid receptors (GR) are important components of crucial nuclear receptors (NR) and are basically transcription factors and ligand inducible. Nuclear receptors prompt clearance and fat production in the liver by regulating an enzyme lipoprotein lipase (Wagner et al., 2011). Glucocorticoid receptor (GR) controls different features of lipid metabolism and lipoproteins in the liver. It is important in managing hepatic disorders like metabolic syndrome, hepatic insulin resistance, non-alcoholic fatty liver disease (NAFLD), and dyslipidemia. Glucocorticoid receptor particularly binds glucocorticoids and steroidal hormones (Heitzer et al., 2007). Glucocorticoids (GCs) show great immunosuppressive plus anti-inflammatory

action in a defined cell-kind approach by affecting cytokines-mediated pathways (Necela and Cidlowski, 2004). Glucocorticoid receptors monitor various physiological functions such as development, proteins, lipids, and carbohydrate metabolism. Metabolic rate is greatly influenced by the elevated concentration of glucocorticoids (GCs), which may generate excessive reactive oxygen species (ROS) by the instigation of oxidative stress (Burraco and Mestre, 2016). Oxidative stress epitomizes a disproportion between antioxidant and oxidant species. Crucial oxidative stress and ROS are two major components in developing and initiating various hepatic disorders, such as hepatitis, cirrhosis, hepatic steatosis, liver fibrosis, and hepatocellular carcinoma. These complex liver diseases are undoubtedly linked to the excessive formation of ROS and oxidative stress (Moro et al., 2016). Lipids and proteins are mostly interrupted in oxidative stress, which further induces hepatocyte death, necrosis, and exaggerated inflammation (Li et al., 2016). Oxidative stress causes damage to each type of liver cell, like Kupffer cells, stellates, hepatocytes, and endothelial cells. This damage ultimately contributes to apoptosis, ischemia, and necrosis. The help of P450 enzymes in the mitochondria of liver cells primarily generates ROS. Hepatocyte DNA, proteins, and lipids are mainly influenced by reactive species of nitrogen and oxygen (Cichoz-Lach and Michalak, 2014). DILI possesses potential severity as well as elusive pathogenesis. It has become a fundamental health issue for regulatory bodies, physicians, and pharmaceutical industries (Andrade et al., 2009). DILI has become a source of substantial concern nowadays. Approximately 20,000 deaths per year occur due to drug-

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induced liver injury. DILI is the primary source of chronic and acute hepatic failure and liver transplantation worldwide (Chen et al., 2015). Drug-induced toxicity of the liver can be predictable (nonidiosyncratic) or unpredictable (idiosyncratic). Amoxicillin, isoniazid, and NSAIDs (nonsteroidal anti-inflammatory drugs) are common sources of DILI (Leise et al., 2014). Acetaminophen recognized as an extensively practiced painkiller. In the United States, one highly prescribed drug is acetaminophen, also known as N-acetyl-4-aminophenol (APAP) or paracetamol (Blough and Wu, 2011). Acetaminophen is metabolized by cytochrome P450 enzyme and converted into a lethal metabolite known as NAPQI (N-acetyl-P-benzo-quinone imine) in the liver (Ji et al., 2013). Acetaminophen causes hepatotoxicity by the generation of oxidative damage. Kupffer cells are stimulated in response to acetaminophen (APAP) induced hepatocellular stress, generating ROS and liberating crucial inflammatory cytokines (Matsura et al., 2006). Acetaminophen hepatotoxicity also results due to excessive formation of superoxide and nitric oxide (NO), which further react to generate peroxynitrite. Generally, peroxynitrite is detoxified by GSH (glutathione), but NAPQI causes depletion or reduction of GSH in acetaminophen overdose (Banerjee et al., 2017). With long-term or prolonged use of acetaminophen, centrilobular necrosis and fibrosis of hepatocytes may occur. APAP proficiently metabolizes in the liver with the aid of cytochrome P-450 into a reactive lethal metabolite. Normally, detoxification of this lethal metabolite occurs with the help of glutathione (GSH). However, in instances of overdose, the level of GSH is reduced due to conjugation of NAPQI with GSH and by covalent binding of NAPQI and cysteine residues, acetaminophen adducts form, which further cause oxidative liver damage by the formation of ROS (Reid et al., 2005). N-acetylcysteine (NAC) is commonly known as a standard antidote for acetaminophen hepatotoxicity and a potent precursor of GSH. But NAC has certain adverse effects, such as vomiting, diarrhea, nausea, fever, drowsiness, headache, and bleeding (Boonruamkaew et al., 2016). Membrane permeability and transport functions are badly affected in acetaminophen-induced liver injury, primarily leading to crucial enzyme leakage. Hence, marked elevation in levels of serum bilirubin and transaminases is observed in acetaminophen-induced hepatotoxicity (Zhang et al., 2015). Nuclear receptors regulate various liver functions like bile secretion, glucose metabolism, homeostasis of bile acids, and drug deposition. Nuclear receptors are crucial for recognizing the pathophysiology and pathogenesis of liver disorders (Trauner and Halilbasic, 2011). GR is involved in various physiological processes like carbohydrate homeostasis, stress adaptation, fat metabolism, immune surveillance, and protein metabolism. Derangements in regulating glucocorticoid receptors promote the pathogenesis and progression of hepatic diseases (Necela and Cidlowski, 2004). Oxidative stress is generally a fundamental conjoint pathological approach, contributing to the progression and activation of hepatic injury. The parenchymal cells of the liver are mainly influenced by oxidative stress. Oxidative stress is essential to liver disorder progression (Li et al., 2015). Redox state manifests a significant background of different inflammatory and metabolic liver diseases. Oxidative stress also prompts the release of crucial inflammatory cytokines, leading to the initiation of

fibrogenic response in the liver (Rolo et al., 2012). Oxidative stress is central in promoting hepatocellular damage associated with non-alcoholic steatohepatitis (NASH) (Chalasan et al., 2012). Oxidative stress is induced by the help of pro-oxidant enzymes in hepatocytes (Cederbaum, 2015). Hepatitis C is regarded as a major chronic hepatic disease comprising 2-3% of the world's population. Induction of ROS by oxidative stress and HCV plays an important part in the pathogenesis of Hepatitis C and inflammation of the liver. HCV infection causes the activation of macrophages and the immune system, which further produces ROS. Liver fibrosis, also known as complex liver dysregulation, is prompted by ROS induction and oxidative stress (Ivanov et al., 2013). Mild to moderate oxidative stress causes the modulation of signal transduction pathways and influences various cellular responses, for example, differentiation, proliferation, etc. (Du et al., 2015). DILI has become a recurrent source for the revocation of many approved drugs (Aithal et al., 2011). Antibiotics and NSAIDs are the major sources of DILI (Bjornsson, 2015). Increased generation of ROS, protein oxidation, MAP kinase stimulation, and peroxidation of lipids are the major effects observed in oxidative stress caused by various drugs (Deavall et al., 2012). Both intrinsic and idiosyncratic mechanisms are involved in DILI. Dose-related DILI is called as intrinsic drug-induced liver injury like acetaminophen while prescription drugs or medications produce idiosyncratic DILI. About 46% of individuals with acute hepatic failure have liver injury linked to acetaminophen (Hamilton et al., 2016). Acetaminophen (APAP) basically causes liver injury by producing massive mitochondrial oxidative damage (Noh et al., 2015). APAP is one of the prime sources of liver damage or failure worldwide. The underlying mechanism of APAP-induced hepatotoxicity or liver injury is the metabolic instigation of acetaminophen by cytochrome P-450, which generates very toxic reactive metabolite in the liver commonly recognized as NAPQI. Excessive formation of NAPQI occurs after overdose of APAP, which causes reduction of cellular GSH, generation of APAP-protein adducts and induction of mitochondrial oxidative stress. This causes fragmentation of nuclear DNA, necrosis and release of crucial pro-inflammatory cytokines (Du et al., 2016). Mifepristone (RU486) is a synthetic steroid which possesses great affinity for glucocorticoid receptors (GR) and progesterone receptors (PR) and shows strong antagonistic activity after binding to these receptors and may antagonize the effect of glucocorticoid receptors at specific therapeutic doses (Ghoumari et al., 2003). Mifepristone (RU486) significantly metabolized by hydroxylation as well as by demethylation in the liver by CYP-450 enzyme. The resultant mifepristone (RU486) proximal metabolites are named as hydroxylated, monodemethylated and didemethylated metabolites, these all retain extensive affinity for glucocorticoid receptors (Heikinheimo et al., 2003). Therefore, the current study was designed to evaluate alone and combined systemic effects of glucocorticoid receptor (GR) ligands, Mifepristone, and Dexamethasone on oxidative damage and liver injury prompted by APAP.

Methodology

The hepatoprotective effects of glucocorticoid receptor ligands Mifepristone and Dexamethasone were examined in

albino mice hepatotoxic models. The experimental procedure of the present study is described below.

Animals

Twenty-five (25) albino mice (healthy) weighing 25-30g were placed at an animal-keeping facility, Agriculture University, Faisalabad. Mice were given a standard pellet ad libitum diet and normal drinking water for 7 days. The experimental animals were given a seasonal diet during the experimental study. The drinking water and diet were available for mice for 24 hours. All mice were placed at a controlled temperature and provided proper ventilation.

Experimental design

Twenty-five albino mice were randomly divided into five groups. All mice were given a normal routine diet for 7 days. Then, they were treated with APAP separately in groups 2,3,4 and 5. Drug administration plan and routine diet in mice throughout the experimental study of 24 hours are shown in Table 1.

Table 1: Drug administration plan and diet in mice throughout the experimental phase (24 hours)

Groups	Treatment
Group 1: Control	Normal saline (1.0 ml/kg body wt. orally)
Group 2: Vehicle + APAP	APAP (200 mg/kg, body weight i.p).
Group 3: Treated group I (Dexamethasone + APAP)	Dexamethasone (Dex) (2 mg/kg, i.p) + APAP (200 mg/kg, i.p)
Group 4: Treated group II (Mifepristone + APAP)	Mifepristone (25 mg/kg, i.p) + APAP (200 mg/kg, i.p)
Group 5: Treated Group III (Dexamethasone + Mifepristone + APAP)	Dexamethasone (2 mg/kg, i.p) + Mifepristone (25 mg/kg, i.p) + APAP (200 mg/kg, i.p).

Collection of blood samples

The blood samples of mice were collected in *gel and clot activator tubes* at 24h of the study. Mice were sacrificed, and from the jugular vein, samples of blood were collected. To get the serum, the centrifugation of samples was performed at the speed of 3000 rpm for 10 minutes. Eppendorf tubes were used to collect serum and stored in a Biomedical freezer at a temperature of -30°C to analyze antioxidant and biochemical parameters.

Biochemical parameters

The clear serum, obtained by centrifugation, was analyzed to estimate liver injury biomarkers such as AST (U/l) and ALT (U/l). The help of biochemical kits and automated Biochemistry analyzer analyzed these factors.

Antioxidant parameters

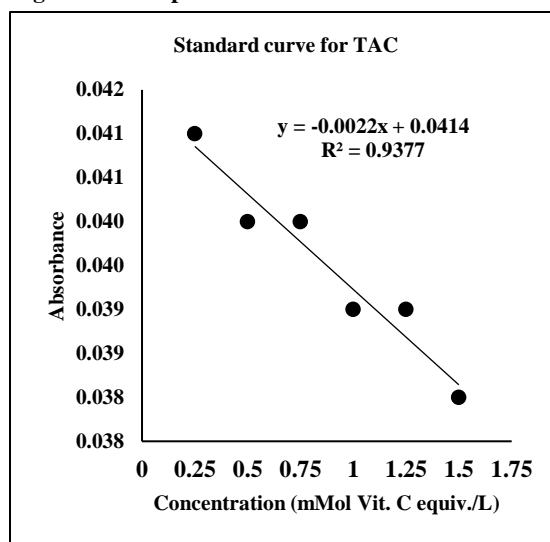
An automated ELISA reader consisting of 96 valve microplate was used for the measurement of parameters of oxidative stress using freshly made lab reagents.

Serum Total Antioxidant Capacity (TAC; mMol Vitamin C Equivalent/liter)

Took 10mL of deionized water and, in this, dissolved 8.8 mg of vitamin C for the preparation of the standard solution. The stock solution of 5mM/L of vitamin C standard was prepared. Then, dilution 1.5, 1.25, 1.0, 0.75, 0.5, 0.25 and 0 mM/L of vitamin C standard Equiv/L were prepared from

this stock solution. After achieving absorbance at the wavelength of 660 nm, absorbance change was further used to calculate the actual concentration to obtain a standard curve. Results were presented in mmol Vit. C Equiv/L is shown in Figure 1 below.

Fig 1: Dose-dependent curve of the true solution of



Vitamin C antioxidant as standard Reagents preparation

A semi-automatic analyzer was used to analyze the serum TAC sample level for spectrophotometric examination. A monochromatic wavelength of 660 nm was chosen, and for warming the particular filter, a 5 5-minute time duration was given. 5µL of standards or serum samples were added in 200µL of R-1 (reagent 1), and the first absorbance was taken. Then 20µL of R-2 (reagent II) was added to this solution, and after incubation for 5 minutes at a temperature of 37°C, 2nd absorbance was also taken. Final dilution was measured by using calibrated standards of Vit. C and presented in terms of mmol Vit. C EquivalentL⁻¹ and also measured delta absorbance of the solution.

Calculation of Oxidative Stress

Serum TOS (Total oxidant status)

Primarily, 10 milliliters of hydrogen peroxide in 35 percent quantity was drawn in a graduated cylinder, and 100mL final volume was made using deionized water. 102.91 mM/Liter of hydrogen peroxide was determined as the solution concentration. Then, this solution took 12.14µL in an empty cylinder, and the final volume equal to 50mL was made using deionized water. Now, the solution's concentration became 250 µM/Liter of H₂O₂. Two-fold sequential dilutions were made until the desired dilutions were attained.

Standard Curve: The Standard curve among a concentration of H₂O₂ and absorbance was drawn by taking absorbance from chronological dilutions. Absorbance was determined at prime wavelength 560nm and, subsequently, absorbance at 800nm wavelength, which is another wavelength of solution (Bichromatic). Delta deviation was calculated to determine the definite concentrations of solution (Absorbance of final minus absorbance of first is equal to delta absorbance). The dose-dependent curve of the

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olution of H₂O₂ (35%) oxidant as standard is presented below in Figure 2.

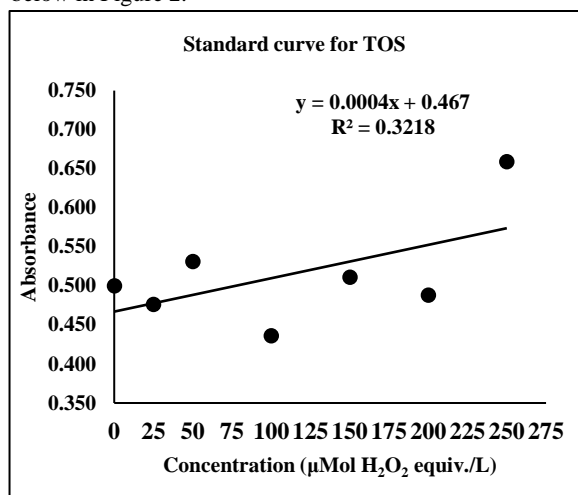


Fig. 2: Dose-dependent curve of the true solution of H₂O₂ (35%) oxidant as standard



Fig.3: Tissues collection for histopathological examination

Histo-pathological Examination

Mice were ultimately sacrificed at the completion of experimental study. The abdomen of experimental animal was opened carefully and then viscera were clearly exposed. Samples of liver were collected from each group of experimental animals after washing the samples by the help of normal saline. 10% neutral buffered formalin was used for fixation purpose and then sections were cautiously cut and stained. Sections were examined microscopically in order to determine histopathological variations (Kanel and Korula, 2005).

Results

Analysis of biochemical parameters Serum ALT (Alanine transaminase):

Experimental results of ALT (U/L) concentrations in the serum of mice are depicted in Table No. 2. ALT serum values were prominently high ($P \leq 0.05$) in the prompted hepatotoxic group of mice. Dexamethasone substantially

declined ALT levels compared to the toxic group (APAP). While Mifepristone alone and combined group (Dex+RU486) also decreased serum ALT (Figure 4).

Table 2: ALT serum levels at 24 hours of a research study (Mean \pm SE) in experimental and control groups.

Groups	ALT levels (7 th Day)
Control	41.855 \pm 3.10559 AB
Vehicle + PAP	135.225 \pm 50.8248 A
Dexamethasone + APAP	21.835 \pm 8.47089 B
Mifepristone + APAP	75.9075 \pm 28.9654 AB
Dexamethasone + Mifepristone + APAP	77.1925 \pm 17.7037 AB

Serum Aspartate Aminotransferase (AST):

Experimental results of AST (U/L) concentrations in mice serum are depicted in Tables 3 and 4. AST serum values were substantially elevated in the prompted toxic group. Mifepristone reduced AST levels considerably compared to the toxic group (APAP). Dexamethasone (Dex) alone and a combined group of GR ligands also reduced serum AST (Figure 5).

Table 3. AST serum levels at 24 hours of research study in experimental and control groups (Mean \pm SE).

Groups	AST levels 7 th Day
Control	118.47 \pm 11.6913 AB
Vehicle + APAP	208.723 \pm 60.0097 A
Dexamethasone + APAP	90.75 \pm 60.9316 AB
Mifepristone + APAP	50.29 \pm 23.4856 B
Dexamethasone + Mifepristone + APAP	75.405 \pm 49.3954 AB

Effects on weight of organs

Liver weight (g)

Liver weight was considerably increased in mice who received acetaminophen (toxic group). (Table no. 4 and 5). In pre-treated groups, liver weight was slightly increased compared to the prompted toxic group of mice (Figure 6).

Table 4. Liver wt. in "g" after 24h of experimental study (Mean \pm SE)

Groups	Liver weight (g) (24h)
Control	1.7336 \pm 0.15136 B
Vehicle + APAP	3.21325 \pm 0.65921 A
Dexamethasone + APAP	2.03828 \pm 0.132481 B
Mifepristone + APAP	1.90968 \pm 0.114515 B
Dexamethasone + Mifepristone + APAP	1.91528 \pm 0.04481 B

There was no prominent change in the mice's weight (g) of spleen given APAP intraperitoneally (200mg/kg). Spleen weight (wt.) was markedly increased in the RU-486-treated group. However, dexamethasone alone and combined group (Dex+RU486) showed a significant decrease ($P \leq 0.05$) in spleen wt. (7).

Table 5: Spleen wt. in "g" (Mean ± SE) after 24 hours of experimental study

Groups	Spleen weight (g) 24h
Control	0.2284 ± 0.067903 A
Vehicle + APAP	0.216475 ± 0.088219 A
Dexamethasone + APAP	0.144525 ± 0.02881 A
Mifepristone + APAP	0.246975 ± 0.040561 A
Mifepristone+ Dexamethasone + APAP	0.129725 ± 0.012748 A

Body weight (g)

The measurement of body wt. in "g" of mice was taken at 0 hours and 24 hours, and there was no considerable variation in body weight in contrast to the control group. But in RU-486 treated mice group, body weight was moderately elevated. However, it was slightly decreased in the dexamethasone and combined group.

Table.6. Variation in mice body wt. in "g" (Mean ± SE) in experimental and control groups at time intervals of 0 hour and 24 hours

Groups	Body wt. 0 hr.	Body wt. 24 hrs.
Control	30.25 ± 1.03078 BC	29.5 ± 1.84842 A
Vehicle + APAP	37.75 ± 2.49583 A	36.75 ± 3.59108 A
Dexamethasone + APAP	34.75 ± 2.49583 AB	33.5 ± 2.75379 A
Mifepristone + APAP	28.5 ± 1.44338 C	29.75 ± 1.31498 A
Mifepristone + Dexamethasone + APAP	35.25 ± 1.54785 AB	33.75 ± 1.03078 A

Assessment of oxidative stress

Serum TAC levels

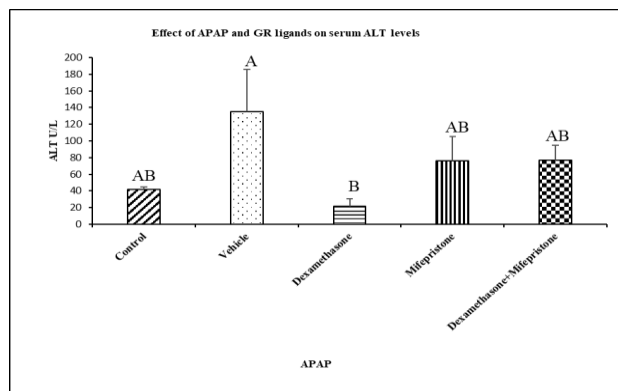


Fig.4: APAP-prompted acute hepatotoxicity and GR ligands effect on ALT (U/L) serum levels. A and B significant difference (P ≤ 0.05)

The results of the experimental study displayed a considerable decrease (P≤0.05) in serum TAC levels in the APAP-induced hepatotoxic group. RU486 significantly increased TAC. However, dexamethasone and the combined group non-significantly increased TAC.

Table 8. TAC serum levels (Mean ± SE) in mice after 24 hours of experimental study

Groups	TAC 24 hrs.
Control	0.167558 ± 0.062561 AB
Vehicle + APAP	0.159095 ± 0.056743 B
Dexamethasone + APAP	0.326653 ± 0.049701 AB
Mifepristone + APAP	0.381377 ± 0.119348 A
Mifepristone + Dexamethasone + APAP	0.35881 ± 0.051336 AB

Serum TOS levels

Results of the experimental study exhibited that TOS level was non-significantly increased in the APAP-induced hepatotoxic group. At the same time, GR ligands pre-treated groups decreased the serum TOS levels in comparison with acetaminophen-induced hepatotoxic group.

Table.9. TOS serum levels (Mean ± SE) in mice after 24 hours of experimental study

Groups	TOS 24 hrs.
Control	76.70462 ± 9.220407 A
Vehicle + APAP	122.5867 ± 34.96329 A
Dexamethasone + APAP	74.30501 ± 10.39496 A
Mifepristone + APAP	67.40959 ± 2.954716 A
Mifepristone + Dexamethasone + APAP	72.44325 ± 3.687727 A

Liver's gross manifestation:

Gross appearance or manifestation of liver in APAP-prompted hepatotoxicity and GR ligands pre-treated groups exhibited distinguished variations in contrast to control group. Additional, alterations in liver and protection by GR ligands were examined by histopathological evaluation.

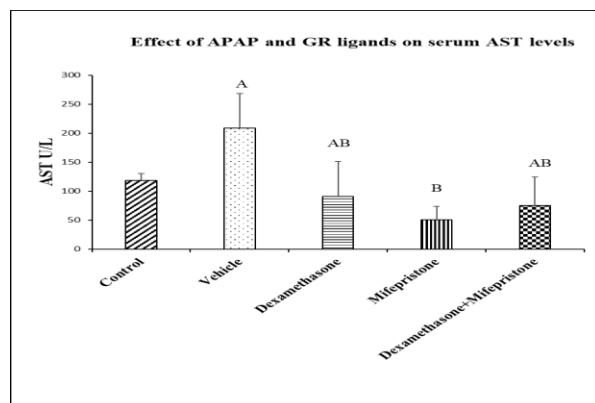


Fig.5: APAP-prompted acute hepatotoxicity and GR ligands effects on AST (U/L) serum levels. A and B significant difference (P ≤ 0.05)

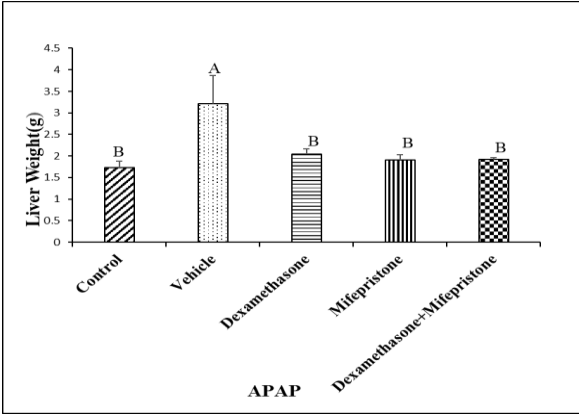


Fig.6. Liver wt. in "g" after 24h of experimental study

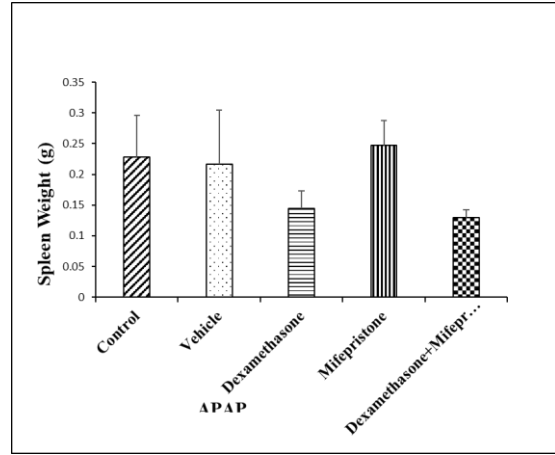


Fig 7 Spleen wt. in "g" after 24h of experimental study

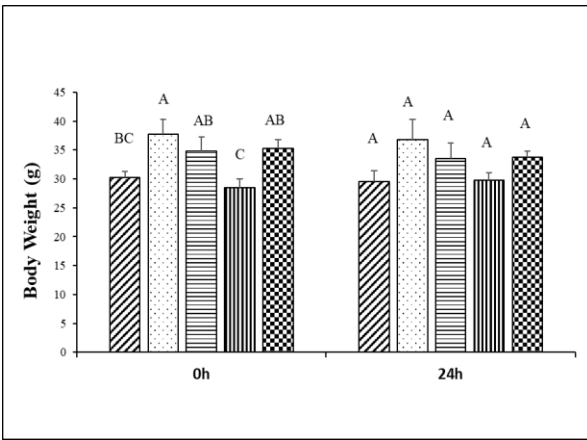


Figure 8: Variation in mice body wt. in "g" in experimental and control groups at time intervals of 0 hours and 24 hours

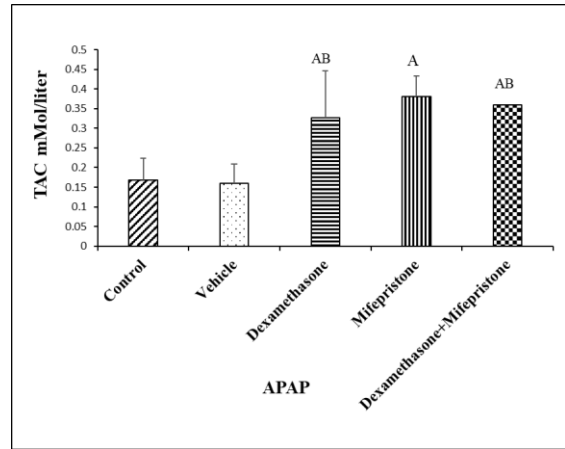


Figure 9: TAC serum levels in mice after 24 hours of experimental study

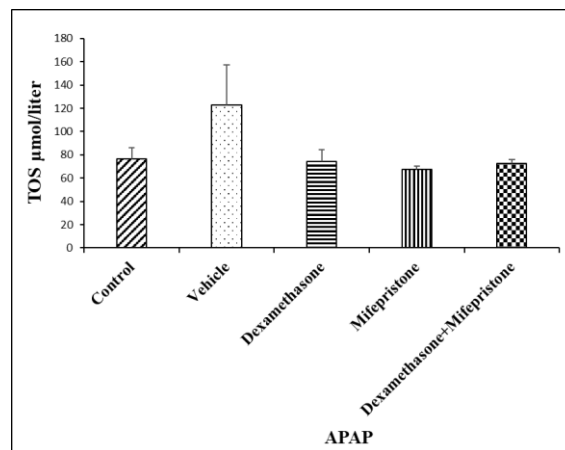


Fig.10: TOS serum levels in mice after 24 hours of experimental study

Histopathological modifications of liver

Acetaminophen prompted liver injury, and fortification by glucocorticoid receptor ligands was prudently noticed by

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the facilitation of histopathological assessment of illustrative livers of each mice group.

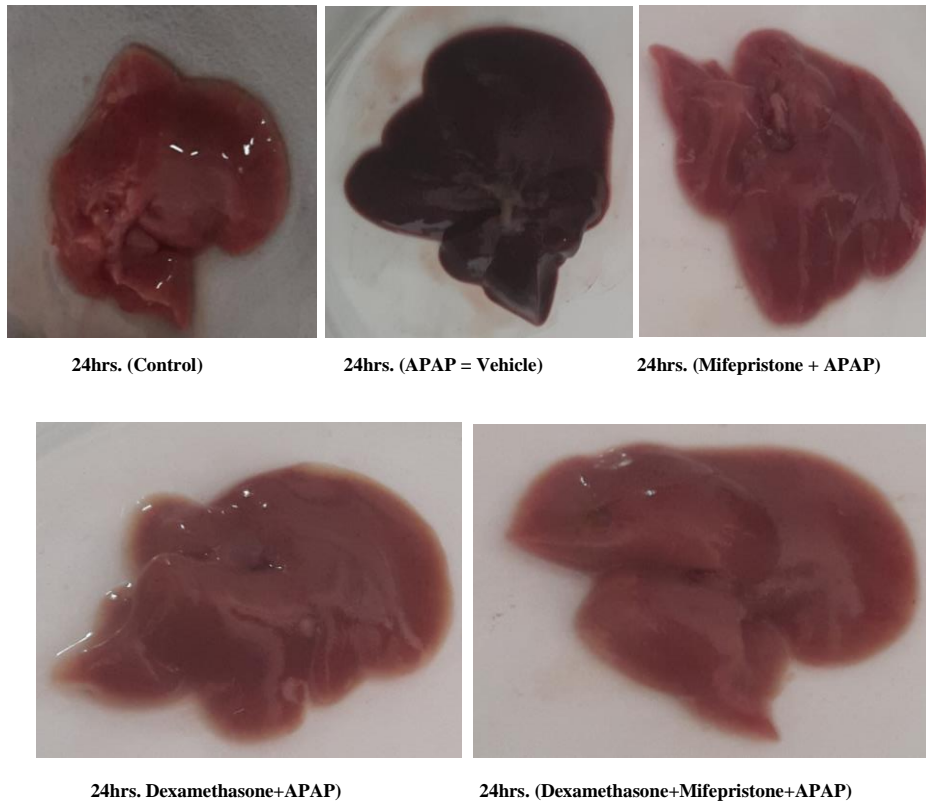


Fig.11. Liver's gross manifestation in vehicle and pre-treated groups after 24 hours of experimental study.

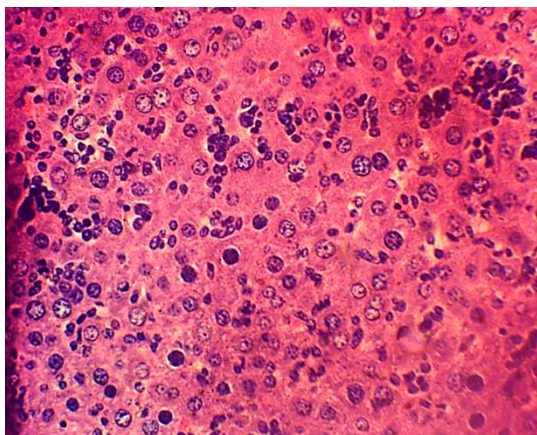


Fig 12: Photomicrograph of illustrative liver displaying normal expression of hepatocytes along with absolute nuclei in the control group (H & E stain) (X-40) H & E stain: Hematoxylin and Eosin

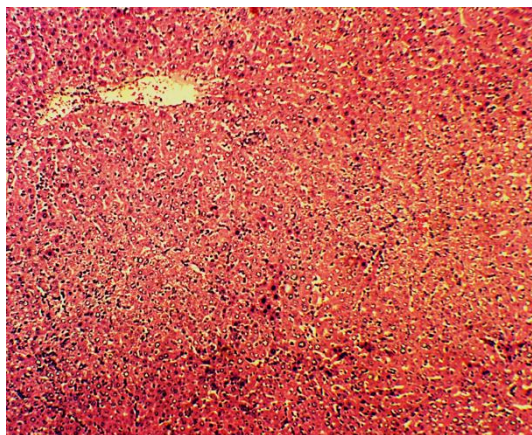


Fig.13. Photomicrograph of illustrative liver displaying hepatocytes normal expression in the control group (X-10)

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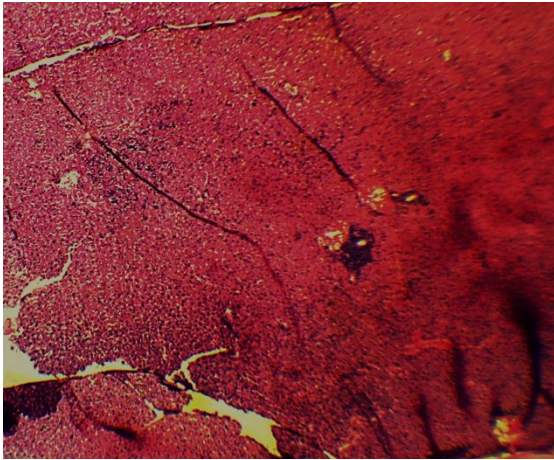


Fig 14. Photomicrograph of illustrative liver of control group displaying intact parenchyma along with comprehensive sinusoidal apertures (X-4)

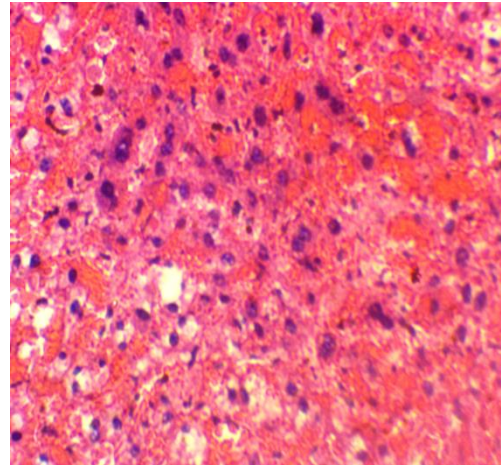


Fig.15. Photomicrograph of illustrative liver of APAP-prompted toxic group displaying necrosis, enlarged hepatocytes, and marked inflammatory cell infiltration at 40 X.

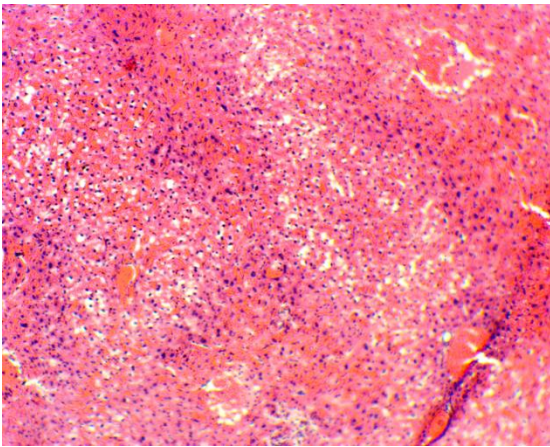


Fig. 16 Photomicrograph of illustrative liver of APAP-prompted toxic group displaying infiltration of cells (mononuclear) within portal regions and centrilobular alterations (X-10)

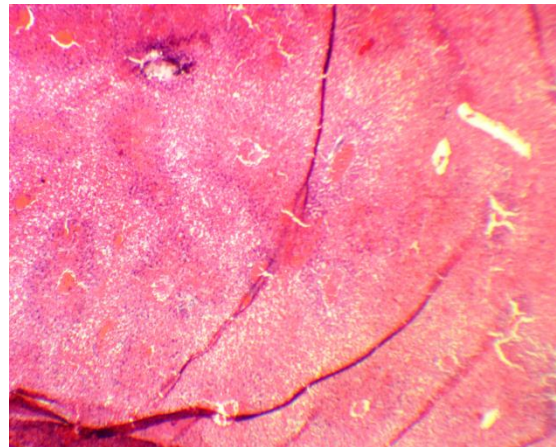


Fig 17. Photomicrograph of illustrative liver displaying centrilobular alterations and slight necrosis in APAP-prompted toxic group at 4 X.

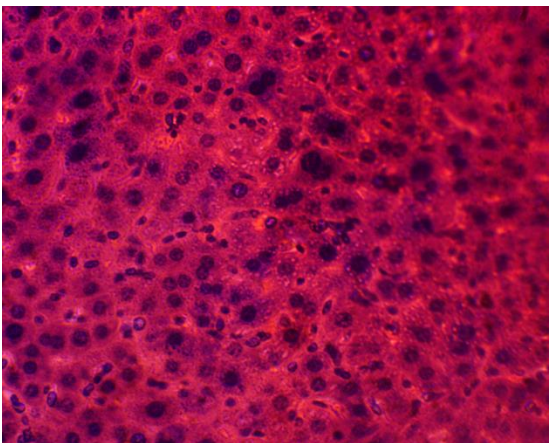


Fig 18. Photomicrograph of illustrative liver displaying normal expression of hepatocytes and absolute nuclei, though slight hepatic damage exists in dexamethasone pre-treated group (X-40).

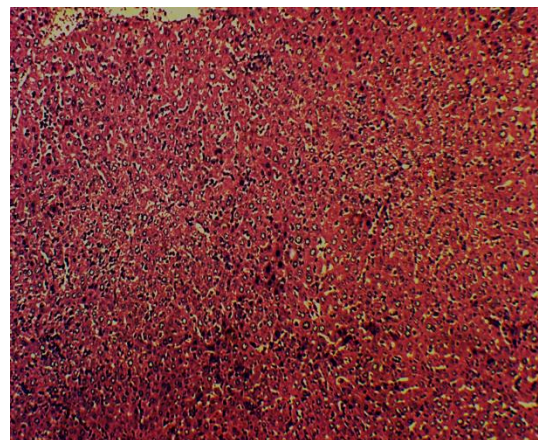


Fig.19. Photomicrograph of illustrative liver of Dexamethasone pre-treated group displaying hepatocytes normal expression with minor liver damage in contrast to toxic group (APAP) (X-10).

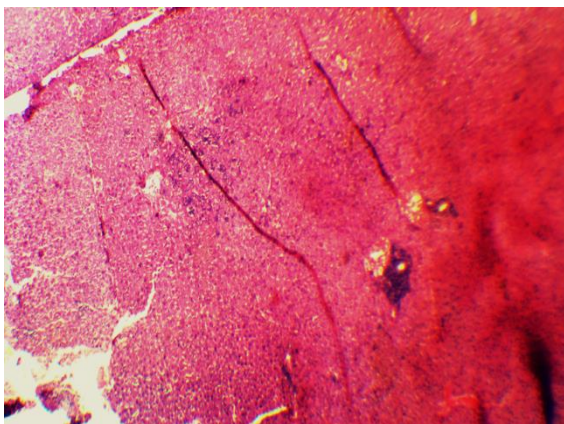


Fig.20. Photomicrograph of illustrative liver displaying intact parenchyma and comprehensive sinusoidal apertures in dexamethasone pre-treated group (X-4).

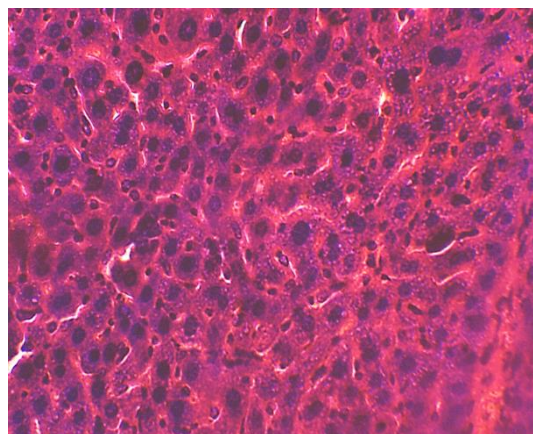


Fig.21. Photomicrograph of illustrative liver displaying an almost normal expression of hepatocytes and absolute nuclei, though slight necrosis exists in the Mifepristone pre-treated group (X-40).

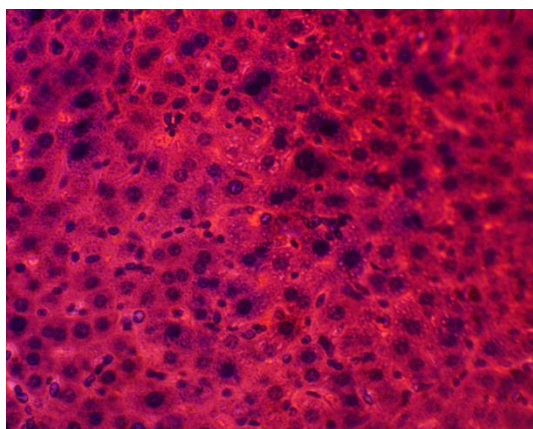


Fig.22. Photomicrograph of illustrative liver displaying intact parenchyma in Mifepristone pre-treated group (X-10).

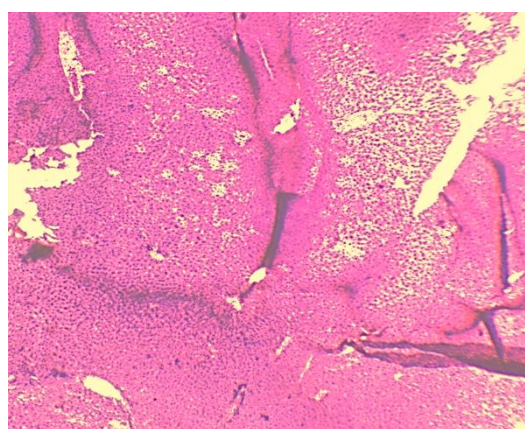


Fig.23. Photomicrograph of illustrative liver displaying intact parenchyma and comprehensive sinusoidal apertures in Mifepristone pre-treated group (X-4).

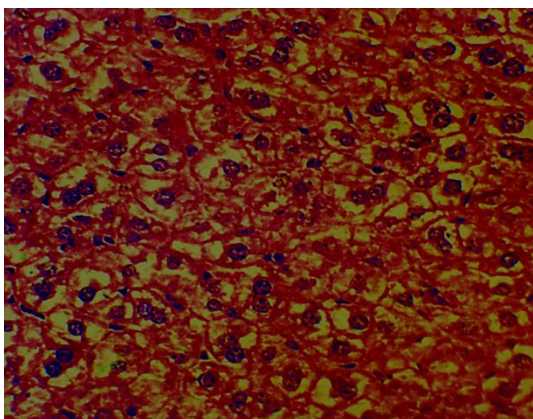


Fig.24. Photomicrograph of illustrative liver displaying virtually normal manifestation of hepatocytes and slight inflammation and necrosis in combined (Dexamethasone + Mifepristone) group (X-40).

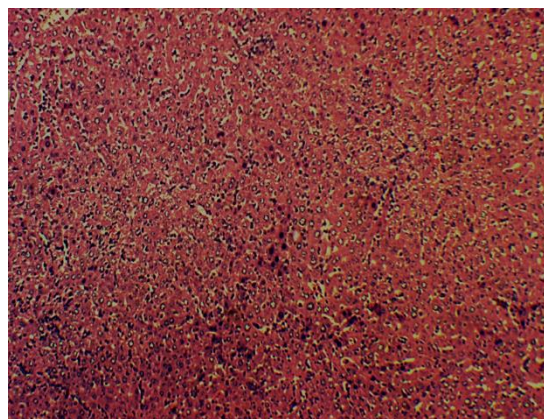


Fig.25. Photomicrograph of illustrative liver of dexamethasone + Mifepristone pre-treated group displaying normal hepatocytes with normal expression with minor liver damage compared to toxic group (APAP) (X-10).

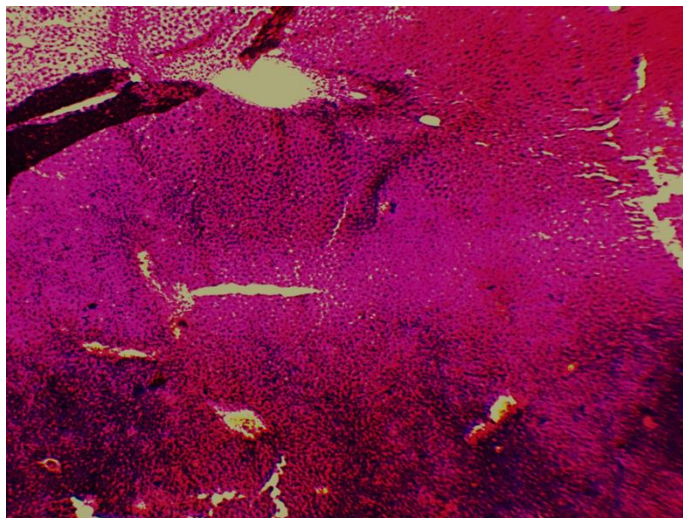


Fig.26. Photomicrograph of illustrative liver displaying intact parenchyma and comprehensive sinusoidal apertures in Mifepristone + Dexamethasone pre-treated group compared to toxic group (X-4).

Discussion

Psychosocial stress is predominant worldwide because it greatly influences the cadence of life in modern society. Different conditions evoke stress, like finances, health, and relationships. When such situations are remarked as stressful, an intricate cascade of neuroendocrine responses and subjective feelings occurs (Reschke-Hernandez *et al.*, 2017). Psychological stress promotes changes in liver functions, plasma cholesterol, insulin resistance, and RNA expression through hypothalamus-pituitary-adrenal axis variations (de Sousa Rodrigues *et al.*, 2017). Glucocorticoid receptors (GR) are transcription factors and ligand inducible. Glucocorticoid receptors exert their action through cellular effects and gene transcription. The signaling of glucocorticoids (GCs) is coupled to various pathways (Eriksen *et al.*, 2017). Glucocorticoids act by binding to glucocorticoid receptors intracellularly. Glucocorticoids (GCs) are primarily steroidal and are synthesized by the help of adrenal glands. The HPA axis exclusively regulates the secretion of GCs and is greatly affected by numerous factors such as GABA, inflammatory cytokines (IL-1, IL-6), etc. Glucocorticoid receptors control several physiological functions like immune responses, reproduction, and development (Dendoncker and Libert, 2017). Glucocorticoid receptor (GR) also monitors different lipid metabolism features and lipoproteins in the liver and plays an important role in managing different hepatic disorders (Khaderi and Hollinger, 2017). Acetaminophen or paracetamol is a broadly used antipyretic and analgesic drug. However, it causes severe liver damage and acute hepatic failure in case of an overdose. Acetaminophen hepatotoxicity is mainly related to the generation of a crucial reactive metabolite (NAPQI), production of adducts of protein, GSH reduction, specifically in mitochondria, oxidative stress causes stimulation of MAP kinase as well as fragmentation of DNA in the nucleus. NAPQI is primarily liable for the induction of lipid peroxidation and causes GSH depletion (Woolbright and Jaeschke,

2017). ALT and serum AST are vital enzymes traced predominantly in the liver, but these primary enzymes are also positioned in heart cells, RBCs, muscles, kidneys, and pancreas (Huang *et al.*, 2006). The extent or level of liver damage is measured by the increased level of crucial serum markers such as ALT and serum AST (Schwimmer *et al.*, 2010). Elevated levels of AST and ALT are observed in Acetaminophen-induced liver damage (Olaleye and Rocha, 2008). Results of the current research study clearly showed that there is a prominent increase ($P < 0.05$) in levels of serum AST and serum ALT in the APAP-prompted hepatotoxic group which was given acetaminophen (200 mg/kg, intraperitoneally), while pre-treated groups 1, 2 and 3 (dexamethasone + APAP, mifepristone + APAP, dexamethasone + mifepristone + APAP) substantially reduced serum ALT and AST concentrations in contrast to APAP-prompted hepatotoxic group. Oxidative stress is crucial in the progression and initiation of liver diseases. Oxidative stress also prompts the discharge of crucial cytokines, initiating a fibrogenic response in the liver. Oxidative stress causes excessive ROS formation or generation, instigating lipid peroxidation. Cell functioning is extensively affected by lipid peroxidation under oxidation-stimulated stress. Oxidation stress is expansively associated with the progression and commencement of hepatic dysfunction by causing DNA damage, necrosis, apoptosis, and mutations (Spahis *et al.*, 2017). Dexamethasone (Dex) is a widely used synthetic glucocorticoid. Dex is primarily practiced for systemic studies of various glucocorticoid effects on different physiological and cellular responses (Cole *et al.*, 2000). Dexamethasone is a potent glucocorticoid receptor (GR) agonist, and it also causes inhibition of phospholipase A2. Dex shows sufficient anti-inflammatory activity (Eken *et al.*, 2006). Mifepristone is a synthetic glucocorticoid receptor (GR) antagonist with anti-glucocorticoid and anti-progesterone activities. Mifepristone (RU486) is efficiently metabolized by the aid of the CYP-450 enzyme in the liver (Reboredo *et al.*, 2008). Albumin is the main protein

component that is synthesized via the liver. The albumin level is decreased in liver disorders due to less production or increased degradation or loss (Gammopathies, 2005). Elevated levels of globulins and reduced albumin levels observed in acetaminophen (APAP) induced hepatotoxicity (McGill *et al.*, 2012). Histopathological assessment of illustrated livers displayed centrilobular alterations, necrosis, and infiltration of cells (mononuclear) within portal regions in the APAP-prompted toxic group. While the Dexamethasone pre-treated group showed hepatocytes normal expression with minor liver damage in contrast to the toxic group (APAP). The mifepristone pre-treated group exhibited intact parenchyma and comprehensive sinusoidal apertures, though slight necrosis was present. However, the Dexamethasone + Mifepristone pre-treated group displayed normal manifestations of hepatocytes along with slight inflammation and necrosis. The current study indicated that acetaminophen provokes oxidative stress in mice, hence serving as a major inducer of hepatotoxicity. A marked reduction in TAC and elevated level of TOS is observed in the APAP-induced hepatotoxic group, while pre-treated groups averted the decline in TAC level and increased TOS level compared to the toxic group.

Conclusion

It was deduced from the current research study that AST, as well as ALT serum values, were considerably high in the APAP-prompted hepatotoxic group. Dexamethasone (GR agonist) substantially declined ALT levels compared to the toxic group (APAP). While Mifepristone alone and combined group (dexamethasone + RU486) also decreased serum ALT (U/L). However, Mifepristone (GR antagonist) significantly decreased AST (U/L) levels compared to the APAP-prompted hepatotoxic group. A marked reduction in TAC and elevation in TOS levels are observed in the APAP-elicited hepatotoxic group, while pre-treated groups showed significant hepatoprotective effects compared to the toxic group (APAP). The results of the present study can be beneficial for the safety evaluation of glucocorticoid receptor ligands (RU-486 and dexamethasone), yet require more investigation to explicate fully the basic underlying process of protection.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned.

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

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