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Evaluation of the Possible Role of Interlukin-1beta (IL-1β) in Diclofenac-Induced Hepatoxicity and the Exacerbative Effect of Progesterone Hormone in Mice

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Abstract Diclofenac (DCLF) is one of the most widely prescribed non-steroidal anti-inflammatory drugs (NSAIDs). DCLF is associated with rare but serious idiosyncratic drug-induced liver injury (IDILI) in humans. It ranges from asymptomatic increase of plasma transaminases to life-threatening fulminant hepatitis with the need for liver transplantation. Mechanisms of DCLF-induced IDILI are not yet clarified, but immune responses are suspected to underlie them. In general, it is believed that women exhibit worse outcomes from DILI than men. The aim of this study is to investigate the involvement of immune reaction in mediating DCLF- hepatotoxicity and to evaluate the exacerbative effect of progesterone on DCLF-IDILI. The study was conducted on 100 albino mice divided into 5 main groups; each group was subdivided into a (female subgroup) and b (male subgroup) each of 10 mice; group I (negative control group), group II (normal saline group), group III (progesterone group), group IV (DCLF group), group V (progesterone and DCLF group). **Results**: DCLF administration, either alone or following progesterone, induced liver toxicity as manifested by abnormalities of hepatic profile (AST, ALT, T.Bil and GGT) together with increased plasma level of immune cytokine interlukin-1beta (IL-1β), with significant higher results encountered in females compared to males. Progesterone pre-treatment led to augmentation of the afro-mentioned indices in female mice only. These changes were substantiated with histopathological observations. From the previous results it can be concluded that an immune factor such as IL-1β is implicated in acute DCLF-induced hepatotoxicity and progesterone inflicts an exacerbative effect on this toxicity.

Introduction

Drug-induced liver injury (DILI) is defined as a liver injury caused by various medications, herbs, or other xenobiotics, leading to abnormalities in liver tests or liver dysfunction with the reasonable exclusion of other etiologies. It can be non-idiosyncratic (predictable), or idiosyncratic (unpredictable). The estimated annual incidence rate of DILI is 13.9-24.0 per 100,000 inhabitants worldwide (Suk and Kim, 2012; Leise et al., 2014). It is the most common cause of the withdrawal of an accredited drug from the market and for cessation of drug development in pharmaceutical companies (Njoku, 2014).

In most cases, the mechanisms of hepatotoxicity of idiosyncratic DILI are not explained, but it is likely to arise from complex risk factors including drug lipophilicity, daily dose, genetic variations, age, sex, diseases, and environmental factors (Chalasani et al., 2014 ; Leise et al., 2014).

Diclofenac (DCLF), the most widely prescribed non-steroidal anti-inflammatory drug (NSAID), is known to be associated with idiosyncratic DILI in humans. It ranges from asymptomatic increase of plasma transaminases to life-threatening fulminant hepatitis (Haque et al., 2016).

Diclofenac had been implicated in more than 250 cases of hepato-cellular damage by the mid-1990s, with a case fatality of approximately 10%. Although rare incidence, yet serious liver injury that may progress to fulminant acute hepatic failure and require liver transplantation can ensue (Lewis and Stine, 2013).

The mechanisms of DCLF-induced liver injury are unknown; however, many theories have been

suggested, including reactive drug metabolites formation, oxidative stress, and mitochondrial injury leading to cell death, but by itself, this does not explain the occurrence of injury only in a minority of patients and no direct evidence of mitochondrial injury in vivo has been reported (Deng et al., 2006; Pandey et al., 2012).

On the other hand, there is clinical and experimental evidence that immune-related reactions may play a role in DCLF-induced liver injury, and that immune mediators might be involved in the pathogenesis (Maiuri et al., 2015). Immune mediators play pivotal roles in the pathogenesis of a variety of human liver diseases (Haque et al., 2016). This action is mediated through the release of a mixture of cytokines, which target liver cells and/or immune cells by activating multiple signaling cascades (Yano et al., 2012)

Generally, women are more susceptible to liver injury by therapeutic drugs than men. Women comprise 74 % of all acute liver failure (Haque et al., 2016). It has been reported that 78% of DILI cases are in women and a significantly greater number of women show DILI than men (Toyoda et al., 2012; Mennecozzi et al., 2015). Women also show worse outcome with 76% underwent liver transplantation and nearly 90% showed fulminant hepatic failure from DILI (Lucena et al., 2009).

Women elicit more vigorous cellular and humoral immune reactions, and experience greater numbers of auto-immune diseases than men (Toyoda et al., 2011). However, there has been vague knowledge concerning the involvement of female sex hormones in DILI (Yokoyama et al., 2005). The circulating levels of estrogen and progesterone fluctuate as a result of the reproductive phase and pregnancy in women (Toyoda et al., 2011). Exogenous manipulation of hormonal level may make some females more prone to hazards of DILI than others for example those using synthetic progesterone preparations for dysfunctional uterine bleeding or for maintenance of pregnancy or as contraception. Estrogen is thought to reduce the severity of various types of liver injuries such as ischemia-reperfusion, trauma-hemorrhage and acetaminophen, but there is little information about the role of progesterone in liver injury (Shimizu et al., 2008; Chandrasekaran et al., 2011; Amacher, 2014).

Aims of this work

Investigate the involvement of the immune cytokine; IL-1β in mediating acute diclofenac (DCLF)-hepatotoxicity and to evaluate the exacerbative effect of progesterone on DCLF-induced liver injury.

Ethical considerations of the study

-The standards of animal care and administration met those required by applicable international laws and regulations.

-Promotion of high standard care and animal well-being at all times.

-Surgical or other painful procedures were performed with appropriate sedation to avoid distress and pain.

Methodology

The drugs

Progesterone (Prontogest 100): was obtained as a 2 ml ampoule containing 100 mg progesteone (Marcyrl Pharmaceutical Industries) and was administrated in a dose of 0.3 mg/mouse subcutaneously (s.c.) once daily for 7 days according to Toyoda et al. (2012).

Diclofenac (Voltaren 75): was obtained as a 3 ml ampoule containing 75 mg diclofenac sodium (Novartis Company) and was administrated as a single toxic dose of 80 mg/kg intraperitonealy (i.p.), 1.5 hours after the last dose of progesterone according to Yano et al. (2012). This dose is less than half of the i.p. LD50. Acute toxicity study demonstrated that the LD50 value of single i.p. administration of DCLF in mice was 250 mg/kg (Yano et al., 2012).

Normal saline: given in a dose of 0.1 ml/mouse i.p.

Animals

This study was carried out on 100 Swiss albino mice with average weight 25 gm. Environmental conditions (e.g lighting, ventilation, temperature and caging as well as water and diet) were similar for all animals. The mice were kept in these cages for one week before starting the experiment to be adapted for any environmental condition.

Animal grouping

The animals were divided into 5 groups:

Group I: (Negative control group): the animals were kept under the same environmental conditions to demonstrate the reference basic parameters: it is further subdivided into two groups:
- **Subgroup Ia:** 10 female mice
- **Subgroup Ib:** 10 male mice

Group II: Normal saline (positive control group): it is further subdivided into two groups:
- **Subgroup IIa:** 10 female mice
- **Subgroup IIb:** 10 male mice

Group III: Progesterone group: it is further subdivided into two groups:
- **Subgroup IIIa:** 10 female mice
- **Subgroup IIIb:** 10 male mice

Group IV: Diclofenac group: it is further subdivided into two groups:
- **Subgroup IVa:** 10 female mice
- **Subgroup IVb:** 10 male mice

Group V: (Progesterone and diclofenac) group: it is further subdivided into two groups:
- **Subgroup Va:** 10 female mice
- **Subgroup Vb:** 10 male mice

Experimental procedure

At the end of the experiment, i.e. 24 hours after DCLF administration, all animals were sacrificed and blood samples were collected and centrifuged to obtain serum to assess hepatic parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) were determined by colorimetric method according to Frankel and Gradwohl

(1970) and total bilirubin (T.Bil) was measured according to Suber (1994). Prothrombin time (PT) according to Tietze (1983). The assay kits were purchased from Alkane Company. The plasma IL-1β levels were measured by ELISA using a kit according to the manufacturer's instructions. Then the abdomen was explored and the liver was removed immediately by dissection and was sent for histopathological examination.

Biochemical Hepatic Profile

- Serum aspartate aminotransferase (AST)
- Serum alanine aminotransferase (ALT).
- Total serum bilirubin (T.Bil).
- Serum gamma glutamyl transferase (GGT).
- Prothrombin time (PT).

Immunological Profile

- Plasma interlukin (IL-1β)

Histopathological examination

Small pieces of mice liver were fixed in 10% neutral-buffered formalin and embedded in paraffin. 5 µ-thick sections were cut and then stained with haematoxylin-eosin (H&E) for histopathological examination by light microscope according to Drury and Wallington (1980).

Statistical analysis

The results were statistically analyzed using the SPSS software, version 15 (SPSS, Inc., Chicago, IL).Quantitative data are described as mean± standard deviation (M±SD). ANOVA one way statistical analysis was used to compare between the groups. P values < 0.05 were considered statistically significant ***(Taylor, 1990).***

Results

1-The biochemical hepatic profile results:

There was non-significant difference between the control groups (Ia,Ib,IIa,IIb) when compared with each other regarding the levels of AST, ALT, T.Bil , GGT and PT. Also, progesterone administration alone did not affect the hepatic profile in either female or male mice (subgroups IIIa and IIIb respectively) as there was non-significant difference when compared with the control group or with each other (Table 1).

Table (2) shows significant elevation of the hepatic parameters (AST, ALT, T.Bil and GGT) in DCLF -treated female mice (subgroup IVa) denoting obvious hepato-cellular damage, yet the elevation in male mice (subgroup IVb) results was still statistically insignificant when compared with the control group. Significant increase of the hepatic profile levels in subgroup IVa as compared with subgroup IVb was also observed. PT in subgroups IVa and IVb showed non-significant difference when compared with the control group or with each other.

The use of progesterone prior to DCLF resulted in highly significant increase of the hepatic parameters in female mice (subgroup Va), while, the increase of the same parameters in male mice (subgroup Vb) was still insignificant when compared with the control group. Also, female mice showed significant liver derangement in subgroup Va when compared with the male mice in subgroup Vb. In addition, females of subgroup Va showed significant higher levels of the liver profile compared to females of subgroup IVa. On the contrary, non-significant difference of results was observed between males of subgroups IVb and Vb. PT in subgroups Va and Vb showed non-significant difference when compared with the control group or with each other (Table2).

2- The immunological results:

There was non-significant difference between the control groups (Ia,Ib,IIa,IIb) when compared with each other regarding the levels of IL-1β. Also, progesterone administration alone did not affect the immunological factor in either female or male mice (subgroups IIIa and IIIb respectively) as there was non-significant difference when compared with the control group or with each other (Table 3).

Table (4) DCLF -administration showed significant elevation of the plasma level of IL-1β in female mice (subgroup IVa), yet the IL-1β increased level in male mice (subgroup IVb) was still statistically insignificant when compared with the control group. Significant difference of IL-1β level in subgroup IVa as compared with subgroup IVb was also observed.

Table (4) also reveals that progesterone pre-treatment to DCLF showed highly significant increase of plasma IL-1β level in female mice (subgroup Va) and insignificant increase of the same cytokine in male mice (subgroup Vb) when compared with control group. The IL-1β level in female mice (subgroup Va) was significantly higher when compared with the male mice (subgroup Vb). Furthermore, females of subgroup Va showed significant higher levels of IL-1β compared to females of subgroup IVa. There was non-significant difference of results between males in subgroups IVb and Vb.

II-Histopathological results

The histopathological findings in all groups were judged in comparison with the controls (groups Ia, Ib, IIa, IIb) that showed unremarkable pathological changes (fig.1). Progesterone treatment alone in either female or male subgroups of group III resulted in unremarkable cellular and architectural appearance of liver (fig.2). The treatment of mice with DCLF induced hepatic lesions in female mice of subgroup IVa, in the form of moderate degree of inflammatory infiltrates seen as one or two large aggregates of neutrophils and micro-vesicular fatty change observed as small, generally spherical to oval non-staining cytoplasmic vacuoles (fig.3,4). These findings were much less in male mice of subgroup IVb with sparse neutrophils seen in between hepatocytes (fig.5). On the other hand, treatment with progesterone prior to DCLF in female mice of subgroup Va induced severe form of hepatic necrosis, severe infiltration with neutrophils and occasional apoptotic cells and microvesicular fatty degeneration (fig.6,7). While in male mice of subgroup Vb mild hepatic changes and mild neutrophilic infilteration were observed (fig.8).

Table (1): ANOVA one way statistical analysis of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total serum bilirubin (T.Bil), serum gamma glutamyl transferase (GGT) and prothrombin time (PT) in group I: negative control group (subgroup Ia: female mice, subgroup Ib: male mice), group II: normal saline group (subgroup IIa: female mice, subgroup IIb: male mice), and group III: progesterone group (subgroup IIIa: female mice, subgroup IIIb: male mice) . Each subgroup consisted of 10 rats.

Groups / Variables	Group I		Group II		Group III		Fc	LSD
	subgroup Ia M ± SD	Subgroup Ib M ± SD	subgroup IIa M ± SD	subgroup IIb M ± SD	subgroup IIIa M ± SD	subgroup IIIb M ± SD		
AST (U/L)	28.8±4.8	25.7±5.9	27.3±8.5	31.5±7.9	29.5±6.6	28.5±6.4	0.83	38.7
ALT (U/L)	36.9±6.6	39.8±5.2	33.5±7.3	39.4±6.7	38.5±6.4	37.9±4.4	1.3	11.8
T.Bil(mg/dl)	0.55±0.04	0.54±0.09	0.58±0.06	0.59±0.08	0.58±0.04	0.57±0.04	0.9	0.35
GGT (U/L)	43.2±7.6	45.5±8.8	39.5±3.2	40.4±2.7	43.2±2.7	43.5±3.2	1.8	10.25
PT(seconds)	12±2.4	11±3.1	14±2.1	13±2.5	14±3.9	11±2.4	2.4	5.3

The data presented as mean ± standared deviation, Fc=variance ratio calculated by ANOVA one way statistical analysis. Ft= tabulated variance ratio =4.184, LSD: least significant difference.

Table (2): ANOVA one way statistical analysis of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total serum bilirubin (T.Bil), serum gamma glutamyl transferase (GGT) and prothrombin time (PT) in group I: negative control group (subgroup Ia: female mice, subgroup Ib: male mice), group IV: diclofenac group (subgroup IVa: female mice, subgroup IVb: male mice), and group V: progesterone and diclofenac (subgroup Va: female mice, subgroup Vb: male mice). Each subgroup consisted of 10 rats.

Groups / Variables	Group I		Group IV		Group V		Fc	LSD
	subgroup Ia M ± SD	subgroup Ib M ± SD	subgroup IVa M ± SD	subgroup IVb M ± SD	subgroup Va M ± SD	subgroup Vb M ± SD		
AST (U/L)	28.8±4.8	25.7±5.9	2041±241.5$^{*\#}$	39.6±11.2	2543±159 $^{*\# \Delta}$	40±9.4	991	42.29
ALT (U/L)	36.9±6.6	39.8±5.2	1050±145.7$^{*\#}$	50±8.5	1258±198.6$^{*\# \Delta}$	64±12.3	326	35.98
T.Bil (mg/dl)	0.55±0.04	0.54±0.09	2.53±0.07$^{*\#}$	1.09±0.13	2.98±0.21$^{*\#\Delta}$	1.06±0.15	663	0.046
GGT (U/L)	43.2±7.6	45.5±8.8	64.5±4.8$^{*\#}$	48.5±5.8	74.3±8.8$^{*\# \Delta}$	50.5±5.3	32.3	9.6
PT(sec.)	12±2.4	11±3.1	14.3±2.2	13±3.9	12±3.1	12±2.7	1.29	7.3

The data presented as mean ± standared deviation, Fc=variance ratio calculated by ANOVA one way statistical analysis. Ft= tabulated variance ratio =4.184, LSD: least significant difference.

** : significant increase as compared to control group (subgroup Ia) p< 0.05*

 # : significant increase as compared to male subgroup within the same group p< 0.05

Δ : significant increase as compared to subgroup IVa p< 0.05

Table (3): ANOVA one way statistical analysis of plasma interlukin-1beta (IL-1ß) in groupI: negative control group (subgroup Ia: female mice, subgroup Ib: male mice), group II: normal saline group (subgroup IIa: female mice, subgroup IIb: male mice), and group III: progesterone group (subgroup IIIa: female mice, subgroup IIIb: male mice). Each subgroup consisted of 10 rats.

Groups / Variable	Group I		Group II		Group III		Fc	LSD
	Subgroup Ia M ± SD	Subgroup Ib M ± SD	subgroup IIa M ± SD	subgroup IIb M ± SD	Subgroup IIIa M ± SD	subgroup IIIb M ± SD		
Plasma IL-1ß (pg/mL)	2.90±0.4	2.66±0.3	2.69±0.5	2.78±0.4	2.98±0.6	3.1±0.5	1.4	0.88

The data presented as mean ± standared deviation, Fc=variance ratio calculated by ANOVA one way statistical analysis,Ft= tabulated variance ratio =4.184, LSD: least significant difference.

Table (4): ANOVA one way statistical analysis of plasma interlukin-1beta (IL-1ß) in group I: negative control group (subgroup Ia: female mice, subgroup Ib: male mice), group IV: diclofenac group (subgroup IVa: female mice, subgroup IVb: male mice), and group V: progesterone and diclofenac (subgroup Va: female mice, subgroup Vb: male mice). Each subgroup consisted of 10 rats.

Groups / Variable	Group I		Group IV		Group V		Fc	LSD
	SubgroupIa M ± SD	SubgroupIb M ± SD	subgroupIVa M ± SD	subgroupIVb M ± SD	subgroupVa M ± SD	SubgroupVb M ± SD		
Plasma IL-1ß (pg/mL)	2.90±0.4	2.66±0.3	36±9.8$^{*\#}$	3.9±0.15	50±11.3$^{*\#\Delta}$	4.7±0.12	116	2.19

The data presented as mean ± standared deviation, Fc=variance ratio calculated by ANOVA one way statistical analysis.
Ft= tabulated variance ratio =4.184, LSD: least significant difference.
* : significant increase as compared to control group (sub group Ia) $p< 0.05$
# : significant increase as compared to male subgroup within the same group $p< 0.05$
Δ : significant increase as compared to subgroup IVa $p< 0.05$

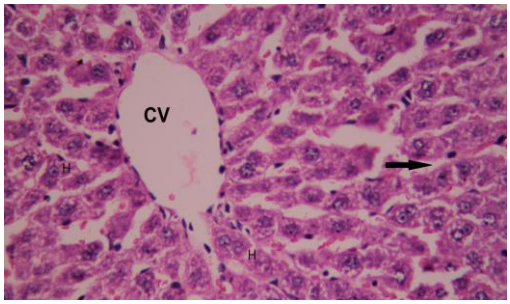

Fig (1): Photomicrograph of sections of albino mice liver from the control group showing normal appearance of hepatic lobule with a central vein (CV). The hepatocytes (H) are arranged in branching cords which are separated by blood sinusoids (→). (Hx&E x400

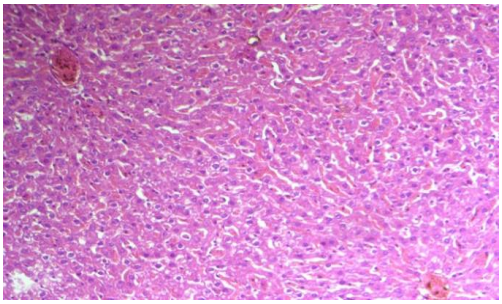

Fig (2): Photomicrograph of sections of female albino mouse liver from the progesterone IIIa subgroup shows relative normal appearance of hepatocytes. The same finding was seen in male liver sections IIIb subgroup. (Hx&E x200).

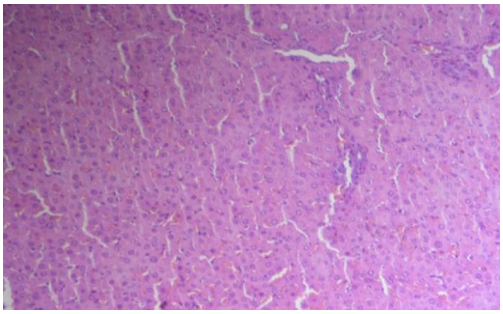

Fig (3): Photomicrograph of sections of female albino mouse liver from the DCLF IVa subgroup showing moderate degree of hepatic inflammatory infiltrates with two adjacent neutrophilic aggregates (→) are detected in one field and micro-vesicular fatty change (→). (Hx&E x200)

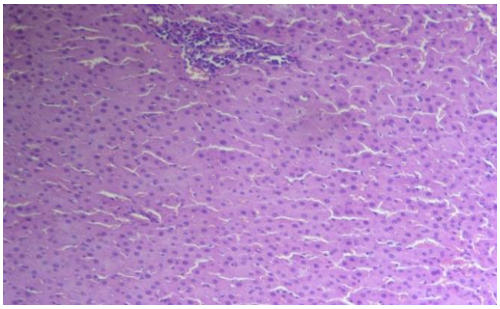

Fig (4): Photomicrograph of sections of female albino mouse liver from the DCLF IVa subgroup showing moderate degree of inflammatory infiltrates with one large neutrophilic aggregate (→)seen at the top of the field. (Hx&E x200).

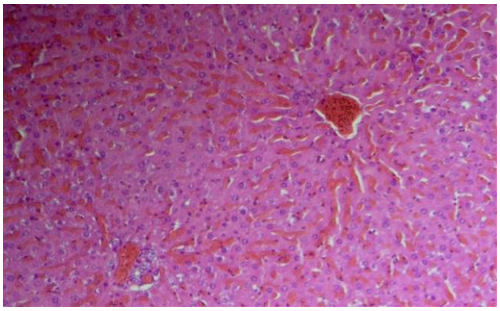

Fig (5): Photomicrograph of sections of male albino mouse liver from the DCLF IVb subgroup shows mild histopathological changes with sparse neutrophils (→) detected in between hepatocytes. (Hx&E x200).

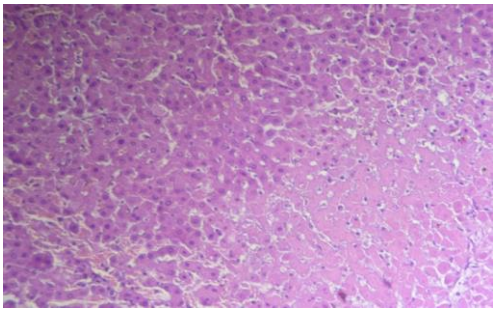

Fig (6): Photomicrograph of sections of female albino mouse liver from the (progesterone and DCLF) Va subgroup showing severe hepatic necrosis with wide area of cellular necrosis (→) is seen to the right of the field, apoptotic cells are detected(→), microvesicular fatty change(→) and neutrophilic aggregates(→) to the left of the photo. (Hx&E x200).

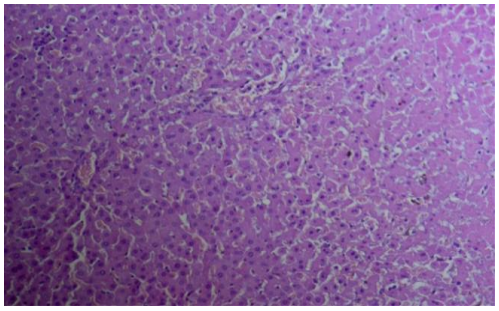

Fig (7): Photomicrograph of sections of female albino mouse liver from the (progesterone and DCLF) Va subgroup showing severe hepatocellular necrosis (→) and hepatic infiltration with neutrophils (→). (Hx&E x200).

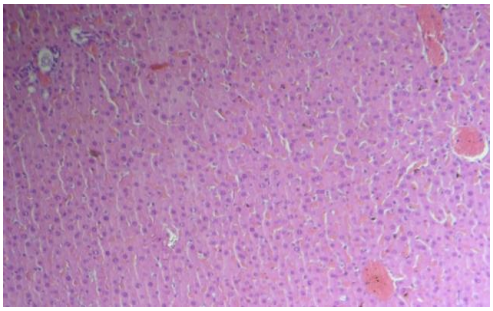

Fig (8): Photomicrograph of sections of male albino mouse liver from the (progesterone and DCLF) Vb subgroup showing mild histopathological changes with minimal neutrophils infilteraion. (Hx&E x200).

Discussion

Diclofenac (DCLF), one of the most commonly prescribed NSAIDs, has caused rare but sometime serious hepatotoxicity in humans, but the exact mechanism remained unclear (Haque et al., 2016).

In this study, liver injury was evidenced with the administration of DCLF (groups 4 and 5) by the increased levels of AST, ALT, T.Bil and GGT, together with abnormal histopathological changes. Similar to this study Deng et al. (2006), reported elevated levels of liver biochemical profile along with liver histopathological changes within 6 hours following DCLF administration to rats at doses of 50 and 100 mg/kg denoting obvious DCLF-induced liver injury. Also, plasma ALT and AST levels slightly increased 1 hour and markedly increased 24 hours after DCLF administration to mice at a dose of 80 mg/kg together with histopathological examination of liver tissues revealing necrosis and apoptosis in a study by Yano et al. (2012).

Diclofenac, more likely than other NSAIDs, is one of the drugs that most commonly causes abnormal serum liver enzyme levels as part of inducing hepatocellular damage (Aragon and Younossi, 2010; Haque et al., 2016). Regarding PT, there was no change in its level in the current study. This can be explained by the fact that prolongation of PT needs longer period of liver damage before prothrombin biosynthetic ability of liver is affected (Giannini et al., 2005).

Induced hepatic injury in human is strongly suggested (Maiuri et al., 2015).

In the present study, the administration of DCLF (groups 4 and 5) significantly increased the plasma Interlukin (IL-1β) levels as well as histopathological evedince of neutrophils infiltration. Similar results were obtained by Yano et al. (2012), who observed increased plasma IL-1β level soon (from 6 to 36 hours; maximum level increase noted 24 hours) after the DCLF administration to mice in a study where cytokines as

immune regulators of drug hepatotoxicity have been invistigated.

Interlukin -1 β is a very potent pro-inflammatory cytokine. It is produced by peripheral monocytes and dendritic cells (Bouman et al., 2005). Hepatocytes and neutrophils also produce IL-1β (Arend et al., 2008). IL-1β acts mainly as a pro-inflammatory agent which mediates a wide variety of immune responses activating and recruiting leukocytes, especially neutrophils, into the liver (Yano et al., 2012). It is illustrated that activated neutrophils release protease such as elastase that can cause tissue damage. The stimulated neutrophils act as effector cells through cytotoxicity within the liver, leading to hepatocyte necrosis (Krenkel et al., 2014).

In several experimental animal studies, accumulated neutrophils in the liver were reported to be involved in the progression and severity of hepatocellular damage as ischemia-reperfusion injury (Ramaiah and Jaeschke, 2007), dicloxacillin (Higuchi et al., 2011), halothane (HAL) (You et al., 2006; Toyoda et al., 2012) and acetaminophen-induced hepatotoxicity (Krenkel et al., 2014; Huebener et al., 2015).

A previous study demonstrated a significant increase of reactive oxygen species (ROS) in hepatocytes treated with DCLF (Gómez-Lechón et al., 2003). Increased ROS levels can trigger inflammasome (a cytosolic multiprotein complex in macrophages) activation which mediates the processing of the pro-inflammatory caspases and cytokines secretion, such as IL-1β (Mehal, 2014) thereby further activates the innate immune system, leading to the recruitment of immune cells to the site of inflammation and advancing hepatocyte necrosis (Krenkel et al., 2014).

Interlukin-1β levels are known to be increased during acetaminophen hepatotoxicity (Cover et al., 2006; Krenkel et al., 2014) and HAL-induced liver injury (Toyoda et al., 2011).Therefore, Yano et al. (2012) believed that IL-1β promotes immune responses to DCLF and enhance DCLF- induced hepatotoxicity.

In this work the elevated transaminases, total bilirubin and GGT levels and hepatic tissue damage as well as increased serum IL-1β level and neutrophils infiltration in DCLF- induced liver injury were exacerbated by progesterone pre-treatment in female mice (subgroup Va) as compared with female mice which received DCLF only in subgroup IVa.

In accordance with this study, Toyoda et al. (2011) observed that progesterone administration (0.3 mg/mouse/day subcutaneously for 7 days) led to increased levels of hepatic biochemical profile and proinflammatory cytokines; IL-1β, tumor necrosis factor (TNF α), and IL-6 and several chemokines in a HAL-induced liver injury model. Also, progesterone exacerbating effect, in thioacetamide (TA), α-naphthylisothiocyanate, and dicloxacillin-induced liver injury in female mice, was reported by Toyoda et al. (2012). It was also reported that estradiol decreased and progesterone increased the production of pro-inflammatory cytokines in oxidative stress-stimulated murine peritoneal macrophage (Huang et al., 2008) and human mononuclear cells (Yuan et al., 2008).

In addition to their effects on sexual differentiation and reproduction, sex hormones appear to influence the immune system (Schumacher et al., 2014). This results in a sexual dimorphism in the immune response in humans. Females produce more vigorous cellular and humoral immune reactions than males (Bouman et al., 2005; Toyoda et al., 2011). Disease expression is also affected by the reproductive status of the female (La Rocca et al., 2014). As for TNF α, differences in IL-1β synthesis at different reproductive stages have been demonstrated with fluctuating progesterone levels (Bouman et al., 2005).

Progesterone, one of the female sex hormones, plays an important role in immune system regulation and has an important effect in DILI (Dugan et al., 2011). The progesterone receptor is expressed on immune cells, natural killer cells, leukocytes as well as Kupffer cells (Gilliver, 2010). Kupffer cells, resident hepatic macrophages seeded in the liver sinusoids, provide important regulatory functions in pathophysiological states of the liver and act as a major source of pro-inflammatory cytokines including TNF α and IL-1β and CXC chemokines under severe stress and various types of liver injury (Adams et al., 2010; Krenkel et al., 2014). Therefore, Toyoda et al. (2012), believe that progesterone augment DILI by activating Kupffer cells and increasing expression of cytokines and chemokines.

It was also reported that the extracellular signal-regulated kinase (ERK) pathway in Kupffer cells has a critical role in the production of the immune response and liver injury (Thakur et al., 2006). A study by Toyoda et al. (2012), postulated that inhibition of ERK significantly decreased the progesterone-induced exacerbation of HAL-induced liver injury and immune responses. It has been reported that inhibition of ERK attenuated Fas-induced CXCL1 in murine epithelial cell line (Farnand et al., 2011). Furthermore, inhibition of the ERK pathway suppressed the inflammatory response and improved the outcome after traumatic injuries and cisplatin-induced renal injury (Jo et al., 2005; Hsu et al., 2009).

So, as Maiuri et al. (2015) believe that DCLF/cytokine-induced hepatic cytotoxicity involve ERK pathway activation, therefore, the stimulation of ERK pathway in Kupffer cells and the subsequent increased expression of cytokines may play an important role in the exacerbation of DILI by progesterone (Toyoda et al., 2011).

The obtained results showed that progesterone administration alone in (group III) did not lead to derangement of liver indices. Similar to this, Toyoda et al. (2011) reported that progesterone administration did not affect the transaminases or the cytokines levels in the control mice indicating that progesterone exacerbates the

severity of DILI rather than causing the liver injury per se.

In this work, progesterone administration prior to DCLF, did not further affect male mice hepatic biochemical or immunological profile (subgroup Vb) as compared with male mice with DCLF only in subgroup IVb. Also, females elicited worse results as compared to males in both groups (IV and V).

In HAL-treated females, plasma concentration of TNFα was greater than in males, and neutrophils were recruited to liver more rapidly and to a greater extent. The liver injury was severe only in females (Dugan et al., 2011). The same sexual diversity was noted by Toyoda et al. (2011 and 2012) where progesterone enhanced different DILI in female mice only not the male ones.

A number of hypotheses have been proposed to explain this sex difference in susceptibility to DILI. Collectively, these hypotheses suggest specific hormonal effects or interactions with immune-modulating agents or signaling molecules and differences in the adverse response of the immune system to some drugs, reactive drug metabolites, or drug-protein adducts (Amacher, 2014).

Male sex hormone, testosterone, have immunosuppressive effects, which may partly account for the sex disparity of autoimmune diseases in humans (Fijak et al., 2011). Therefore, females have a higher incidence compared to males to develop autoimmune diseases such as rheumatoid arthritis (RA), myasthenia gravis, multiple sclerosis (MS), systemic lupus erythematosus (SLE), or autoimmune hepatitis (Hughes et al., 2013). It is thought that testosterone may partly suppress progesterone induced immune activation in male mice and play a protective role against flaring of DILI (Toyoda et al., 2011 and 2012). Lastely, Mennecozzi et al. (2015) stated that there is an overall higher sensitivity of female primary hepatocytes to hepato-toxicants than males.

Conclusion

Diclofenac (DCLF) induced acute liver injury in mice with increased levels of AST, ALT, T.Bil and GGT. Furthermore, it was demonstrated that immunological factors such as IL-1β appeared to be involved in the early onset of DCLF -induced hepatic injury. Moreover, progesterone exacerbated the severity of immune mediated liver injury induced by DCLF.

The mechanism of the exacerbation by progesterone appears to involve immune responses such as the increased production of cytokines as IL-1β and neutrophils infiltration which is thought to be via the activation of ERK pathway and Kupffer cells. These findings may shed light on one of the crucial mechanisms involved in IDILI and point out to the vulnerability of females in general who might have one of the IDILI risk factors (ie. genetic predisposition, liver dysfunction, etc.) or during late pregnancy or under progesterone therapy to DCLF-hepatotoxicity .

Further studies are recommended to enclose the effect and the role of other sex hormone as well as other immunological factors in DCLF-induced liver injury. Extra studies with different DCLF dose leveling and longer durations are suggested. It is recommended to use the lowest effective dose of DCLF for the shortest duration consistent with individual patient treatment goals. Physicians should measure transaminases and immune profile periodically in patients receiving long-term therapy with DCLF, because severe hepatotoxicity may develop without a prodrome of distinguishing symptoms. The potential clinical application of progesterone receptor antagonist in immune-mediated responses in DILI is suggested. It is advisable that women, who are pregnant or using synthetic progesterone injections, should be prohibited from using DCLF owing to increased susceptibility to develop serious DILI.

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الملخص العربى

تقييم للدورالمحتمل للانترلوكين ١بيتا فى السميه الكبديه المحدثه بالديكلوفيناك والتأثير المفاقم لهرمون البروجستيرون فى الفئران

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الديكلوفيناك هو احد أكثر العقاقير اللا ستيرويديه المضاده للالتهاب الموصوفه للعلاج. يرتبط الديكلوفيناك نادرا ولكن بشكل جدى بإصابه الكبد الناتجه عن العقاقيرفى الانسان , والتى تتراوح بين زياده غير عرضيه لانزيمات الكبد الى التهاب كبدى مداهم وخطر على الحياه مع الحاجه لزرع كبدى. آليات الديكلوفيناك المحدثه لإصابه الكبد الناتجه عن العقاقيرليست واضحه بعد ولكن يشتبه ان تكمن وراءها استجابه مناعيه. عموما, يعتقد ان النساء أكثر عرضه من الرجال للتداعيات الأسوأ لإصابه الكبد الناتجه عن العقاقير.

الغرض من هذه الدراسه هو بحث تدخل رد الفعل المناعى فى احداث التسمم الكبدى بالديكلوفيناك و تقييم التأثير المفاقم لهرمون البروجستيرون على إصابه الكبد الناتجه عن العقاقير بالديكلوفيناك.

تم اجراء هذه الدراسه على عدد ١٠٠ من الفئران البيضاء مقسمه الى خمس مجموعات رئيسيه: كل مجموعه قسمت لمجموعه فرعيه أ (اناث) و مجموعه فرعيه ب (ذكور) تحتوى كل منها على عشره فئران. المجموعة الاولى : مجموعة ضابطة سلبية والمجموعة الثانية: اعطيت محلول ملح طبيعى/فسيولوجى والمجموعة الثالثة: أعطيت بروجستيرون والمجموعة الرابعة: أعطيت ديكلوفيناك والمجموعة الخامسة: اعطيت (بروجستيرون وديكلوفيناك).

النتائج: نتج عن اعطاء الديكلوفيناك, وحده او بعد البروجستيرون, سميه كبديه تجلت باختلال وظائف الكبد (انزيمات الكبد والبيلليروبين الكلى والجاما جلوتاميل ترانسفريز) بالاضافه لزياده بمستوى السيتوكاين المناعى انترلوكين ١بيتا فى البلازما مع تسجيل نتائج بزياده ذات دلاله احصائيه فى الاناث بالمقارنه بالذكور. ادى العلاج المسبق بالبروجسترون الى مضاعفه جميع القياسات المسبق الاشاره اليها فى اناث الفئران فقط. وكانت هذه التغيرات مدعمه بملاحظات هيستوباثولوجيه. من النتائج السابقه يمكن استنتاج ان عامل مناعى مثل الانترلوكين ١بيتا متدخل بالسميه الكبديه المحدثه بالديكلوفيناك وان البروجسترون يوقع تاثير مفاقم على هذه السميه.

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