Research Article

Glutathione S-Transferase (A1) as a marker of Acute Acetaminophen Induced Hepatotoxicity and Nephrotoxicity, and the Protective Role of Dexmedetomidine

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Abstract
Background: acetaminophen (APAP) is an important drug that cause nephrotoxicity and hepatic toxicity in high dose. Glutathione S-transferase A1 (GSTA1) is a marker that used to early diagnosis of this toxicity in rats. Dexmedetomidine (DEX) is an antioxidant, so it play an important role in treatment of liver and renal affection caused by high dose of APAP. **Methods**: 100 rats divided into 5 groups, group I received normal saline, group II received APAP, group III received APAP + NAC, group IV received APAP + DEX, and group V received APAP + NAC + DEX. Blood samples were drawn from group I after 24hours, from group II after 6, 8, 10 hours, from group II, III, IV, V after 24 hours. By these samples we estimated Alanine Transferase (ALT), Aspartate transaminase (AST), serum urea, serum creatinine, Glutathione S-transferase A1 (GSTA1), Catalase enzyme (CAT), Glutathione peroxidase (GSH-Px), Malondialdehyde (MDA). Liver and kidney of each rat were dissected for histopathology after scarification. **Results:** the levels of ALT, AST, urea, and creatinine were significantly increased in group of APAP, and significantly decreased in group APAP + NAC, and APAP + NAC + DEX. The levels of glutathione peroxidase, and catalase (antioxidants) were significantly decreased in group of APAP, and significantly increased in group APAP + NAC, and group APAP + NAC + DEX. The levels of MDA that excreted due to lipid peroxidation were significantly increased in group APAP. NAC alone or NAC + DEX induced a significant decrease in a level of MDA but DEX alone could not induce a statistically significant reduction in MDA level. In group of APAP, ALT and AST were statistically significant after 8 hours and 10 hours of giving APAP but GSTA1 started to be statistically significant after 6 hours. **Conclusion**: GSTA1 is considered a marker for early diagnosis of hepatic injury and renal affection of acute acetaminophen toxicity, also DEX had an important role in its treatment.
Keywords: GSTA1, Dexmedetomidine, hepatotoxicity, nephrotoxicity, acetaminophen (APAP), liver markers,

Introduction
Acetaminophen (N- acetyl- para-aminophenol, APAP, paracetamol, Tylenol) is now a ubiquitous and highly utilized over-the-counter medication for relief of pain and fever. Although acetaminophen is believed to be safe at therapeutic doses, it is a dose-related toxin and tends to produce a centrilobular hepatic necrosis at toxic doses because of its toxic metabolites that cause severe organ toxicity (Nikravesh et al., 2018).

Paracetamol toxicity is caused by intentional (overdose) and unintentional (excessive use) use of the drug. Most people with acetaminophen toxicity have nonspecific symptoms within the first 24 hours of overdose including nausea and vague abdominal pain. With time, signs of acute hepatic failure may develop including low blood glucose, low blood PH, kidney failure, blood clotting problems and hepatic encephalopathy (Yoon et al., 2016).

Hepatic failure and renal failure are the most critical condition in these patients, so in this study we aim to detect the role of Glutathione S-Transferase (A1) as a marker for early diagnosis of liver and renal affections followed APAP overdose, also study the role of Dexmedetomidine in treatment them

Materials and methods
This experimental study was carried on in laboratories of The Departments of Forensic Medicine & Clinical Toxicology, and Clinical Pathology, Faculty of Medicine, Minia University during the period from 1st of June to 15th of July 2020.

Materials:

I. Animals:

One hundred male albino rats with an average weight of 200-250 grams were included in this study. Animals were obtained from Minia University laboratory animals growing center.

Animals were acclimatized to the laboratory conditions for a week before the onset of the experiment to exclude any possible stress. Experimental study was conducted in accordance with the recommendations and guidelines of care and usage of laboratory animals authorized by the ethical committee of Faculty of Medicine.

Rats were divided into five groups, 20 rats per each group Group (I): The control group. Rats were received saline by oral route through orogastric tube to exclude stressful condition, Group (II): Rats were received 1g/kg paracetamol diluted in 3ml volume of 0.9% NaCl via gavage, Group (III): Rats were received 400mg/kg N- acetylcysteine (NAC) by intra peritoneal injection 1 hour after administration of the same APAP dose, Group (IV): Rats were received 100 µg/kg dexmedetomidine (DEX) by intra peritoneal injection z after the same APAP dose, and Group (V): Rats were simultaneously received both 100 µg/kg DEX and 400 mg/kg NAC by intra peritoneal injection after the same APAP dose.

Paracetamol (APAP) was given to rats according to McGill et al., (2016), N- acetylcysteine (NAC) was given to rats according to Tas et al., (2019), and Dexmedetomidine (DEX) was given to rats according to Tas et al., (2019).

Blood samples were collected from rats in group (I) 24 hours after saline intake, from rats in group (II) which received 1 g/kg paracetamol at 6, 8 and 10 hours post drug intake, and from rats in group (II), (III), (IV) and (V) 24 hours of drugs intake. Then rats were scarified by cervical decapitation for histopathological studies.

Blood samples were taken from the retro-orbital plexus of veins by using capillary pipette in heparinized tubes containing 5000 I.U/ml heparin sodium and centrifuged at 3000 rotations per minute for 15 minute (Schubert et al., 2007), Serum was separated and then stored at -70°C till required. Each sample was subjected for estimation of:

Alanine Transferase (ALT):

According to Gaze (2007), ALT was measured by using kinetic method for determining ALT without perodoxal-phosphate which is optimized in accordance with IFCC recommendations using Elitech Flexor Proxl apparatus (France, BEU04434).

Aspartate transaminase (AST):

According to Paraskevas et al., (2011), AST was measured using kinetic method for determining alanine amino transferase without perodoxal- phosphate which is optimized in accordance with IFCC recommendations using Elitech Flexor Proxl apparatus (France, BEU04434).

Serum urea:

According to Fawcett & Soctt (1960), Serum urea was measured using Urease-Berthelot method which is based on formation of ammonium ions via the action of urease enzyme on urea and water, these ions were measured by using Berthelot reaction. The blue dye indophenol product reaction absorbs light ranging between 530 nm and 560 nm proportional to the initial urea concentration.

Serum creatinine:

According to Levey et al., (2006) and Allen et al., (2012), Serum creatinine was measured by using Colorimetric method, creatinine reacts with picric acid to form a yellow - orange color complex under alkaline conditions. The rate of colour formation is proportional to the creatinine quantity in the sample.

Glutathione S-transferase A1 (GSTA1):

According to Habig et al., (1974), GSTA1 was measured by using UV method, The Glutathione S- Transferase Assay Kit measures total GST activity by measuring the conjugation between 1- chloro - 2, 4 - dinitrobenzene and reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. In the sample, the rate of this increase is directly proportional to the GST activity.

Catalase enzyme (CAT):

According to Fossati et al., (1980) and Aebi (1984), Catalase enzyme was measured by using Colorimetric method, catalase reacts with a known quantity of hydrogen peroxide forming a chromophore with a color intensity inversely

proportional to the catalase amount in the original sample.

Glutathione peroxidase (GSH-Px):
According to Paglia & Valentine (1967), GSH-Px was measured by using UV method, the GPx assay is performed indirectly when a cell or tissue homogenate is added to a mixture of solution containing glutathione, glutathione reductase, and NADPH as its oxidation is accompanied by a decrease in absorbance at 340 nm (A340), providing a spectrophotometric means to monitor the activity of GPx enzyme. The enzyme reaction is initiated by adding the hydrogen peroxide substrate and then the A340 is recorded. The decline rate in the A340 is directly proportional to the GPx activity in the sample.

Malondialdehyde (MDA):
According to Satoh (1978) and Ohkawa et al., (1979), MDA was measured by using Colorimetric method, Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) in an acidic medium at temperature of 95°C for 30 minutes to form thiobarbituric acid reactive product. The absorbance of the resulting pink product can be measured at 534 nm.

Histopathological studies:
After scarification of rats by cervical decapitation, liver and kidney of each rat were carefully dissected under complete aseptic conditions and were prepared for histological examination.

The tissues were fixed in a 10% neutral buffered formalin solution followed by dehydration in increasing or ascending alcohol grades, infiltrated with paraffin wax to make the block sufficiently rigid for uniformly thin section about 5 μm thickness and ready for cutting, trimming and finally staining with hematoxylin and eosin (H&E) stain preparing for light microscopic examination (John et al., 2002).

The examination of the liver and kidney was carried out using a light microscope with an attached camera to photograph these sections (Olympus BX51, Tokyo, Japan), in Pathology department, Faculty of Medicine, Minia University.

Statistical analysis:
The collected data was coded, tabulated, and statistically analysed using SPSS (Statistical Package for Social Sciences) program software, version 25. Graphical Presentations were created by using Microsoft office Excel 365, version 2016.

For parametric (normally distributed) quantitative data, descriptive statistics were carried out by mean, Standard deviation (SD), minimum and maximum of range. Distribution of the data was performed by using Shapiro Wilk test.

Analyses were performed for quantitative data between the five groups by using One - Way ANOVA test which was preceded by Post Hoc Tukey's analysis between each two groups.
Analyses were carried out for quantitative data between different times in the same group by using Repeated Measures ANOVA test that was preceded by Post Hoc LSD analysis between each two times. Analyses were performed for quantitative data between two groups by using Independent Samples T test.
The level of significance was considered at P value ≤ 0.05.

Results

ANOVA test revealed significant statistical increased in levels of ALT, AST, urea, and creatinin in APAP group compared with the control group, and in (APAP & DEX) group compared with APAP group. NAC administrations induce significant reduced ALT, AST, urea, and creatinin levels in comparison between APAP group and (APAP & NAC) group as shown in table 1,2, 3, 4

Table (1): ALT level changes between the examined groups

	Group I Control	Group II APAP	Group III APAP+NAC	Group IV APAP + Dex	Group V APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
ALT (U/L) ***Range*** ***Mean ± SD***	(29.5-62.1) 44±9.2	(90.2-168.1) 128.9±25.8	(40.2-65) 51.8±8.2	(90.2-125) 107.8±12.9	(33.5-67.7) 48.4±9.9
Control (I)		**<0.001***	0.457	**<0.001***	0.879
Paracetamol (II)			**<0.001***	**<0.001***	**<0.001***
APAP +NAC (III)				**<0.001***	0.950
APAP + Dex (I V)					**<0.001***
APAP,NAC+Dex(V)	0.879	**<0.001***	0.950	**<0.001***	

Table (2): AST level changes between the examined groups

	Group I Control	Group II APAP	Group III APAP+NAC	Group IV APAP + Dex	Group V APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
AST (U/L) ***Range*** ***Mean ± SD***	(125.2-225.6) 156.8±28.1	(248.6-412.5) 351.6±62	(141.9-185.3) 164.9±13.2	(178.6-350.6) 275.9±59.7	(110.5-170.2) 138±19.4
Control (I)		**<0.001***	0.973	**<0.001***	0.615
Paracetamol (II)			**<0.001***	**<0.001***	**<0.001***
APAP+ NAC (III)				**<0.001***	0.258
APAP + Dex (IV)					**<0.001***
APAP,NAC+Dex(V)	0.615	**<0.001***	0.258	**<0.001***	

Table (3): Urea level changes between the examined groups

	Group (I) Control	Group (II) APAP	Group (III) APAP+NAC	Group (IV) APAP + Dex	Group (V) APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
Urea (mg/dl) ***Range*** ***Mean ± SD***	(30.5-57.5) 43.9±8.8	(50.9-72.1) 61.5±7.4	(33.1-54.8) 45.3±7	(37.9-81.5) 53.6±12.5	(34.2-54.2) 44.9±6.5
Control (I)		**<0.001***	0.989	**0.006***	0.996
Paracetamol (II)			**<0.001***	**0.043***	**<0.001***
APAP +NAC (III)				**0.024***	1
APAP + Dex(IV)					**0.018***
APAP,NAC+Dex(V)	0.996	**<0.001***	1	**0.018***	

Table (4): Creatinine level changes between the examined groups

	Group (I) Control	Group (II) APAP	Group (III) APAP+NAC	Group (IV) APAP+Dex	Group (V) APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
Creatinine(mg/dl) ***Range*** ***Mean ± SD***	(2-2.3) 2.1±0.1	(2.5-3.6) 3±0.4	(2-2.7) 2.3±0.3	(2.2-3) 2.6±0.3	(1.9-2.6) 2.2±0.3
Control (I)		**<0.001***	0.217	**<0.001***	0.617
Paracetamol (II)			**<0.001***	**<0.001***	**<0.001***
APAP + NAC (III)				**0.005***	0.954
APAP + Dex (IV)					**<0.001***
APAP,NAC+Dex(V)	0.617	**<0.001***	0.954	**<0.001***	

Table 5, 6 showed significant statistical reduced level of glutathione peroxidase and catalase enzyme in APAP group compared with all other groups, NAC intake induced a significant increased glutathione peroxidase and catalase level in (APAP & NAC) and (APAP, NAC & DEX) groups compared with APAP group, DEX administration could induce a significant increased GSH-Px level in (APAP & DEX) group and (APAP, NAC & DEX) group compared with APAP group, but it could induce significant statistical reduced catalase levels in (APAP & DEX) group compared with control group, (APAP & NAC) and (APAP, NAC & DEX) group.

Table (5): Glutathione peroxidase (GSH-Px) level changes between the examined groups

	Group (I) Control	Group (II) APAP	Group (III) APAP+NAC	Group (IV) APAP+Dex	Group (V) APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
GSH-Px (pg/mL) ***Range*** ***Mean ± SD***	(276.1-450.1) 341±59.4	(131.4-210.1) 175.8±24.8	(240.4-340.3) 287.2±26.9	(271.4-340.4) 299.7±26	(280.9-360.6) 315±25.4
Control (I)		**<0.001***	**<0.001***	**0.003***	0.142
Paracetamol (II)			**<0.001***	**<0.001***	**<0.001***
APAP + NAC (III)				0.793	0.100
APAP + Dex (IV)					0.648
APAP,NAC+Dex(V)	0.142	**<0.001***	0.100	0.648	

Table (6): Catalase level changes between the examined groups

	Group (I) Control	Group (II) APAP	Group (III) APAP+NAC	Group (IV) APAP+Dex	Group (V) APAP+NAC+Dex
	N=20	N=20	N=20	N=20	N=20
Catalase(KU/L) ***Range*** ***Mean ± SD***	(45.5-78.2) 62.9±9.4	(33.1-51.9) 40.7±6.2	(40.4-68.2) 58.6±8.3	(36.1-64.1) 49.9±11.3	(74.5-76.6) 60.4±10.5
Control (I)		***<0.001****	*0.591*	***<0.001****	*0.917*
Paracetamol (II)			***<0.001****	***0.021****	***<0.001****
APAP + NAC (III)				***0.032****	*0.972*
APAP + Dex (V)					***0.005****
APAP,NAC+Dex(V)	*0.917*	***<0.001****	*0.972*	***0.005****	

As regard Malondialdhyde levels, ANOVA test revealed a significant statistical increased level of MDA in APAP group compared with all other groups. DEX couldn't induce a statistically significant reduced MDA level in comparison between (APAP & DEX) group with groups (APAP & NAC) and (APAP, NAC & DEX) group. NAC administration induced a significant reduced MDA level in (APAP & NAC) and (APAP, NAC & DEX) group compared with APAP group as shown in table 7.

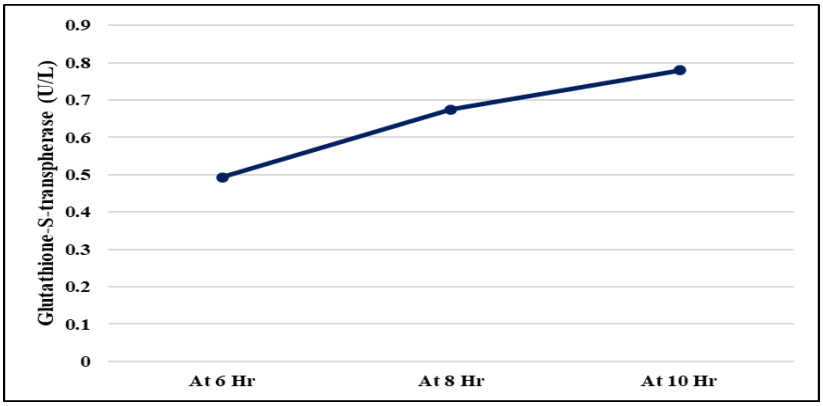
Table (7): Malondialdhyde (MDA) level changes between the examined groups

	Group (I) Control	**Group (II) APAP**	**Group (III) APAP+NAC**	**Group (IV) APAP+Dex**	**Group (V) APAP+NAC+Dex**
	N=20	**N=20**	**N=20**	**N=20**	**N=20**
MDA(µmol/L)					
Range	(0.24-0.29)	(0.42-0.71)	(0.26-0.37)	(0.4-0.45)	(0.3-0.4)
Mean ± SD	0.27±0.01	0.54±0.1	0.32±0.04	0.41±0.01	0.35±0.03
Control (I)		***<0.001****	***0.014****	***<0.001****	***<0.001****
Paracetamol (II)			***<0.001****	***<0.001****	***<0.001****
APAP + NAC (III)				***<0.001****	*0.287*
APAP + Dex (IV)					***0.002****
APAP,NAC+Dex(V)	***<0.001****	***<0.001****	*0.287*	***0.002****	

By measuring GSTA1 in APAP group after 6, 8, 10 hours, ANOVA test revealed significant statistical increased levels of glutathione S-transferase A1 at 8 and 10 hours in comparison to its level at 6 hours after drug intake (table 8) and (figure 1, 2). But a comparison between both ALT, AST and the GSTA1 levels in the APAP group at different time intervals (0 hr as control, 6, 8 and 10 hours) shown in table 9 revealed significant statistical increased ALT, AST and GSTA1 levels after 8 and 10 hours of APAP toxicity compared with the control group. APAP administration could induce a highly significant increased GSTA1 levels at 6 hours post drug intake compared with the control group. Meanwhile, both ALT and AST levels were not of statistical significant increased levels at 6 hours post drug intake compared with the control group.

Table (8):GSTA1 level changes at different time intervals after APAP administration in group (II)

APAP group	**At 6 hrs**	**At 8 hrs**	**At 10 hrs**
	N=20	**N=20**	**N=20**
GSTA1(U/L)			
Range	(0.45-0.53)	(0.62-0.71)	(0.73-0.82)
Mean ± SD	0.49±0.03	0.67±0.02	0.78±0.03
At 6 hrs		***<0.001****	***<0.001****
At 8 hrs			***<0.001****
At 10 hrs	***<0.001****	***<0.001****	



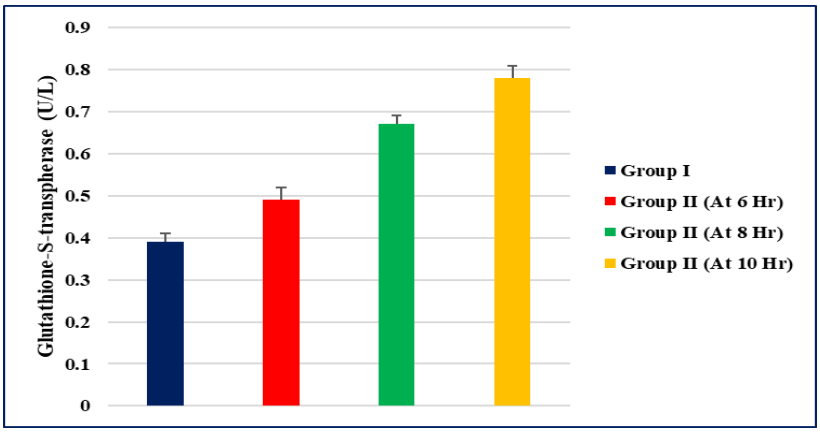


Figure (1, 2): Showing GSTA1 level changes at different time intervals after APAP administration in group (II)

Table (9): Showing ALT, AST and GSTA1 level changes at different time intervals after APAP administration in group (II)

	Control	APAP group			P value vs. 1oControl		
	At 0 hr	At 6 hrs	At 8 hrs	At 10 hrs			
	N=20	N=20	N=20	N=20	At 6h	At 8h	At 10h
ALT(U/L)							
Range	(29.5-62.1	(31.9-64.2)	(49-91.1)	(242.5-503.3)	*0.984*	***<0.001****	***<0.001****
Mean ± SD	44±9.2	44.1±9.3	67.2±15	373.4±77.3			
AST(U/L)							
Range	(125.2-225.6	(111-258.7)	(195.3-325.7)	(245.2-427.5)	*0.486*	***<0.001****	***<0.001****
Mean ± SD	156.8±28.1	164.8±42.5	254.1±47.1	365.5±59.9			
GSTA1(U/L)							
Range	(0.35-0.42)	(0.45-0.53)	(0.62-0.71)	(0.73-0.82)	***<0.001****	***<0.001****	***<0.001****
Mean ± SD	0.39±0.02	0.49±0.03	0.67±0.02	0.78±0.03			

Histological evaluation:

The study in brief revealed that, regarding to the effect of DEX administration on the histopathological results, it is shown that although there was a well observed histopathological improvement of the liver tissues obtained from rats receiving (APAP and DEX), there was no similar improvement in the histological scores of kidney tubular injury. This directs us to the consideration that there may be a different mechanism of renal injury still has to been found out.

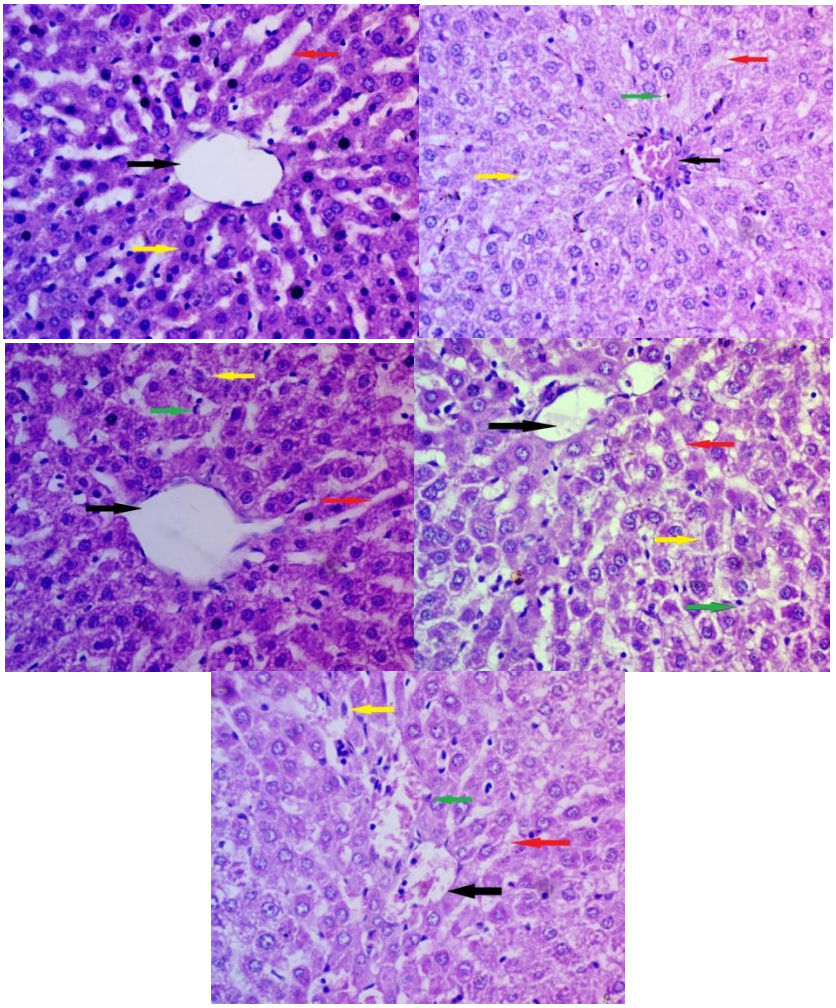


Fig. 3. (A) Hepatic histology of the Control group showing normal hepatocytes (yellow arrow), sinusoids (red arrow) and central vein (black arrow). (B) Group APAP showing enlarged hepatocytes with vacuolated and enlarged cytoplasm (yellow arrow) and small pyknotic nuclei (green arrow), congested central vein (black arrow) and congested sinusoids (red arrow). (C) Group NAC have a few hepatocytes with dark acidophilic cytoplasm (yellow arrow) and pyknotic nuclei (green arrow) and a little dilated sinusoids (red arrow) and mild congested central vein (black arrow). (D) Group DEX has most hepatocytes with dark stained acidophilic cytoplasm (yellow arrow) and pyknotic nuclei (green arrow). Some sinusoids are markedly dilated (red arrow) with congested central vein (black arrow). (E) Group NAC+DEX showing a few hepatocytes with dark acidophilic cytoplasm (yellow arrow) and pyknotic nuclei (green arrow) and little dilated sinusoids (red arrow) and central vein (black arrow).

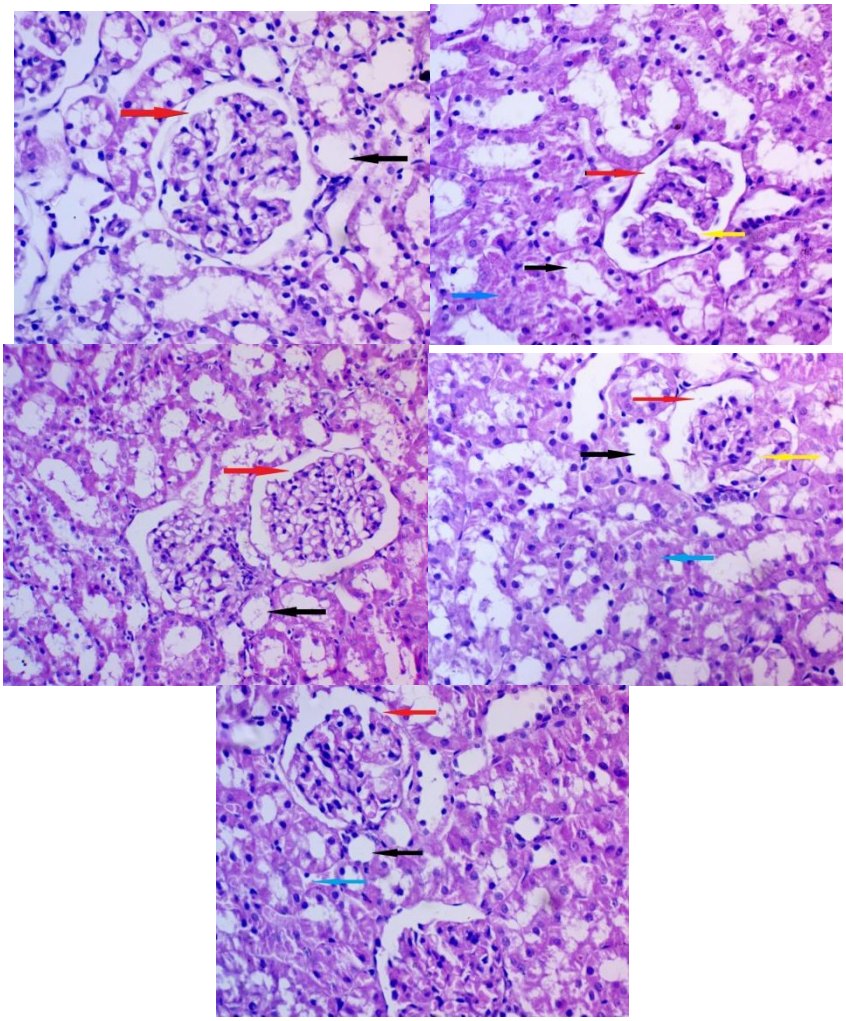


Fig. 4. (A) Control group showing normal glomeruli (red arrow) with normal surrounding tubules (black arrow). (B) Group APAP; dilated tubules (black arrow), sclerotic glomeruli (red arrow), marked loss of glomerular capillaries (yellow arrow) and massive accumulation of extracellular matrix in glomeruli (blue arrow). (C) Group NAC; showing minimal glomerular sclerosis (red arrow) and mild tubular dilatation (black arrow). (D) Group DEX; showing dilated tubules (black arrow), sclerotic glomeruli (red arrow), loss of glomerular capillaries (yellow arrow) and accumulation of extracellular matrix in glomeruli (blue arrow). (E) Group NAC+DEX; minimal glomerular sclerosis (red arrow) and mild tubular dilatation (black arrow) with minimal extracellular matrix accumulation (blue arrow).

Discussion

Acetaminophen is one of the most commonly utilized analgesic and antipyretic medication world - wide that has a wide safety profile at the recommended therapeutic dosing. However, it may cause hepatotoxicity and nephrotoxicity after overdosing either by unintentional ingestion or intentional misuse in both humans and animals (Lee, 2012 and Yoon et al., 2016).

Acetaminophen induced hepatotoxicity occurs as a result of saturation of sulfate and glucuronide pathways, and depletion of the endogenous glutathione stores that lead to accumulation of excess amount of NAPQI that binds covalently to cellular biological macromolecules such as proteins, lipids and nucleic acids causing increased oxidative stress, structural, metabolic and enzymatic systems dysfunctions and mitochondrial dysfunction resulting into necrotic cell death (Hinson et al., 2010 and Foufelle & Fromenty, 2016).

Recent researches documented a pathogenic role of mitochondrial dysfunction occurring due to excess NAPQI production that binds to

mitochondrial proteins results in cytotoxic protein adducts that cause cellular necrosis. Mitochondrial dysfunction also leads to ATP depletion, overproduction of reactive oxygen species, increased lipid peroxidation and oxidetive stress which may lead to reduced filtration across renal glomeruli resulting into retention of urea and creatinine in the body, renal tubular damage and acute renal failure in both human and animals (Hua et al., 2018 and Iqbal et al., 2018).

The diagnosis of acetaminophen toxicity is commonly based on history of acetaminophen ingestion and the serum APAP level in addition to the commonly used biochemical markers of hepatic injury such as serum ALT and AST which unfortunately peak within 24-48 h post ingestion while a severe liver injury has been already done, so there is a great necessity for new diagnostic, more sensitive and specific markers that can early detect the damaging process (Antoine et al., 2013 and Camilleri, 2015).

Glutathione S-transferase A1 (GSTA1) is a substantial enzyme for detoxification and cytoprotection; as being one of the phase II drug metabolizing enzymes, it catalyzes the conjugation of reduced glutathione to the phase I modified compounds including drugs and oxidative stress products to make them more non-toxic to be excreted safely. Also GSTA1 can exhibit glutathione peroxidase activity that protects the cells from the damaging effects of oxidative stress and products of lipid peroxidation (Ma et al., 2017 and Allocati et al., 2018).

Dexmedetomidine (Dex) is known to be a highly selective α2 adrenergic receptor agonist with sedative, antioxidant and anti-inflammatory effect, it can reduce the inflammatory and oxidative responses in tissues thus reduces their histological damage. In addition, it causes a significant increase in the antioxidant enzymes as glutathione peroxidases, catalases and superoxide dismutases (Zhou et al., 2018).

In accordance with Mahesh et al., (2009) and Ozer et al., (2008) study which showed a significant increase in serum ALT and AST levels in rats exposed to APAP toxicity. They explained the increase in liver enzymes by their liberation from the hepatocytes as when liver cells are damaged or necrosis occurred, the hepatocellular membrane integrity is destroyed and the hepatocellular contents are released extracellularly into serum.

NAC administration in the current study results showed statistical significant reduced liver enzyme levels in groups received the antioxidant; (APAP and NAC) and (APAP, NAC and DEX), coinciding with a study performed by Alipour et al., (2013).

DEX administration in the current study showed significant improvement in liver enzyme levels in (APAP and DEX) group as serum ALT and AST showed significant reduced levels in DEX treated group compared with APAP group, coinciding with Şen et al., (2014).

The current results were coinciding with Firozian et al., (2020), where rodents were challenged with an oral dose of APAP (2g/kg), then administered NAC and NAC-loaded niosome 4 hrs later then sacrificed after 48 hrs. Their microscopic features of APAP induced hepatotoxicity were significantly improved in tissues of rats received NAC and NAC-loaded niosome.

The study of Li et al., (2017) wasn't coinciding with the current study as it approved that ALT and AST levels showed significant increase at 10 hrs after APAP overdose but GSTA1 level showed significant increase 2 hrs before; it means that the increase of GSTA1 level can be detected earlier than the ALT and AST levels. Besides, ALT and AST levels increased significantly at the APAP concentration higher than that of GSTA1 content increased at. The study clarified the sensitivity and specificity of GSTA1 by being abundant in liver with high concentrations in the centrilobular area which is the most liable area for toxic changes, unlike ALT and AST.

In the current study, NAC administration resulted into significant reduced urea and createnine levels in groups received the antioxidant; (APAP and NAC) and (APAP, NAC and DEX) in a matter coinciding with a study performed by Ucar et al., (2013).

In accordance with Shin et al., (2016) study, the microscopic picture of kidney tissues obtained

from APAP treated rats showed increased casts, tubular dilation, necrosis, and degeneration.

Our results revealed that there was a significant increase in MDA levels in the paracetamol administered group and significant decrease in the antioxidant enzymes including SOD, CAT, and GSH-Px, coinciding with Kuvandik et al., (2008)

The recent study findings as regard MDA, GSH-Px and catalase levels in DEX administered group were according to Tas et al., (2019) study, who found that administration of DEX for APAP toxicity was identified to cause a significant increase in GSH-Px and catalase enzyme, and cause a significant decrease in MDA levels.

In accordance with the study of Firozian et al., (2020), where rats were challenged with an oral dose of APAP (2g/kg), followed by administration of NAC and NAC-loaded niosome 4 hrs later, rats were sacrificed after 48 hrs. The findings revealed significant reduced levels of GSH, GSH-Px, SOD and CAT activity and significant increase in MDA levels in rats received APAP, while significant reversal of these levels occurred following the NAC therapy.

These results clarify the role of GSTA1 as an early indicator and predictor of acute liver injury after APAP intoxication

Conclusion

GSTA1 is an early indicator and predictor of acute liver injury after APAP intoxication

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