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Assessment of the Effects of Lacteal Forte® and Vitamin B12 versus N-acetylcysteine in the Treatment of Acetaminophen-induced Hepatorenal Toxicity

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Abstract

Acetaminophen (APAP) is one of the most frequently used analgesics and antipyretics worldwide, however, an overdose of paracetamol is a leading cause of hepato-renal injury. N-acetylcysteine (NAC) has been used as a remedy for paracetamol-induced toxicity, however several adverse effects of long-term use of NAC have been reported. Therefore, we assessed the ameliorating effect of lacteol forte® and vitamin B12 alone or in combination against APAP-induced hepatorenal injury in rats and compare it with that of NAC. Fife groups of rats were given APAP (400 mg/kg BW, i.p.), while the control group was treated only with 0.9% saline. Treatment with NAC, lacteal forte®, and vitamin B12 was done 24hrs after APAP. Serum biochemical parameters related to liver and kidney injury, including, AST, ALT, HDL, urea, and creatinine were estimated in all groups, hepatic and renal lipid peroxidation, and antioxidant biomarker levels were also determined. The expression of the tumor necrosis factor (TNF- α), TNFR-1, COX-1 and COX-2 genes was investigated. Finally, sections of the liver and kidney of all rats were examined for any histopathological changes. Results: Compared to control rats, APAP induced acute hepatic and renal toxicity manifested by significant increases in serum biochemical parameters related to liver and kidney injuries. Also, APAP significantly increased hepatic, and renal lipid peroxidation and decreased antioxidant biomarker levels. APAP upregulated the expression of TNF-α and TNFR-1 genes, while it downregulated the expression of COX-1 and COX-2 genes. It also produced marked histopathological lesions of liver and kidney. The test treatment with lacteal forte and vitamin B12 combination had improved serum parameters and gene expression, enhanced endogenous antioxidant status, reduced lipid peroxidation, and histopathological lesions in a comparable fashion to that of NAC effects. Conclusions: Our data confirmed that treatment with vitamin B12 and lacteal forte® combination has exerted potent antioxidant and free radical-scavenging activities comparable to that NAC. Suggesting that combination of vitamin B1 and lacteal forte® may be considered a safer alternative to NAC in ameliorating APAP induced toxicities. More research will be required to estimate the level of toxic metabolite, NAPQI, to determine the actual mode of action of these medications in protecting against APAP toxicity.

Keywords: N-acetylcysteine, Lactobacillus LB, Vitamin B12, acetaminophen, rats.

1. Introduction

Paracetamol (acetaminophen, N-acetyl-p-aminophenol [APAP]) is described as an analgesic (pain reliever) and antipyretic (fever reducer). The toxicity of paracetamol is due to its metabolite, N-acetyl-p-benzoquinoneimine (NAPQ1) [1]. In overdoses, larger amounts of paracetamol are metabolized and consequently liver and kidney diseases will be developed [2].

Liver damage is the most noticeable marker in paracetamol overdose. Acute overdoses cause potentially acute hepatic injury [3]. liver damage caused by paracetamol overdose is more common than renal damage [4]. Studying the effect of protective drugs is crucial to avoid its potential hazards on the liver and kidney.

N-acetylcysteine (NAC) has been used for several decades as the antidote of choice in treating paracetamol-induced hepatotoxicity [5]. It possesses an antioxidant effect in many tissues [6]. However, this drug could induce many adverse effects, for instance, allergy, anaphylaxis, angioedema, and

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bronchospasm [7]. Recently, there has been a great interest to discover novel agents that may have a potential effect in treating paracetamol-induced toxicity with less hazard [8].

Vitamin supplementation is known to have substantial benefits in the prevention of many diseases. B vitamins have been investigated in a variety of experimental and clinical models of oxidative stress. Vitamin B12, cyanocobalamin, has neurotronic effect [9] and is well known in hematopoiesis function [10], boosts immune function, and has a beneficial role in the treatment of atherosclerosis and other cardiac diseases, also, it lowers homocysteine levels [11].

Probiotic bacteria are well known to have an antioxidant and anti-inflammatory effect [12], for instance, Lactobacilli exhibits detoxifying properties [13] and neutralizes toxins in humans and animals [14]. Moreover, Arpita et al [15] evoked that the probiotic L. plantarum AD3 strain exhibits a great antiuremic effect in APAP-induced toxicity through modification of intestinal flora to refrain generation of toxins [16].

To the best of our knowledge, no published data are available about the possible ameliorating effect of lacteol forte® and vitamin B12 alone or in combination vs NAC against APAP-induced hepatorenal injury in rats. Rats are considered a reflective model for human developmental risk assessment. Therefore, the purpose of the present study was to evaluate the potential effect of these drugs against APAP-induced toxicity through molecular, biochemical, and histopathological examination.

2. Materials And Methods

2.1. Drugs:

Paracetamol® was obtained from Sigma Company (Sigma, St. Louis, Mo, USA) in a liquid preparation as a 1 mg/ml sterile concentrate. Acetylcysteine® was obtained from SEDICO Company, ARE in 200 mg/sachets. Vitamin B12 (Depovit B12®) (AMRIYA Pharmaceuticals Company4, Egypt) as 1 ml ampoules of hydrocoblamin, 1000 µ/ml. Lacteol fort® was purchased as sachets each sachet contains 200 mg of Lactobacillus LB. corresponding to Lactobacillus delbruekii and Lactobacillus fermentum each contains 5 billion colony forming unit of probiotic CFU, manufactured by Tenth of Ramadan for Pharmaceutical Diagnostic Reagents (Rameda), 6th of October, under License of APTALIS PHARMA SAS-France.

2.2. Kits for biochemical analysis

Prepared serum samples were examined for AST, ALT, urea, creatinine, LDH, total protein, and albumin with a semi-automatic spectrophotometer (BM-Germany, 5010) using commercial test kits (Randox Co. UK and Bio-diagnostic, Egypt) according to enclosed pamphlets. MDA, SOD, and NO concentrations in hepatic and renal tissue homogenates were analyzed using commercial kits (Bio-diagnostic Co, Egypt).

2.3. Experimental animals

Sixty healthy male albino rats weighing 200 to 220 g were used in this study. Animals were purchased from the animal house in Helwan and kept in a well-ventilated room under standard laboratory conditions in the Department of Pharmacology, Faculty of Veterinary medicine, Mansoura University. Animals were acclimatized for ten days, then randomly assigned to six equal groups as illustrated in Table, (1).

Ethics Statement: This study was conducted following the requirements set out in the Ethical Committee of the National Research Center, Egypt. Registration number (09/189). Animals were treated under the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

2.4. Sample collection

At the end of the experiment, all rats were bled from the retro-orbital plexus after light anesthesia using ether. Blood was centrifuged at 3000 rpm for 15 min. Serum was separated and frozen at −80 °C until use.

After blood collection, six rats of each group were sacrificed by decapitation. Tissue samples from the liver and kidney were collected. Part of each tissue sample was used to prepare tissue homogenates (10% w/v) in PBS for the determination of SOD, MDA, and NO levels. The other part was excised and fixed in 10% neutral buffered formalin for histopathological examination.

2.5. Biochemical analysis of serum

levels of AST and ALT were estimated according to Reitman and Frankel [21]. Total protein and albumin levels were determined colorimetrically [22]. LDH activity was measured kinetically using a commercial kit (Egyptian Company for Biotechnology) according to Young and Friedman [23]. Urea and creatinine levels were measured with enzymatic colorimetric methods respectively [24, 25].

2.6. Assessment of oxidative stress markers in liver and kidney homogenates

SOD and MDA Levels in liver and kidney homogenates were assayed spectrophotometrically as described by Ohkawa et al [26]. The activity of NO was measured colorimetrically as described by Montgomery and Dymock [27].

2.7. Reverse transcription and quantitative real-time PCR analysis.

Liver and kidney total RNA was extracted from samples using the RNeasy Mini Kit (Qiagen, Heidelberg, Germany). The concentration and purity of extracted RNA were assessed with a NanoDrop® ND-1000 spectrophotometer. The c-DNA synthesis used a high-capacity cDNA reverse transcription kit (Applied Biosystems) following the manufacturer's instructions. Quantitative analysis of the transcriptional level of target genes was carried out by a Rotor-Gene Q cycler (Qiagen, Heidelberg, Germany) with QuantiTect SYBR Green PCR kits (Qiagen, Heidelberg, Germany). Relative expression of mRNA level was assessed for each gene as described previously [29]. The sequence of the used primers was illustrated in (Table 2)

RT-PCR data were analyzed using the method of relative gene expression as described in Applied Biosystems User Bulletin No. 2. Gene expression data are presented as fold change that are normalized to the endogenous reference β-actin gene using the 2−DDct method according to Livak and Schmittgen [30].

2.8. Histopathological examination

Formalin-fixed tissue specimens were prepared for histopathological examination according to the method of Mohamed and Laurence [31]. Histopathological lesions in liver and kidney were assessed and graded semi-quantitatively: no change - 0 (no distinguishable change, 0%); mild change - 1 (initiation of changes, up to 30%); moderate change - 2 (patent changes, 31-60%); severe change - 3 (widespread changes, 61-100%).

2.9. Statistical analysis

One-way analysis of variance (ANOVA). Data were analyzed using statistical SPSS (version 20, USA). Differences between groups' means ±standard error of mean (±SEM) were compared using Duncan's multiple range test. Differences were considered significant if P-value for the effect was < 0.05. Histopathological scores were estimated using Kruskal-Wallis tests followed by Dunn's method to compare means.

3. Results

3.1. Markers of liver and kidney function:

AST and ALT levels in APAP-treated rats (group II) were significantly higher than those of control rats. NAC significantly regulates the increased activity of both two enzymes (group III) followed by lacteal Forte® and vitamin B12 combination (group VI). However, non-significant changes between vitamin B12 (group IV) and Lacteal Fort® (group V) in treated rats (Table 3).

Serum urea and creatinine levels showed marked changes in APAP-treated rats (group II) compared to the other groups ($p < 0.05$). Moreover, rats treated with NAC (group III) were better than other treated groups in restoring normal serum concentrations of urea and creatinine followed by lacteal forte® and vitamin B12 combination (group VI) which is more effective than each drug alone (Table 3).

3.2. LDH and protein profile findings

Results showed significant elevation ($p \leq 0.05$) of lactate dehydrogenase (LDH) in the circulating blood of APAP intoxicated rats compared to control animals. While there was a significant decrease in LDH level in NAC treated group (group III), followed by combined vitamin B12 and Lacteal Fort® treated rats (group VI). However, non-significant changes between vitamin B12 (group IV) and Lacteal Fort® (group V) in treated rats. Furthermore, levels of total protein and albumin significantly declined in APAP-treated rats. Levels of total protein were significantly higher in all treated groups compared with the APAP group (group II). The highest total protein levels were observed in rats in NAC treated rats (group III), followed by combined vitamin B12 and Lacteal Fort® treated rats (group VI). However, non-significant changes between vitamin B12 (group IV) and Lacteal Fort® (group V) in treated rats (Table 4).

3.3. Oxidative stress markers

Concentrations of MDA were significantly raised in liver and kidney homogenates in APAP-treated rats (group II) compared to the control rats. MDA levels were significantly decreased in all treated groups to be the lowest in group given NAC (group III) followed by lacteol forte® and vitamin B12 combination (group VI) (Table 5).

Levels of SOD were significantly decreased in APAP-treated rats (group II) compared to the control. These levels were significantly increased in all treated groups; however, the greatest response was observed in the group administered NAC (group III) followed by rats given lacteal Forte® and vitamin B12 combination (group VI) (Table 5).

NO levels were significantly increased in kidney and liver homogenates in APAP-treated rats (group II) comparing the control group. NO levels were lower in all treated groups. The greatest response was observed in (group III) followed by (group VI) (Table 5).

3.4. Gene expression findings

Analysis of gene expression in the kidney tissues revealed that mean mRNA expression of the TNF-α gene was significantly upregulated in APAP-treated rats (group II). However, the were no significant changes in expression between groups IV and V. Meanwhile, the expression was significantly downregulated in groups III and VI when compared to group I. (Fig. 1A).

Table 1: Experimental groups:

Group	Treatment / Dose	References
Group I	A single dose of 0.9% saline (i.p.)	-
Group II	A single i.p. injection of paracetamol (400 mg/kg body weight)	**17**
Group III	A single i.p. injection of paracetamol (400 mg/kg body weight) then after 24hrs, N-acetyl cysteine was given orally to each rat at a dose 1g/kg body weight daily for 2 weeks	**18**
Group IV	A single i.p. injection of paracetamol (400 mg/kg body weight) then after 24hrs rats lacteal forte® was orally given 5 billion CFU /kg body weight daily for 2 weeks	**19**
Group V	A single i.p. injection of paracetamol (400 mg/kg body weight) then after 24hrs, vitamin B12 was given by i.p. injection (15g/kg) twice a week for 2 weeks.	**20**
Group VI	A single i.p. injection of paracetamol (400 mg/kg body weight) then after 24hrs, lacteal forte®+ vitamin B12 were given by their doses as mentioned before.	

Table 2: Primer sequences used for RT-PCR

Gene	Forward (5--3)	Reverse (5--3)	Accession number	Reference
COX-1	CATCCATCTACTCCCAGAGTCATGAG	GAGGGCTGGGGATAAGGTTGGACCGC	S67721.1	**28**
COX-2	GCTGTGCTGCTCTGCGCTTGCCCTGGC	GATCTGGACGTCAACACGTATCTCATG	S67722.2	
TNF-α	GCATGATCCGCGACGTGGAA	AGATCCATGCCGTTGGCCAG	NM_012675.3	**29**
TNFR1	CCGGGCCACCTGGTCCG	CAAGTAGGTTCCTTTGTG	NM_013091.2	
Nephrin	CGGAGAAGACTGAGGCGCCTT	TCACACCAGATGTCCCCTCAG	NM_022628.1	
Desmin	TCAAGGGCACCAACGACT	GGTCTGGATCGGAAGGTTGAT	NM_022531.2	
β-actin	TCCTCCTGAGCGCAAGTACTCT	GCTCAGTAACAGTCCGCCTAGAA	NM_031144.3	**30**

Table 3: Serum levels of LDH, total protein and albumin (Mean ±SE).

Parameters	Groups					
	Group (I)	**Group (II)**	**Group (III)**	**Group (IV)**	**Group (V)**	**Group (VI)**
LDH (U/L)	84.31±5.1^{e}	439.33± 40.0^{a}	109± 3.5^{d}	200.15± 5.1^{b}	218.00± 79.5^{b}	144.07± 12.2^{c}
Total protein (g/dl)	6.49±0.86^{a}	0.69±0.19^{e}	4.37±0.47^{b}	3.08±0.81^{d}	3.50±0.50^{d}	5.24±0.61^{c}
Albumin(g/dl)	4.76 ± 1.05^{a}	0.02 ± 0.19^{e}	3.67±1.03^{b}	1.84 ± 0.58^{d}	1.78 ± 1.41^{d}	2.51 ± 0.56^{c}

Values represents Mean ±SEM. Values with the different letters in the same column are significantly different at (P< 0.05).

Table (4): Serum levels of AST, ALT, Urea and Creatinine

Parameters	Groups					
	Group (**I**)	Group (**II**)	Group (**III**)	Group (**IV**)	Group (**V**)	Group (**VI**)
AST (U/L)	11.63±0.7^{e}	137.38± 14.2^{a}	14.70± 1.5^{d}	88.61± 25.5^{b}	96.90± 34.4^{b}	61.89± 12.43^{c}
ALT (U/L)	14.40±1.5^{e}	44.69±0.19^{a}	23.97 ±0.97^{d}	35.73±1.85^{b}	33.00 ±19.0^{b}	25.30±18.05^{c}
Urea (mg/dl)	35.93±4.9^{e}	99.97 ± 9.96^{a}	39.17±4.510^{d}	49.03±10.8^{b}	47.69± 5.0^{b}	41.42±12.64^{c}
Creatinine (mg/dl)	0.59± 0.19^{e}	1.93± 0.96^{a}	0.65±0.05^{d}	1.16±0.38^{b}	1.13± 0.47^{b}	1.730± 0.24^{c}

Values represents Mean ± SEM. Values with the different letters in the same column are significantly different at (P< 0.05).

Table (5): Levels of MDA, SOD and NO in liver and kidney homogenates

Group	MDA(K) nmol/g	SOD(K) U/g	NO (K) (µmol/g)	MDA(L) nmol/g	SOD(L) U/g	NO (L) (µmol/g)
Group I	12.16±0.80^{e}	41.20±1.22^{a}	34.16±0.24^{e}	34.23± 1.13^{e}	3.93±0.11^{a}	45.06±0.04^{e}
Group II	45.49±1.34^{a}	10.53± 1.25^{e}	86.13±0.30^{a}	59.20± 0.47^{a}	1.70± 0.27^{e}	91.61±0.13^{a}
Group III	16.53±0.37^{d}	39.41± 0.98^{b}	34.10±0.52^{d}	36.83±0.09^{d}	3.67±0.56^{b}	52.34±0.02^{d}
Group IV	25.70±0.39^{b}	24.22±1.02^{d}	55.03±0.37b	44.46± 0.79^{b}	2.03±0.46^{d}	75.23±0.17^{b}
Group V	24.80±0.96^{b}	25.66 ±1.36^{d}	52.53±0.48^{b}	46.34±0.66^{b}	2.36±1.10^{d}	73.52±0.18^{b}
Group VI	19.96± 1.02^{c}	33.50± 0.33^{c}	40.50±0.63^{c}	40.26± 0.69^{c}	3.01±0.70^{c}	67.50±0.29^{c}

- L: Liver & K: Kidney, Values are Mean ± SEM. Values with the different capital letters in the same column are significantly different at (P< 0.05).

However, the expression of the TNFR-1 gene was significantly upregulated in group II when compared to group I, followed by groups IV and V. However, there were no significant changes in the expression between groups III and VI and they showed the lowest expression compared to the control group (group I) (Fig. 1B).

The fold change of desmin gene expression was significantly ($P < 0.063$) elevated in APAP-treated rats (group II) when compared to control rats (group I). Two weeks post-treatment, the fold change of desmin expression was significantly decreased in all treated groups with the least expression in groups III and VI. (Fig. 1C).

Consequently, the expression of the nephrin gene in kidney tissue was significantly ($P<0.046$) upregulated in group II. After two weeks of treatment, the fold change of the nephrin gene expression was significantly decreased in all treated groups to various degrees with the least expression observed in groups III and VI. (Fig. 1D).

Analysis of gene expression in the liver tissues revealed that the mean mRNA expressions of COX-1 and COX-2 genes showed significant downregulation in group II, meanwhile, it was upregulated in all treated groups to be the highest in groups III and VI followed by groups IV and V with non-significant changes between them (Fig. 2. A, B).

3.5. Histopathological findings

Livers from control animals showed normal hepatic cords radially arranged around central veins, portal areas, and hepatic sinusoids (Fig. 3A). Acute liver injury was marked by hepatocyte degeneration, congestion, and portal inflammation degeneration was observed in rats of APAP treated group (Fig. 3B). Mild hepatocyte degeneration was observed in livers of NAC treated rats (group III) (Fig. 3C). Mild to moderate hepatocyte degeneration, congestion and portal inflammation were present in lacteal Forte® treated group (Fig. 3D&E) and vitamin B12 treated group (Fig. 3F&G). Mild hepatocyte degeneration was observed in rats given lacteal Forte® and vitamin B12 combination (Fig. 3H). Qualitative analysis of hepatic histopathological scores indicated substantial efficacy of NAC in the treatment of hepatic injury induced by APAP (Fig. 3I).

Kidneys from control rats showed normal renal corpuscles, proximal and distal convoluted tubules, and interstitial tissues (Fig. 4A). Acute kidney injury was characterized by glomerular shrinkage and degeneration, tubular dilatation, tubular epithelial degeneration, vacuolization, prominent desquamation of the lining epithelium and interstitial leukocytic cell infiltration was observed in the kidney of APAP treated group (Fig. 4B-D). Renal tissue displayed normal histology in NAC-treated rats (group III) (Fig. 4E).

Moderate histopathological lesions were recorded in Lacteal Forte® treated rats (group IV) (Fig. 4F) and B12 (group V) including tubular dilatation with cast formation (Fig. 4G). Mild histopathology was observed in the kidneys of (group VI) including tubular dilatation with cast formation (Fig. 4H). Qualitative analysis of renal histopathological scores indicated substantial efficacy of NAC treatment in APAP-induced renal injury (Fig. 4I).

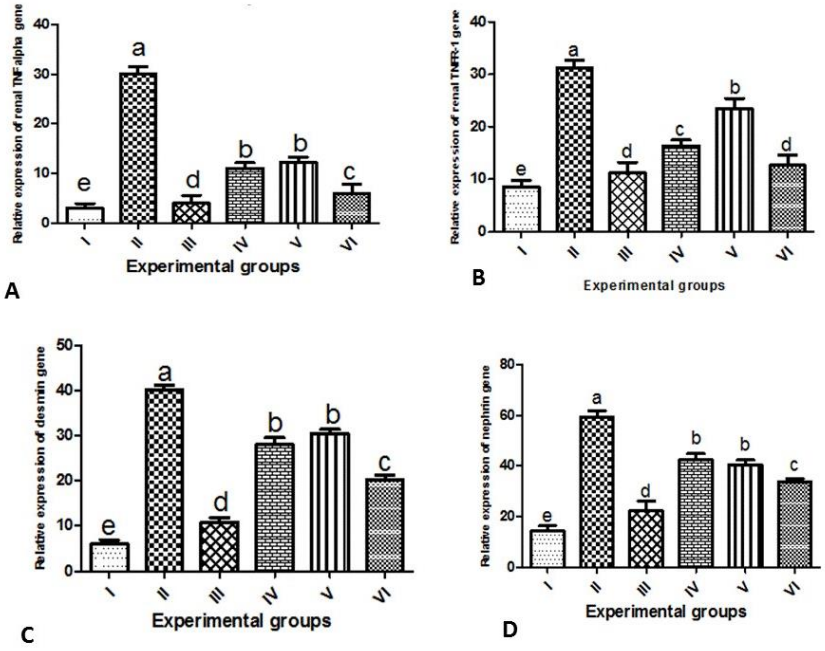

Figure. 1: Diagram showing fold changes of TNF-α (**A**), TNFR-1 (**B**), desmin (**C**) and nephrin (**D**) expression levels in kidneys

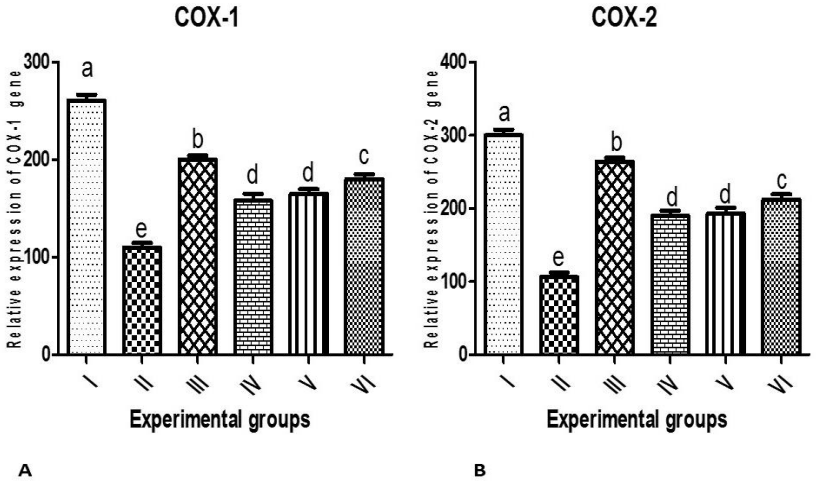

Figure. 2: Diagram showing fold changes of COX-1 (**A**) and COX-2 (**B**) expression levels in liver. Values are Mean ± SEM

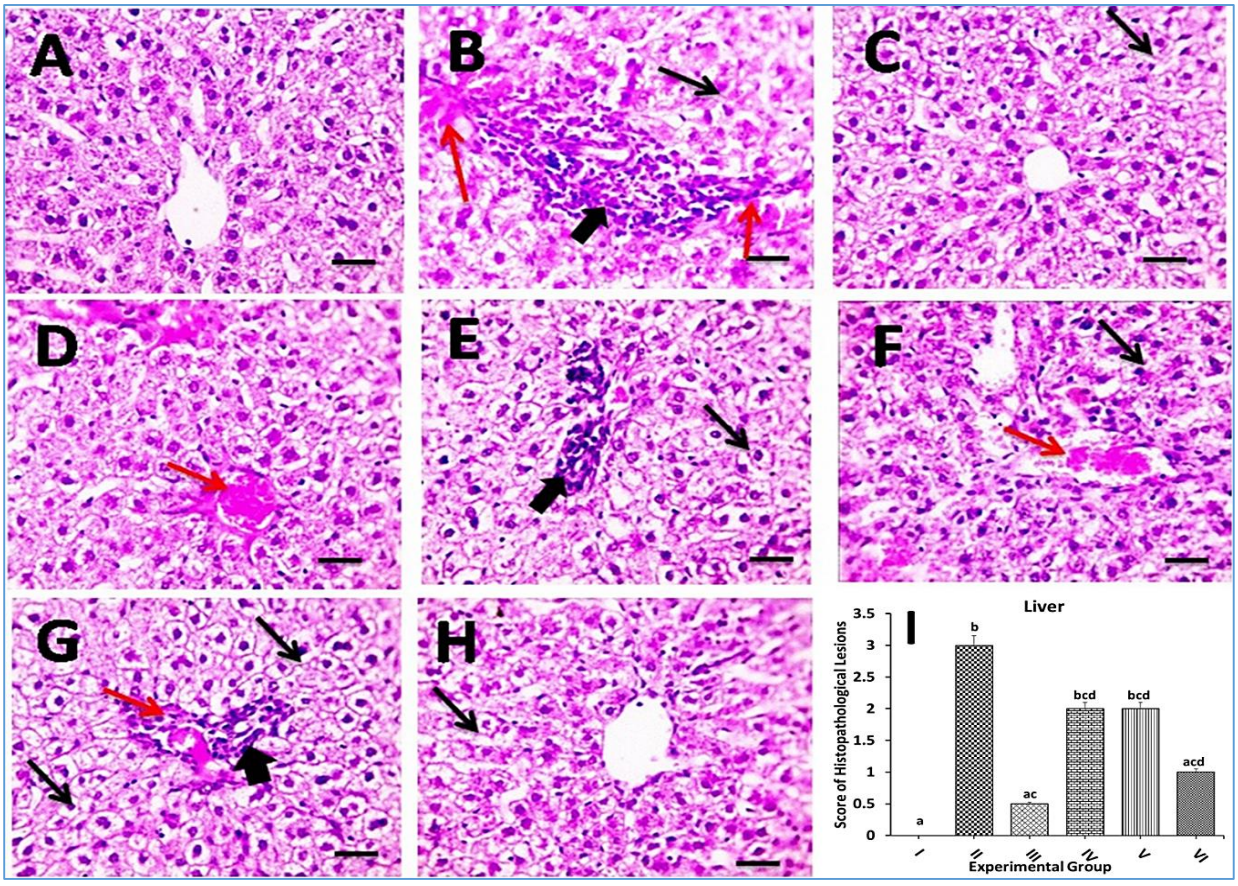

Figure. 3: Microscopic representations of H&E-stained liver sections in control group showing normal hepatic cords radially arranged around central veins, portal areas and hepatic sinusoids (**A**), marked hepatocytes degeneration, congestion, portal inflammation in II group(**B**), mild hepatocytes degeneration in group III (**C**), mild hepatocytes degeneration, congestion, and portal inflammation in groups IV (**D&E**) and V (**F&G**), mild hepatocytes degeneration in group VI (**H**). Statistical analysis shows decreased hepatic histopathological scores (mean ± SEM) (**I**). Hepatocytes degeneration (thin black arrow), congestion (red arrows) and portal inflammation (thick black arrow). X:400 bar 50

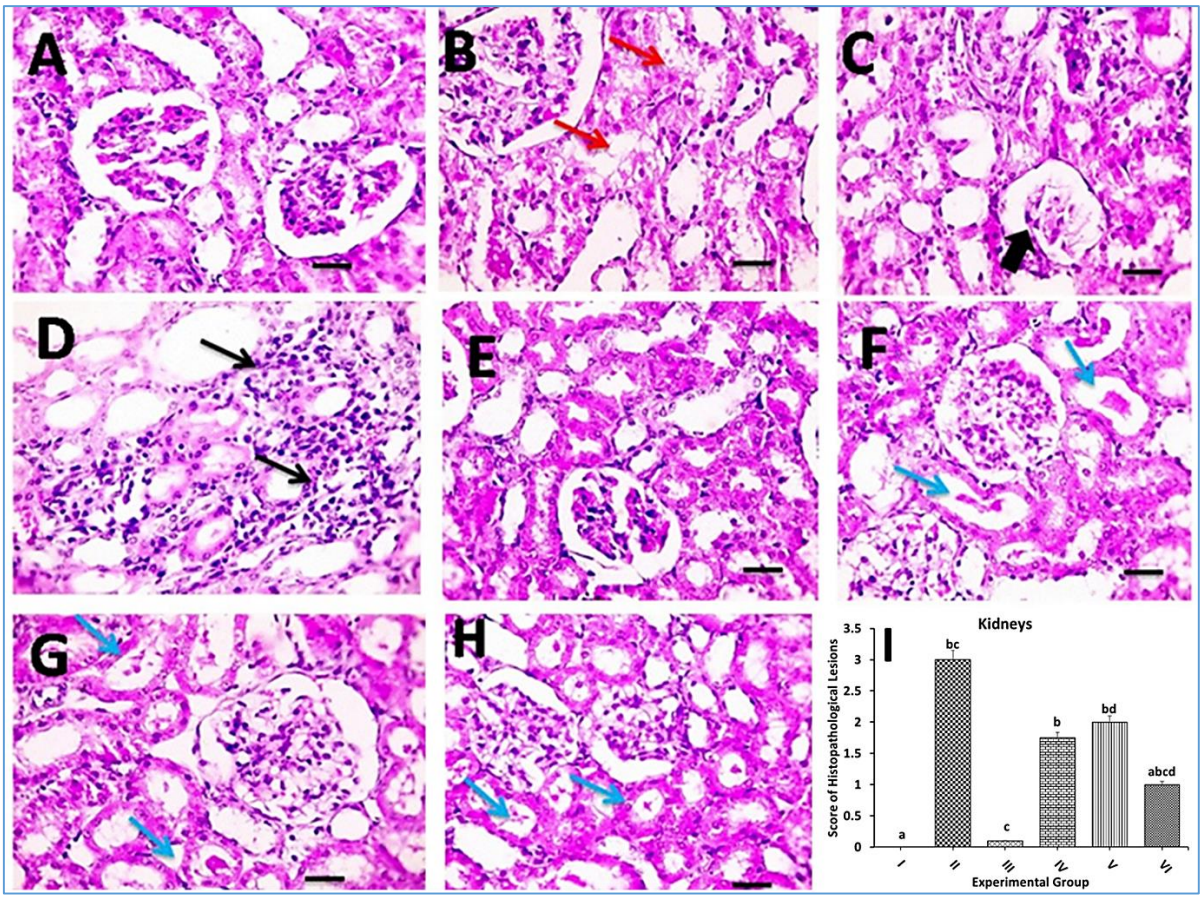

Figure. 4: Microscopicpictures of H&E stained kidney sections in the control group showing normal renal corpuscles, proximal& distal convoluted tubules and interstitial tissues (**A**), tubular dilatation, tubular epithelial degeneration, vacuolization (**B**), glomerular shrinkage (**C**), interstitial leukocytic cells infiltration (**D**), normal tubules and glomeruli in group III (**E**), moderate tubular dilatation with cast formation in groups IV (**F**) and V (**G**), mild tubular dilatation with cast formation in group VI (**H**). Statistical analysis shows decreased renal histopathological scores (mean ± SEM) (**I**). Interstitial inflammation (thin black arrow), tubular degeneration (red arrows) and glomerular shrinkage (thick black arrow). X:400 bar 50

4. Discussion

APAP overdose was first recognized as a cause of acute liver failure which is predisposition to death [32, 33].

In the last decades, NAC is used as the treatment of choice for APAP intoxication [34]. NAC has long been recognized as an effective APAP-antidote, minimizing the risk of acute liver injury in APAP intoxication. Despite this, there is a possibility of occurrence of adverse effects after NAC administration [7].

In our study, quantitative evaluation of APAP induced structural and functional alterations in the livers and kidneys were performed by biochemical, gene, and histopathological analyses to determine potential beneficial effects of Lacteal fort and vitamin B12 alone or in combination vs NAC on APAP-induced hepatorenal damage.

It has been reported that hypoproteinemia is a feature of liver damage due to a significant reduction in protein synthesis, as well as serum albumin concentration decreased in liver diseases [35]. Our findings demonstrated a significant decline in total protein and albumin in APAP-treated rats compared to the non-treated rats. This may be attributed to damage to hepatocytes; due to the arylation of proteins by NAPQI, a highly reactive intermediate metabolite produced during APAP overdose [36]. NAPQI depletes 70% glutathione resulting in oxidative stress. Moreover, it causes lipid peroxidation in addition to hepatic necrosis [37]. These results were inconsistence with those of Abdulkhaleq et al. [38] who declared a significant decline in serum albumin levels in rats who received APAPA at a dose of 2,800 mg/kg. Our finding revealed that NAC significantly increased serum total protein and albumin levels indicating the ability of NAC to attenuate liver damage caused by paracetamol overdose [39].

LDH is an intracellular enzyme localized in the cytoplasm and is released when cells are damaged. The high LDH level in serum of APAP intoxicated

animals is an indicator of liver damage [40]. Our results showed a significant elevation of serum LDH levels in APAP-treated rats. This may be attributed to the leak of LDH from the cytoplasm of injured hepatic and renal cells into the blood [38].

Additionally, liver enzymes, ALT, and AST, remain the major signal to assess any liver damage and are extensively considered as the biomarkers of choice [41]. In the current study, both ALT and AST were significantly increased in the APAP group compared to control rats indicating liver injury. While treatment of rats with NAC significantly regulates the increase of both ALT and AST serum levels. These results were inconsistent with those of Aycan et al. [42] who stated that the activity of serum ALT and AST significantly increased in APAP-treated rats (500 mg/kg). This may be related to the loss of functional integrity of hepatocytes and cellular leakage and hence these enzymes are released into the blood [43]. However, lowering liver enzymes in treated groups indicates the membrane-stabilizing activity and might indicate their ability to regenerate the damaged hepatic cells, therefore reducing the escape of these enzymes into the circulation. Prescott39 mentioned that NAC minimized liver damage resulting from paracetamol overdosage. On the same hand, Wong et al. [44] evoked that, the use of probiotics may decrease the levels of liver fat and serum aminotransferases. Moreover, reduction of cellular deterioration in liver and kidney in treated groups was confirmed by the histopathological examination which revealed mild to moderate hepatocyte degeneration, congestion, portal inflammation, and decreased hepatic and renal histopathological scores.

In addition, the obtained results showed elevated urea and creatinine levels in APAP treated (group II). The increase in serum urea levels indicates renal disease as the rate of serum urea production exceeds the clearance rate manifested by uremia [45]. The present study suggested that renal injury was regulated in all treated groups indicating the renal protective effect of all treatment regimens with a more powerful effect of NAC and the combined lacteal fort and vitamin B12.

Oxidative stress is defined as the imbalance between the production of reactive species and antioxidant defense which therefore leads to disturbances in cellular biology [46]. It is well known that liver tissue is rich in polyunsaturated fatty acids, which are very sensitive to peroxidation [47]. Peroxidation of polyunsaturated fatty acids leads to the production of MDA, a biomarker of oxidative stress and consequently necrosis [48]. In the current study, APAP intoxication induced a significant increase in levels of MDA and NO in hepatic and renal tissues, as well as a significant decline in SOD activity. The toxic effect of APAP is related to NAPQI, which is a toxic metabolite of APAP, detoxified by conjugation with glutathione. However, when glutathione levels are depleted, thus cellular necrosis in hepatocytes and the sinusoidal endothelial cell will accrue resulting in oxidative stress [49], this explains the increased levels of oxidative stress markers, MDA and NO, in liver and kidney homogenates in APAP compared to the control non treated rats. Our results agreed with Abdulkhaleq et al. [38] who noticed the elevated MDA in APAP-treated rats. Moreover, Aycan et al. [42] observed an elevation in MDA in both sera and tissues of the APAP medicated group compared with those of control group. However, our finding of decreased SOD activity contradicts that of Dadkhah et al. [17] who detected elevation of SOD in the erythrocytes of rats treated with a high dose of APAP. This discrepancy may be related to the difference in the tissues analyzed.

The increase in MDA level was significantly regulated by NAC [group III) indicating that it could counter the tissue damage caused by oxidative stress [50]. Numerous studies mentioned that NAC can inhibit oxidative stress and DNA damage [51]. NAC is actively transported into hepatocytes and renal tissue where it serves as a precursor to GSH [52] as sufficient GSH levels could neutralize NAPQI and obviate oxidative stress [34]. Moreover, rats treated with both lacteal fort and vitamin B12 combination showed significant regulation of MDA and NO levels, this protective effect may be due to the anti-oxidative capacity of these supplements, while vitamin B12 can maintain the sulfhydryl level under oxidative conditions despite lack of radical scavenging, in addition to, cobalt complex present in vitamin B12 could effectively inhibit the inflammation and fibrosis of hepatocytes and improves the mitochondrial integrity [53]. Moreover, Chan et al. [54] illustrated that B12 may possess antioxidant properties and have a direct superoxide scavenger. In the same line, Antelava et al [55] clarified that vitamin B12 could protect hepatocytes of rats from carbon tetrachloride-induced hepatotoxic effect. Additionally, lacteal forte revealed a potent antioxidant effect and prevent endothelial apoptosis caused by oxidants [56]. Moreover, Patra et al. [57] observed that probiotics could reduce membrane damage through scavenging lipid peroxidation. Aboderin and Oyetayo [58] showed that rats administered Lactobacillus Plantarum have signs of better health based on their hematological status and weight gain. In a previous study, Zhou et al. [59] reported that four weeks of L. rhamnosus, L. acidophilus, and Bifidobacterium lactis administration caused beneficial effects on general health status, hematological picture, blood biochemistry, and histology of gut mucosa.

In addition to the hepatoprotective effect of NAC, vit B12, and lacteal forte illuminated in this study, they appeared to have a renal protective effect and

significantly decreased serum levels of urea and creatinine. As well as these medications significantly declined MDA and NO levels in renal tissue and returned nearly to normal levels. Similar results mentioned by Chan et al. [54] evoked a significant reduction of superoxide bursts in retinal ganglion cells of Long-Evan rats after vitamin B12 therapy resulting in increased cell survival. On the same hand, Kaizu et al. [60] demonstrated that lactobacilli have antioxidative activity and decrease the generation of reactive oxygen species.

TNF-α is a proinflammatory cytokine that activates a cascade of interleukins like IL-1 b, IL-6, and IL-8 [61]. It is elevated in many diseases where apoptosis is involved as renal damage caused by paracetamol [62]. Nephrin is an important podocytes' protein in the kidneys that maintain glomerular ultrafiltration. Moreover, it plays an important role in cell adhesion and regulates the structure and function of podocytes [63]. Therefore, this protein is important for maintaining the autoregulatory process in the kidney [64]. On the other hand, desmin is a key cytoskeleton protein on the glomerular membrane cells. When podocytes are damaged, the expressions of desmin protein increase, and the phenotypic desmin transformations occur [65]. In the current study, APAP induced a significant elevation in the expression of desmin, nephrin, and TNF-α genes. This point to kidney injury was detected in histopathological examination of the renal tissue. This nephrotoxicity is related to NAPQI, a highly reactive intermediate metabolite [66] that was correlated to interstitial leukocyte infiltration in renal tissue [67]. These findings were in the same line as those of Devkar et al. [68] and El-Boshy et al. [69] who declared that administration of paracetamol-induced elevation of TNF-α. While there was a significant down-regulation in TNF-α, desmin, and nephrin expressions in all treated groups. These results were following those of Patra et al. [57] who mentioned that probiotic supplementation could reduce uremia. Also, Nowak et al. [70] noticed that Lactobacilli Spp could enhance TNF-α response. For instance, Kano et al. [71] mentioned that skimmed milk fermented with L. Delbrueckii helped in the treatment of collagen-induced arthritis and reduced secretion of interferon-γ in mice.

COX-1 is one of the important contributors to inflammatory processes [72]; it is responsible for initial prostanoid responses to inflammatory stimuli [73] and involved in the resolution of inflammation [74]. COX-2 has recently been identified as the major implication in prostanoid synthesis as inflammation progresses73. COX-2 is an enzyme mainly produced by pro-inflammatory cytokines, mitogens, tumor promoters, and growth factors in many cell types, such as monocytes [75]. In the present study, a large dose of APAP decreased COX-1 and COX-2 expressions in liver tissue. The reduction of COX-1 and COX-2 activity resembled the action of NSAID that inhibit COX by competing with arachidonic acid. Paracetamol may act as a reducing agent within the peroxidase active site that inhibits a protoporphyrin radical cation. The latter typically generates a tyrosine radical in the COX active site that is essential to the oxidation of arachidonic acid [76].

Biochemical and ultrastructural studies showed that overdoses of paracetamol caused morphological and functional changes in the hepatic mitochondria [77]. In rodents, binding of the toxic metabolite to proteins causes mitochondrial dysfunction and nuclear DNA fragmentation resulting in necrotic cell death. Our histopathological results confirmed hepatorenal damage confirmed the biochemical analysis. Liver sections from the APAP group exhibited marked congestion and hydropic degeneration, as previously reported by Lim et al. [78] who mentioned that rats intoxicated with the APAP showed hepatocytic degeneration and portal inflammation. In the same respect, the study of Dadkhah et al. [17] exhibited focal necrosis in hepatic cells in rats that received high APAP [450 mg/kg BW]. Additionally, APAP administration induced renal injury including severe congestion and tubular degeneration as previously reported by Chinnappan et al [36]. These histological alterations in hepatic and renal tissues were recovered in all treated groups confirming the results of blood and tissues biochemical assays and the immune gene expressions as well. Meanwhile, many studies suggest that probiotics have a beneficial role in liver function during cirrhosis, whereas Macbeth et al. [79] suggested that Lactobacillus acidophilus might have a helpful effect in the therapy of cirrhosis and hepatic encephalopathy. Moreover, Ouattara et al. [80] mentioned that B12 improves hepatic function which might reduce metabolic stress in dairy cows.

The adverse effects of NAC are a long-term process [7], longer than the period of the current experiment that fits with our rat model (ten days), which is considered a limitation towards assessing the long-term NAC side effects.

5. Conclusions

In conclusion, vitamin B1 and lacteal forte® combination may be considered as a potential safe alternative to N-acetylcysteine in ameliorating hepato-renal injury caused by APAP or other chemotherapeutic drugs. More research will be required to estimate the level of toxic metabolite, NAPQI, to determine the actual mode of action of these medications in protecting against APAP toxicity.

6. Conflicts of interest

"There are no conflicts to declare".

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