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Health Risk Assessment of Co-administration of the Non-steroidal Anti-inflammatory Drugs and Proton Pump Inhibitors on Hepatic and Renal Structures, Experimental Study

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This study aimed to evaluate the effect of acetylsalicylic acid (ASA) and omeprazole combination on the histopathological and histomorphometry structures of hepato-renal tissues in male Swiss albino mice. Forty male Swiss albino mice were randomly divided into four groups: control group; ASA treated group; omeprazole treated group and ASA -omeprazole treated group. Histopathological and histomorphometric studies were conducted for evaluation. Combined administration of ASA with omeprazole resulted in several histopathological lesions and histomorphometric alterations in hepato-renal tissues as revealed by light microscopic examinations compared to the ASA / or omeprazole-treated group. The ASA-Omeprazole treatment group showed severely dilated and congested blood vessels in the liver tissues, the inflammatory cells were also severely aggregated and most hepatocytes were severely vacuolated with condensed pyknotic nuclei.

Interestingly histomorphometry analysis showed that combination of ASA acid and omeprazole resulted in a significant decrease in both the corpuscle area and glomerulus area and induced a substantial increase in the ratio of corpuscle area/glomerulus in the renal tissues. Conclusions: The co-administration of ASA and omeprazole led to significant histopathological and histomorphometric changes liver and kidney tissues. We recommend avoiding the combined use of ASA and omeprazole; as an alternative, they should be administered at spaced intervals.

Keywords: Histopathological; kidney; Liver; acetylsalicylic acid; Omeprazole.

1. Introduction

Non-steroidal anti-inflammatory drugs such as Acetylsalicylic acid (ASA) are widely used for the treatment of rheumatoid arthritis and the prevention of cardiovascular thrombotic diseases. It is considered one of the cheapest drugs that is commonly used as an anti-inflammatory, anti-platelet, analgesic and antipyretic drug. However, its use triggers gastrointestinal ulcers; including mucous ulcers, bleeding, and perforation [1-4]. Additionally, it causes a reduction of gastric juice pH and fluctuation in the volume of gastric juice as well as its acid output [5-6], which leads to ulcer formation. Hence, proton pump inhibitors as omeprazole is efficacious for treating gastric ulcer syndrome [7], and are considered as the main choice in gastric ulcer treatment [8]. Omeprazole was promoted for healing Non-steroidal anti-inflammatory drugs-associated ulcers, as they provide potent and long-lasting inhibition of gastric acid secretion [9]. It is suggested that co-administration of non-steroidal anti-inflammatory drugs with proton-pump inhibitors, such as omeprazole, reduces upper gastrointestinal tract adverse events by preventing mucosal injury [10,11]. This combination is presumed to provide significant protection for the gastric mucosa and increase mucosal blood flow [12]. This effect was confirmed by previous studies reporting that omeprazole reduces recurrent ulcers and erosions in patients using low-dose ASA over 24 weeks [13]. Thus, our study aimed to evaluate the effect of the

combined administration of ASA and omeprazole on the hepatic and renal structure of Swiss albino mice through histological examinations and histomorphometric analysis.

2. Materials and Methods

2.1 Animals

Swiss male albino mice (40) weighting 25 -30 gm

source of Animal House is the National Organization for Drug Control and Research (NODCAR) in Giza, Egypt. Before starting the experiments, we provided animals with standard commercial diet pellets and water (Ad-labium), and housed the animals in plastic cages for 7 days to acclimate to laboratory conditions.

Animals for experiments were grouped and housed according to the guidelines of the institutional animal ethics committee of the National Organization for Drug Control and Research (NODCAR). In addition, all experimental procedures were conducted following the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at NODCAR (approval no. NODCAR/III/42/2019).

2.2 Chemicals

Combination treatments was prepared as following; ASA (Egyptian company for Chemical & Pharmaceuticals S.A.E) 25mg/kg body weight bw was used according to the dose recommended for arthritis treatment by Somasundaram *et al*., [14]. Omeprazole (Sigma-Aldrich Company U.S.A) was used as 10 mg/kg bw according to the dose recommended as a standard treatment for upper gastrointestinal mucosal by Kuramoto *et al*., [15].

2.3 Experimental design

Swiss albino mice (25–30 g) were kept for 1 week for proper acclimatization to the animal house conditions (12- hour, lighting cycle and 25 ± 2 °C temperatures) with free access to standard rodent chow and water. The animals were then divided randomly into four groups of 10 animals: The first group orally administrated distilled water (1ml/kg bw) and served as a control group; ASA treated group: orally administrated ASA (25mg/kg bw/ day); Omeprazole treated group: orally administrated omeprazole (10mg/ kg bw / day). The fourth group of animals treated with both ASA (25mg / kg bw / day) and omeprazole (10mg/ kg bw / day). All tested drugs were administrated via an oral tube for four days.

2.4 Histopathogical examination of the treated groups

The animals were subjected to cervical dislocation, dissection. Hepatic-renal tissue were freshly collected and immediately transferred to 10 % formaldehyde for fixation for 48 hours. The specimens were washed then washes, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Five micron thick paraffin sections were prepared, mounted on clean slides and stained with Ehrlich's haematoxylin-eosin (H&E) [16] histopathological examinations was then performed under Olympus microscope.

For the histomorphometrical analysis of hepatic lesions, hepatocytes showing vacuolar degenerative and pyknosis were counted, area of blood vessels and areas of infilammatory cells aggregation for each image were also counted instinctively [17]. For the histomorphometrical analysis of renal lesions, twenty renal corpuscles were analyzed randomly for each group to measure renal corpuscle area (μm²), glomeruli –area (μm²) and the ratio of corpuscle area/ Glomerulus area [18].

2.5 Statistical analysis

All statistical analysis were performed using the Analysis Of Variance (ANOVA) and GraphPad Prism software 5.01 (La Jolla, CA, USA) to determine differences between group means and standard deviation Mean value was stated as means ± standard deviation (SD) for ten samples for each group. $P < 0.05$ was considered as statistically significant, while *P* values "$P < 0.001$ was considered as highly significant.

3. Results

3.1 Histopathological examination of the hepatic tissues

Histopathological examination of hepatic tissues from the control group showed typical normal hepatic architecture; hepatic cell cords radiated from the central vein and separated by narrow blood sinusoids lined by Kupffer cells. Each hepatocyte contains one or two vesicular round nuclei and acidophilic cytoplasm (figure 1a). ASA-treated group only showed prominent areas of mild vacuolated changes with hepatocytes radiated from the intact central vein (figure 1b). The central vein showed RBCs hemolysis, most hepatocytes with pyknotic nucleus. Kupffer cells and mild aggregation of inflammatory cells in hepatic tissues were also detected (figure 1c). Omeprazole treatment resulted in intact hepatocytes and central vein with mild focal inflammatory cells aggregations (figure 1d). Mild vacuolated hepatocytes with pyknotic nuclei radiated from a normal appearance central vein were seen in some regions (figure 1e).

Combine administration of ASA with omeprazole resulted in several histopathological lesions including; hepatocytes with pyknotic nucleus and homogenized cytoplasm, severely dilated, congested hyalinized central veins, and dilated

sinusoids filled with inflammatory cells. In other regions, severely dilated central veins with hyalinized walls were seen. Scattered un-nucleated hepatocytes, perivascular dense aggregation of inflammatory cells and scattered necrotic areas were more prominent (figure 1g). Most portal areas showed marked dilated, congested portal veins, proliferated bile ducts and perivascular aggregation of inflammatory cells (figure 1h). Hepatocytes with severe vacuolated cytoplasm and pyknotic nucleus, mildly dilated sinusoids infiltrated by a few inflammatory cells and hyalinized extracellular materials encircling the wall of hepatocyte were also noticed (figure 1i).

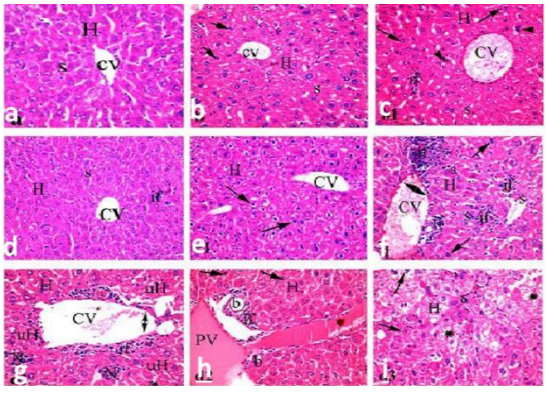

Figure 1: Photomicrographs of liver sections: (a) Control mice showing hepatocytes (H), central vein (CV), and sinusoids (s); (b) Mice treated with ASA, displaying nuclear condensation in hepatocytes (arrow); (c) Mice treated with ASA showing pyknotic nuclei in hepatocytes (arrow), proliferated Kupffer cells (arrowhead), and inflammatory cells (if); (d) Mice treated with omeprazole; (e) Mice treated with omeprazole showing mildly vacuolated hepatocytes (H) with pyknotic nuclei (arrow) radiating from a normal-appearing central vein (CV); (f) Mice treated with ASA and omeprazole; (g) Mice treated with ASA and omeprazole displaying severely dilated central veins (cv) with hyalinized walls (double arrowhead), scattered necrotic areas (N), and scattered anucleate hepatocytes (uH); (h) Mice treated with ASA and omeprazole, showing proliferated bile ducts (bd) and hepatocytes with pyknotic nuclei (arrow); and (i) Mice treated with ASA and omeprazole, displaying hepatocytes (H) with pyknotic nuclei (arrow) and hyalinized extracellular materials (star). (H&E, x20).

3.2 Histopathogical Parameters Alteration of the liver in treated Groups

The liver pathology results from mice treated with ASA, Omeprazole, and the combination of ASA-Omeprazole demonstrate distinct changes across various histopathological parameters when compared to the control group.

Vacuolar Degeneration: In the control group, vacuolar degeneration was absent, indicating normal hepatocyte structure. However, treatment with ASA alone resulted in a vacuolar degeneration score of 10.5, indicating mild cellular changes and initial stress on hepatocytes. The Omeprazole-treated group displayed an increased vacuolar degeneration score of 14.5, showing a moderate level of cellular disruption. Notably, the combined ASA-Omeprazole treatment group exhibited a significant vacuolar degeneration score of 19.8, highlighting substantial structural compromise in hepatocytes. This pattern suggests a progressive increase in vacuolar degeneration severity with the use of ASA, Omeprazole, and especially their combination.

Pyknosis of Hepatocytes: Pyknosis, a sign of nuclear condensation typically associated with cell injury or death, was absent in the control group, confirming the lack of cellular distress. In contrast, ASA treatment induced a pyknosis score of 4.25, suggesting mild cellular injury. The Omeprazole-treated group showed a slightly higher pyknosis score of 5.5, indicating further cellular stress compared to ASA alone. The combination treatment resulted in a marked increase in the pyknosis score to 13.6, indicating significant hepatocyte injury. This trend underscores the cumulative cellular stress and potential cytotoxicity introduced by combined ASA-Omeprazole treatment, leading to greater nuclear condensation within hepatocytes.

Blood Vessel Area (%): Blood vessel dilation and congestion were minimal in the control group, with an area of 339 μm^2. In the ASA-treated group, there was a significant increase in blood vessel area to 6149 μm^2, reflecting notable dilation and congestion likely due to inflammatory responses or vascular stress. Omeprazole treatment alone resulted in a blood vessel area of 3035 μm^2, indicating moderate vascular changes. However, the combined ASA-Omeprazole group displayed a dramatic increase in blood vessel area to 11332 μm^2, demonstrating pronounced vascular dilation and congestion. This escalation highlights a strong vascular response to the combination therapy, suggesting synergistic effects on hepatic blood flow and vessel integrity when both drugs are administered together.

Inflammatory Cell Aggregation Area (%): No inflammatory cell aggregation was observed in the control group, indicating a lack of immune response or tissue injury. The ASA-treated group showed an inflammatory cell aggregation area of 1114 μm^2, suggesting a mild immune response within liver tissues. In the Omeprazole-treated group, the area of inflammatory cell aggregation was slightly higher at 1186 μm^2, indicating a moderate level of

immune cell infiltration table 1. The combination treatment resulted in the highest inflammatory cell aggregation area of 1517 µm², indicating substantial immune response and tissue inflammation. This result supports a heightened inflammatory reaction with the combination of ASA and Omeprazole, potentially due to increased hepatocyte injury and vascular changes.

Table 1: Effects of ASA, Omeprazole, and Combined ASA-Omeprazole Treatment on Liver Pathology in Male Swiss Albino

Pathological parameter	Control Group	ASA Treated Group	Omeprazole Treated group	ASA-Omeprazole Treatment
Vacuolar Degeneration	0±0 [a]	10.5±2.8 [b]	14.5 ± 2.1 [c]	19.8±3.6 [d]
Pyknosis Hepatocyte	0±0 [a]	4.25 ± 1.4 [b]	5.5 ± 2.5 [b]	13.6 ±2.4 [c]
Blood Vessels Area % (µm²)	339±338 [a]	6149±16 1 [b]	3035± 731 [b]	11332±590 [c]
Inflammatory Cells Aggregations Area % (µm²)	0 ± 0 [a]	1114±48 6 [b]	1186± 616 [b]	1517± 15 [c]

Data are represented as mean ±S.D. Means with same letter are not significantly. (P< 0.05) is significant and (P< 0.01) is highly significant.

3.3 Histopathological examination of the Renal tissues

Histological examination of the control group renal section showed normal architecture of the kidney tissues, normal appearance of the glomerulus with its capillary tuft surrounded by Bowman's space and normal structure of renal tubules lining with epithelial cells (figure 2a) ASA treatment results in a mild reduction of glomerulus's size, with some renal tubules has mild degenerative changes in the epithelial lining of renal tubules and others have hyaline cast in their Lumens. Additionally, mild inflammatory cell infiltrations were noticed (figure 2b). Omeprazole treatment retained the normal appearance of most glomerular and renal tubules. A mild atrophy of the glomerulus with a few renal tubules showing mild vacuolar degenerative changes either of the epithelial lining renal tubules or hyaline casts in their lumen together. This treatment has also led to mild inflammatory cell infiltrations (figure 2c).

Combined treatment of ASA and omeprazole resulted in a vacuolated glomerulus with narrow space, interstitial hemorrhage and hyalinization of the corpuscle wall. Hemosiderin deposits and hyaline cast in the lumen of renal tubules were also detected (figure 2d1). Areas of the lobulated atrophied glomerulus, narrow Bowman's space, diffused hemosiderin deposits together with severe vacuolated renal tubules, and marked degenerative epithelial lining of other renal tubules with pyknotic nuclei were considered (figure 2d2). This treatment also resulted in prominent areas of loss of normal architecture as evidenced by severely atrophied glomerulus, narrow Bowman's space, dilated congested blood vessels, wide areas of interstitial hemorrhage and marked inflammatory cell aggregations. Additionally, the combination of both drugs resulted in the degenerative epithelial lining of the renal tubules with pyknotic nuclei and necrotic area in renal tubules.

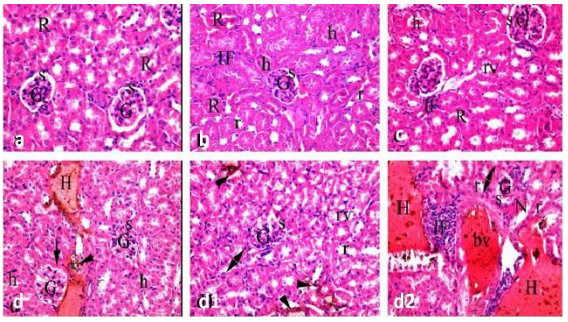

Figure 2: A photomicrograph of kidney section. (a) control mice; glomerulus (G) Bowman's space (S) renal tubules (R); (b) ASA; degenerative change of epithelial lining (r) hyaline cast (h). inflammatory cellss (IF); (c) Omeprazole treatment vacuolar degenerative changes (rv) (d) Combined ASA and omeprazole treatment; narrow space (s) hyalinization of wall corpuscle (arrow). Interstitial hemorrhage (H), hemosiderin deposited (head arrow). (d1) Combined ASA and omeprazole treatment; degenerative epithelial lining of other renal tubules (r) pyknotic nuclei (two head arrow). Diffused hemosiderin deposited (arrow head). (d2) Combined ASA and Omeprazole treatment; congested blood capillary (bv), and interstitial hemorrhage (H) Marked degenerative epithelial lining of renal tubules (r) with pyknotic nuclei (two head arrow) necrotic area (N) (H&E, X20).

Histomorphometrically analysis of renal lesions from male Swiss albino mice treated with ASA, Omeprazole and ASA-Omeprazole is summarized in table 2.This study assessed renal parameters across four groups: a negative control group, an ASA-treated group, an omeprazole-treated group, and a combined ASA-omeprazole treated group. The parameters examined included the corpuscle and glomerulus areas, as well as the ratio of corpuscle area to glomerulus area.

In terms of corpuscle area, the negative control group displayed an average area of 4954 ± 340 µm², representing the baseline. In contrast, the ASA-treated group showed a significantly reduced corpuscle area of 2590 ± 366 µm². Similarly, the omeprazole-treated group had a decreased corpuscle area at 3844 ± 336 µm², although it remained higher than that of the ASA-treated

group. The combined ASA-omeprazole treated group had the lowest recorded corpuscle area, with an average of 1543 ± 545 µm², suggesting a potentially additive effect of both treatments in reducing corpuscle area.

For the glomerulus area, the negative control group maintained a mean area of 3949 ± 230 µm². However, treatment groups exhibited reductions, with the ASA-treated group showing a significant decrease to 1657 ± 203 µm². The omeprazole-treated group recorded a glomerulus area of 2702 ± 436 µm², indicating a lesser reduction than the ASA-treated group. The combined ASA-omeprazole treated group displayed the most pronounced reduction in glomerulus area, averaging 886 ± 337 µm². This substantial reduction in glomerulus area within the combined treatment group aligns with the observed decreases in corpuscle area, suggesting potential cumulative effects on renal structure.

The ratio of corpuscle area to glomerulus area further highlights these differences. The negative control group showed a baseline ratio of 1.257 ± 0.07. In comparison, the ASA-treated group displayed an increased ratio of 1.563 ± 0.09, while the omeprazole-treated group had a slightly lower ratio at 1.422 ± 0.39. Notably, the ASA-omeprazole treated group recorded the highest ratio of 1.74 ± 0.17, indicating that the relative reduction in glomerulus size was more significant than in corpuscle size, particularly under combined treatment conditions.

Table 2: Effects of ASA, Omeprazole, and Combined ASA-Omeprazole Treatment on Renal Pathology in Male Swiss Albino

Parametrs / Groups	Corpuscle Area %(µm²) mean± SD	Glomerulus Area % (µm²) mean± SD	Ratio of Corpuscle Area / Glomerulus Area% mean± SD
Negative Control	4954±340[a]	3949±230[a]	1.257±0.07[a]
ASA	2590±366[c]	1657±203[c]	1.563±0.09[c]
Omeprazole	3844±336	2702±436[b]	1.422±0.39[b]
ASA-Omeprazole	1543±545	886±337[d]	1.74±0.17[d]

Data are represented as mean ±S.D. Means with same letter are not significantly. (P< 0.05) is significant and (P< 0.01) is highly significant

4. Discussion

The potential risks associated with combined ASA and Omeprazole therapy on liver and renal structure, was assessed throughout this study. Histological examinations and histomorphometric analysis was applied to study various histopathological parameters.

Histopathologically and histomorphometrical changes observed in the hepaic-renal tissues of animals treated only with ASA could be attributed to generate free radicals, decrease activity of antioxidant enzymes [19] and inhibit cyclooxygenase enzymes [20-21]. Reactive oxygen species in turn are capable of initiating and promoting oxidative damage [22]. Productions of these radicals play an important role in the initiation and/or progression of various diseases [23, 24]. Additionally these free radicals also might affect pathophysiology of various diseases [23] as gastrointestinal disorders, small intestinal diseases, hepatic and kidneys injury [17, 25-28].

Cytochrome P450 inhibition considered as one mechanism, which explained the histoathological lesions and histomorphometrical alterations observed in the hepatic-renal tissues of animals treated only with omeprazole [29]. Cytochrome P450 (CYP450) enzymes are necessary for the detoxification of foreign chemicals and the metabolism of drugs [30-31]. The hepatic CYPs are also involved in the pathogenesis of several liver diseases [32]. Another reason for these observed changes could be attributed to the ability of proton pump inhibitors to increase oxidative stress and to also limit the capacity of regenerative in liver and kidney [33-35]. Utilization of proton pump inhibitors are always correlated with acute kidney injury and chronic renal [36, 37].

In the present work, the histopathological examination of hepato-renal tissues from male Swiss albino mice treated with ASA combined with omeprazole revealed marked pathological lesions. Hepatic tissues showed severe dilation, congested blood vessels, and dense aggregation of inflammatory cells. Additionally, vacuolated cytoplasm and pyknotic nucleus of hepatocytes was characterized as compared to control group/ omeprazole treated group/and ASA treated group. Renal tissues showed also distorted histopathologically architectures, lobulated atrophied glomerulus, narrow Bowman's space and hyalinization corpuscle wall. These resulted coincide by others [38] and could be attributed to drug-drug interaction [39]. Drug-drug interactions represent a widely distributed health problem [40]. Another reason explained these histological changes are the observed severe aggregation of inflammatory cells in the kidney and liver tissues that caused excessive production of reactive oxygen species. Excessive-induction of free radicals weaken the defense system, and caused oxidation and disrupted of cellular integrity [19, 41].

Hemosiderin deposited in kidney tissues could be

attributed to loss of the capacity of formation of Haptoglobin-hemoglobin (Hp–Hb complex). Haptoglobin-hemoglobin (Hp–Hb complex) is a large complex not filtered by the glomerulus but it is catabolized in liver, spleen, and bone marrow. When plasma haptoglobin (Hp) levels decline due to increased consumption, free hemoglobin (Hb) accumulates in the plasma, preventing the formation of the haptoglobin-hemoglobin (Hp–Hb) complex. Consequently, free Hb is easily filtered through the glomerulus and deposited in the renal tubules, leading to the accumulation of ferric ions (hemosiderin) within these tubular cells. [42].

The observes accumulation of hemoglobin (hemosiderin) in the kidney sections of animals treated with both ASA and omeprazole was proposed as the cause of necrosis of renal tubular, vacuolar degenerative of renal tubules and aggregation of inflammatory cells through accelerated generation of oxygen reactive species, apoptosis, and inflammation [43].

5. Conclusion

This study demonstrated that treatment with ASA and Omeprazole, particularly in combination, induces significant liver pathology characterized by increased vacuolar degeneration, hepatocyte pyknosis, blood vessel dilation and congestion, and inflammatory cell aggregation. The results underscore the potential risks associated with combined ASA and Omeprazole therapy on liver structure, which may have implications for hepatotoxicity in clinical use. ASA-omeprazole combined treatment had a severe renal damaging effect; reducing both corpuscle and glomerulus areas, as well as increasing the corpuscle-to-glomerulus area ratio, highlighting a potential cumulative impact of the treatments on renal structure. This study suggested usage of ASA and omeprazole at spaced intervals to prevent the side effects of their combined use.

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7. References

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