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Influence of different aspirin doses on kidney tissue and blood parameters in rats

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Abstract

Acetylsalicylic acid (ASA), usually referred to aspirin, is a nonsteroidal anti-inflammatory medication (NSAID) used as an anticoagulant to treat pain, fever, inflammation and to treat a variety of inflammatory disorders, including Kawasaki disease, pericarditis, and rheumatic fever. In addition to being an antithrombotic, aspirin also lowers the risk of heart attacks. We investigated the effect of aspirin on RBC, Hb, and PCV and renal functional enzyme (urea, Creatinine and uric acid) using different doses of aspirin in healthy adult rats and at the same time clarify its effect on renal tissue. This study included 50 Wistar adult rats were divided into five groups as following; control, G1, G2, G3, and G4, rats in control group drenched distilled water daily, while the rest groups drenched daily with aspirin 30, 70, 100, and150 mg/kg using gastric lavage sequentially. Blood samples were collected from heart for blood parameter and kidney functional enzymes assessment. Kidney tissues were collected for histological process. The results showed decreased in the serum level of RBC, Hb, and PCV; likewise, there was increase in urea and creatinine level. While, there was a decrease in uric acid serum level. In tissue samples, results showed mild atrophy to extensive necrosis in renal tissue samples with cystic dilation in convoluted tubules of the kidney. In summary, current research showed that the use of different aspirin doses over a period of time has a negative impact on blood parameters and renal functional enzymes. Furthermore, the use of aspirin in this way had an adverse effect on renal tissue.

Key words: Aspirin, kidney, necrosis, RBC, and creatinine

Introduction

Aspirin is the most widely medication used in medicine, it is one of the oldest medicine in use **(**1**)**. In 2007, the Agency for Healthcare Research and Quality (AHRQ) reported that nearly 20% of adults in the United States reported taking aspirin daily or every other day, with this number increasing to nearly 50% in those aged 65 and older **(2)**. However, aspirin as we know it today was not introduced for public use until 1904, following a series of attempts at extraction and purification of salicylic acid from willow bark and subsequent modification to acetylsalicylic acid to reduce the unpleasant side effects **(**3**).** In addition to its anti-inflammatory properties, aspirin was also observed to increase bleeding time, and later studies demonstrated that it was effective as an antithrombotic drug (4)**.** *In vitro* and *in vivo* studies showed that aspirin at high doses caused necrosis of the blood vessel tissues (5) and long-term therapeutic use of aspirin has been associated with nephrotoxicity, hepatotoxicity, gastrointestinal ulcerations, and renal cell cancer and adverse effects to multiple organ (6).

Materials and Methods

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Ethical approval

The procedures for research regarding the current study were approved by the Ethical Committee at the College of Veterinary Medicine, University of Al-Muthana, Iraq.

Experimental design

Fifty mature Wistar rats (aged 90 days and weighted 150±10 g) were assigned to a five equal groups (10 each) and treated for 30 days as follow; Control (C), rats were drenched with distilled water daily. Group 1 (G1), rats were drenched with aspirin (30 mg/ kg b.w) daily. Group two (G2), rats were drenched with aspirin (70 mg/ kg b.w) daily. Group three (G3), rats were drenched with aspirin (100 mg/ kg b.w) daily. Group 4 (G4), rats were drenched with aspirin (150 mg/ kg b.w) daily. Rats have been monitored throughout the experimental periods and at the end of experiment, rats were anaesthetized (by injection of 0.3 ml ketamine + 0.1 ml of xylazine/ kg b.w. *ip*). Blood samples were collected from the heart in non-heparinized tubes and EDTA tube. Blood with EDTA assessment for CBC and blood serum samples were separated (by centifugation at 3000 rpm for 5 minuts) and kept at –20 ºC until assessment of creatinine, urea and uric acid. Tissue samples from kidney were collected and kept in 10% formalin to tissues processing (H and E).

Results

Blood parameters

RBCs, Hb and PCV

The results in figure (1) showed a significant difference decrease ($p<0.05$) between experimental groups at period of treatment in RBCs in G1 (4.20125±0.009), G2 (3.10875±0.018), G3 (2.3675±0.020), and G4 (1.56375±0.015), compared with control group (6.6125±0.020).

Furthermore, the results in figure (2) illustrate a significant difference decrease ($p<0.05$) between experimental groups at period of treatment in Hb; G1 (10.42875±0.025), G2 (7.44875±0.047), G3 (5.77625±0.030), and G4 (3.63±0.034) sequentially, compared with control group (13.3275±0.020).

At the same time, there was a significant difference decrease ($p<0.05$) between experimental groups in PCV, in G1 (35.3825±0.059), G2 (27.0825±0.123), G3 (19.3575±0.106), and G4 (14.35375±0.128), compared with control group (41.7975±0.083), which showed in figure (3).

In summaries, the results of the present study showed a significant decrease in RBCs, Hb and PCV, wherever rise of dose of aspirin according to groups of the study.

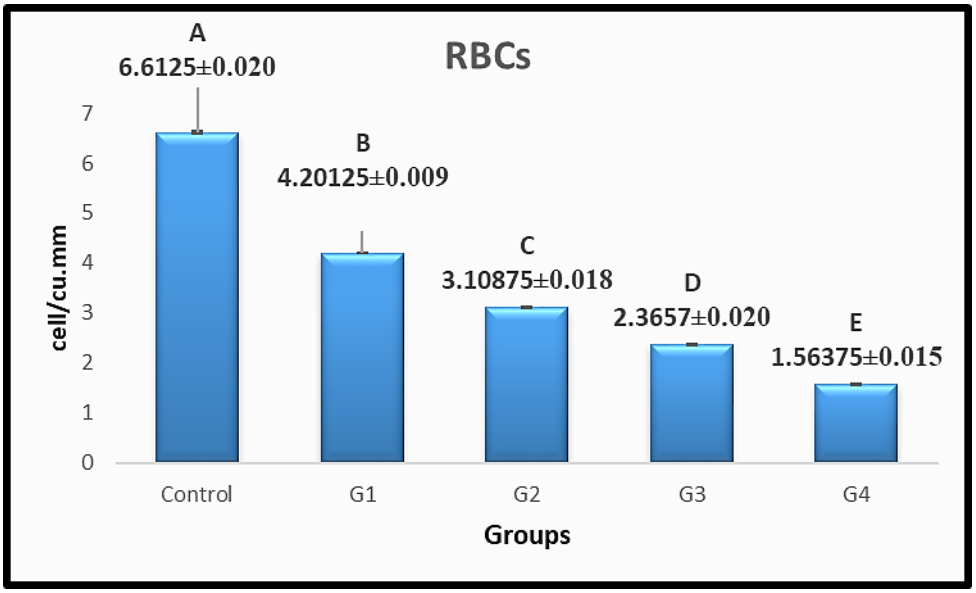

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Figure 1: Effect of different doses of aspirin on RBCs, the results represented as mean ± SE. Similar capital letters denotes the absence of significant differences (P<0.05) between period . Control (C):- rats were drenched with drinking water daily. G1:-rats were drenched with aspirin (30 mg/ kg b.w) daily. G2:- rats were drenched with aspirin (70 mg/ kg b.w) daily. G3:-rats were drenched with aspirin (100 mg/ kg b.w) daily. G4:- rats were drenched with aspirin (150 mg/ kg b.w) daily.

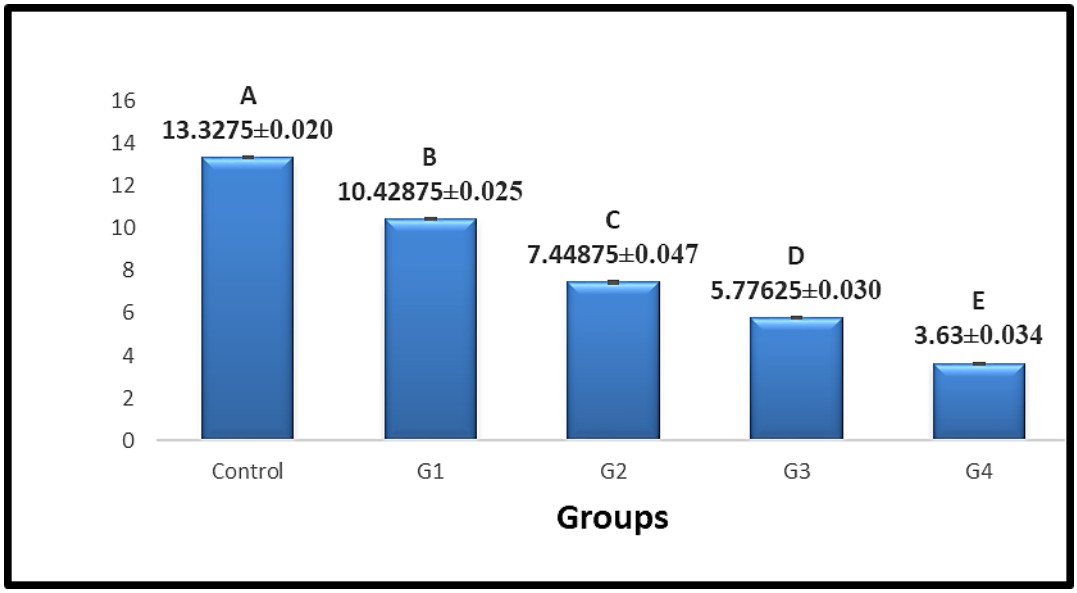

Figure 2: Effect of different doses of aspirin on Hb. Similar capital letters denote the absence of significant differences (P<0.05) between period. Control:- rats were drenched with drinking water daily. G1:-rats were drenched with aspirin (30 mg/ kg b.w) daily. G2:- rats were drenched with aspirin (70 mg/ kg b.w) daily. G3:-rats were drenched with aspirin (100 mg/ kg b.w) daily. G4:- rats were drenched with aspirin (150 mg/ kg b.w) daily.

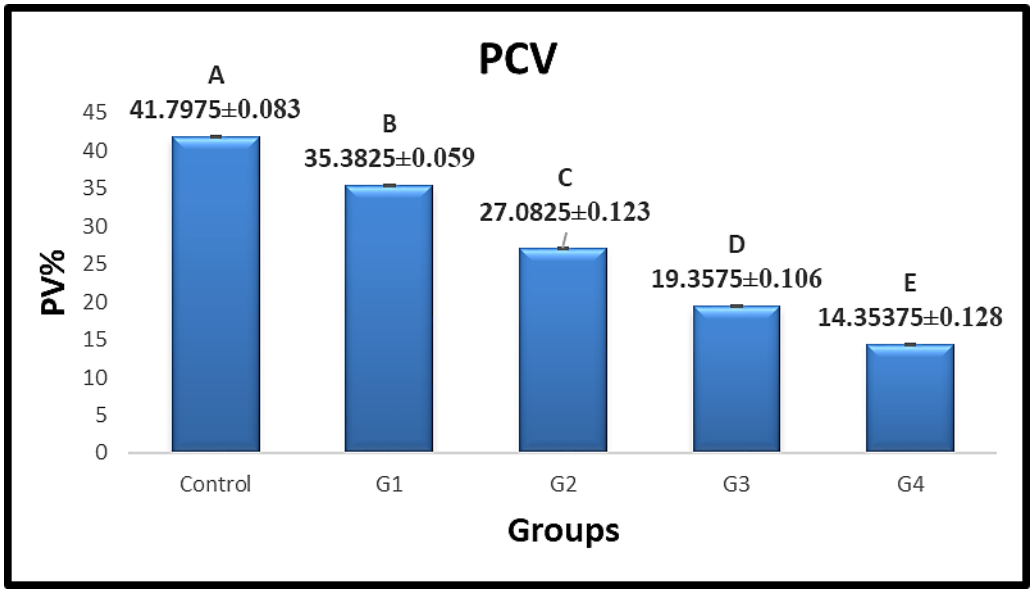

Figure 3: Effect of different doses of aspirin on PCV. Similar capital letters denote the absence of significant differences (P<0.05) between period. Control :- rats were drenched with drinking water daily. G1:-rats were drenched with aspirin (30 mg/ kg b.w) daily. G2:- rats were drenched

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with aspirin (70 mg/ kg b.w) daily. G3:-rats were drenched with aspirin (100 mg/ kg b.w) daily. G4:- rats were drenched with aspirin (150 mg/ kg b.w) daily.

Kidney Functions

The results illustrated in figure (4) showed a significant difference increase ($p<0.05$) between experimental groups at period of treatment in urea, in G1 (37.17375±0.27), G2 (47.4325±0.31), G3 (60.11375±0.30), and G4 (70.79625±0.29), compared with control group (33.455±0.033). At the same time, in figure (5), the results showed a significant increase ($p<0.05$) in creatinine in G1 (0.798625±0.010), G2 (1.211875±0.011), G3 (1.838375±0.018), and G4 (2.518±0.029), compared with control group (0.63575±0.007). While, the results showed a significant decrease in uric acid ($p<0.05$) as G1 (1.04±0.003), G2 (0.79±0.011), G3 (0.4425±0.008), and G4 (0.23625±0.007), compared with control group (1.81125±0.003), figure (6).

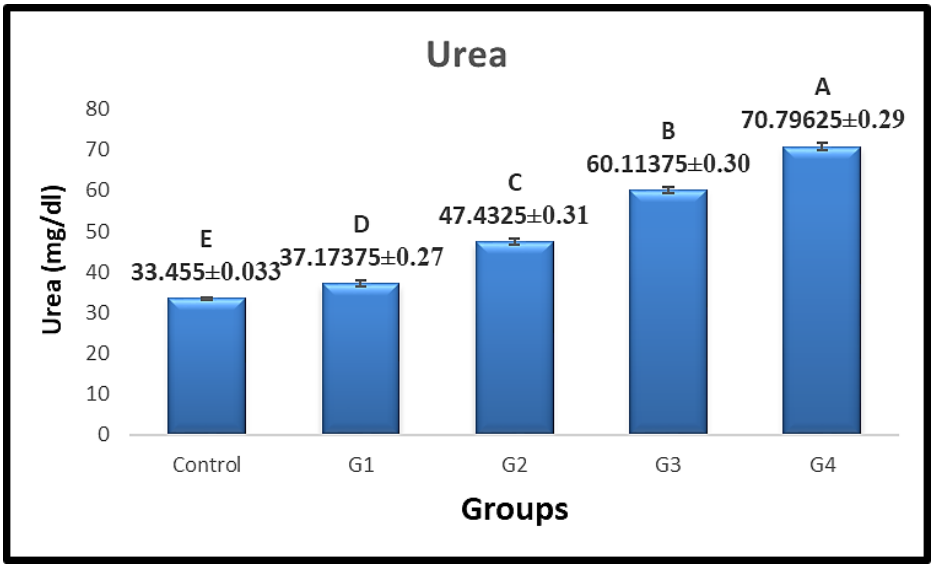

Figure 4: Effect of different doses of aspirin on urea.

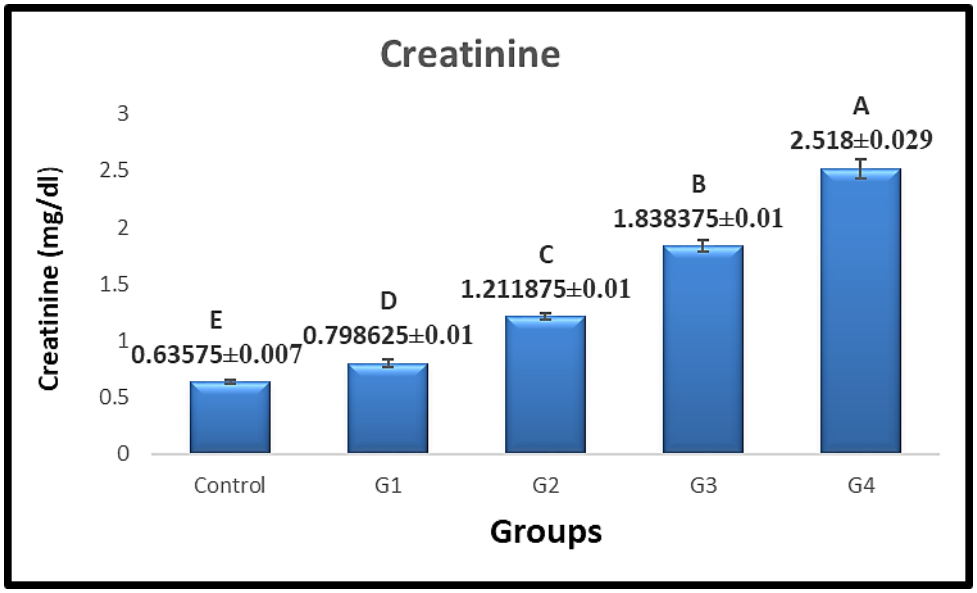

Figure 5: Effect of different doses of aspirin on creatinine.

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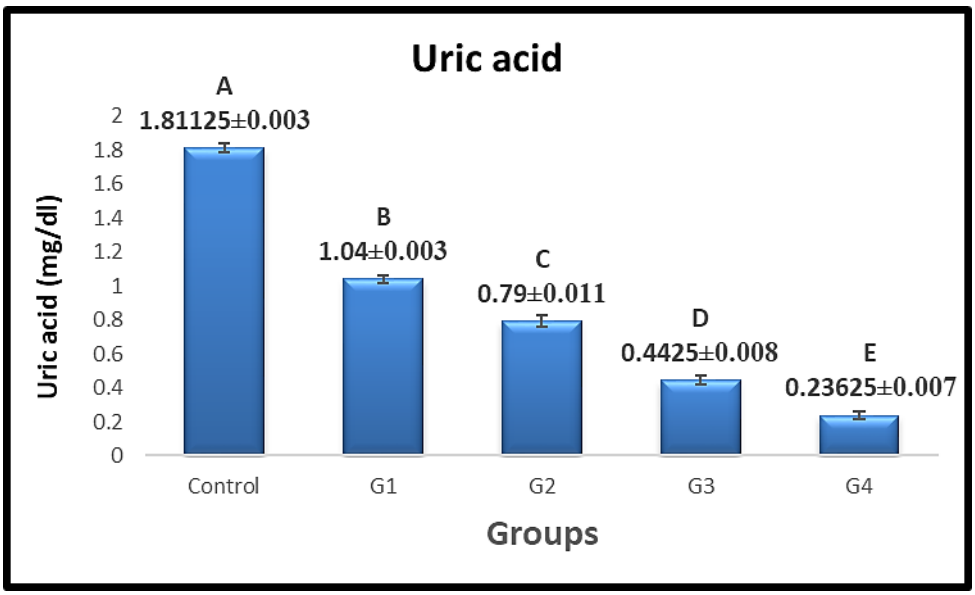

Figure 6: Effect of different doses of aspirin on uric acid.

Histology of kidney tissues

Control group

Histological section, obtained from control rat revealed the presence of normal proliferation glomeruli which lining with endothelial cells, also normal renal convoluted tubules which lining normal epithelial cells, figure (7, A and B).

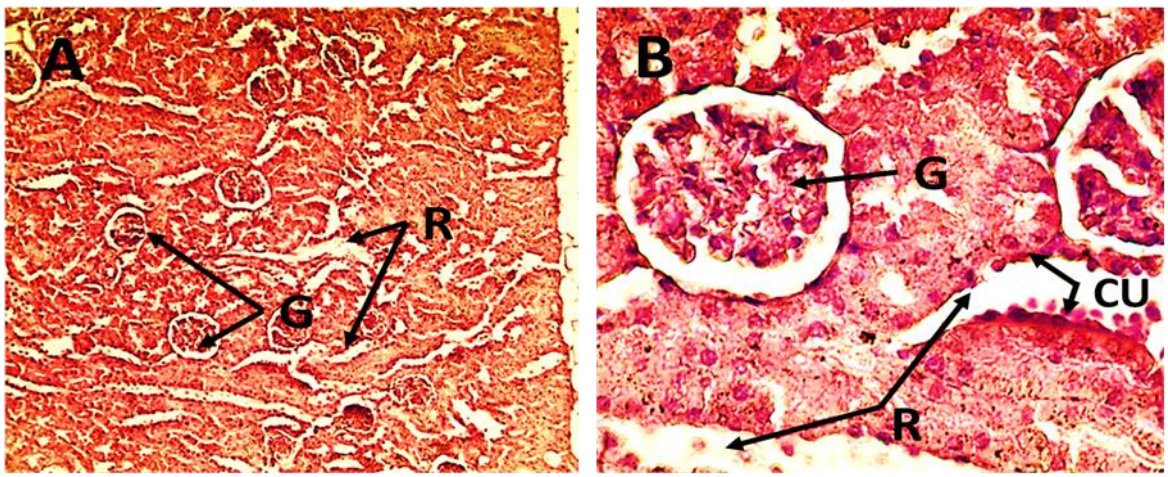

Figure 7: Section of kidney tissues in rats of control group drenched distil water. A: there is no any changes in renal tissue. Normal glomeruli (G) and normal renal convoluted tubules (R). 10x H and E. B: normal circled glomeruli with normal convoluted tubules which lined by cuboidal epithelium (CU). 40x H and E.

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Group 1 (30 mg/kg)

Histological section of kidney from rats in G1, showed mild atrophy of glomeruli with a few dilation of renal convoluted tubules with degeneration of epithelial cells which lining these tubules, figure (8, A). Furthermore, figure (8, B), showed an atrophy of glomeruli with sloughing and degeneration of epithelial lining of renal convoluted tubules.

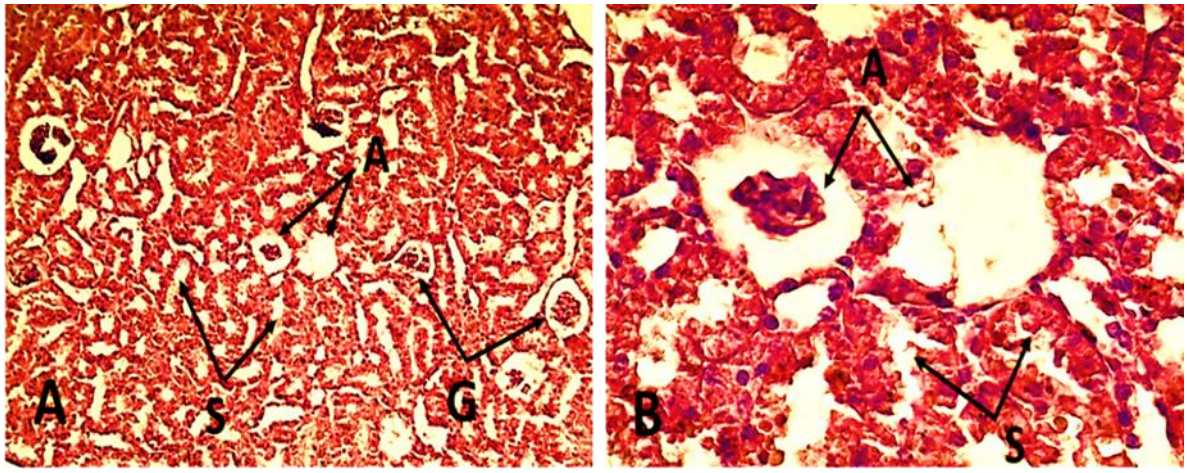

Figure 8: Section of kidney tissues rats drenched with aspirin (30 mg/kg suspended in 0.5 ml of drinking water). A:10x and B:40x A: some glomeruli showed atrophied (A) and others appeared normal (G). Mild dilatation of renal convoluted tubules (D) .10 x H and E. B: higher magnification marked with atrophy of glomeruli (A) with sloughing of renal convoluted tubules epithelium (S). 40 x H and E.

Group 2 (70 mg/kg)

In figure (9, A) the histological section presented a complete distraction and atrophy of a glomeruli with cystic dilation in the renal convoluted tubules. Moreover, there was a complete distraction of glomeruli tubule with necrosis and sloughing of epithelial cells with lining the dilation tubules, figure (9, B).

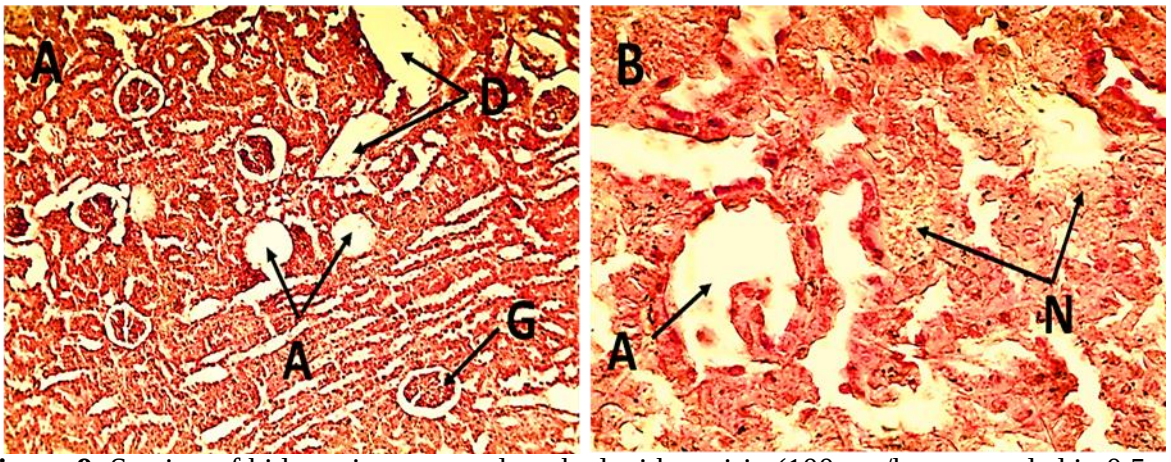

Figure 9: Section of kidney tissues rats drenched with aspirin (100 mg/kg suspended in 0.5 ml of drinking water). A: 10x and B: 40x A: some glomeruli showed complete atrophy (A) and others showed normal shape (G). Cystic dilation of renal convoluted tubules (D). 10 x H and E. B: complete atrophy of glomeruli (A) with sever necrosis of renal convoluted tubules epithelium (N).40 x H and E.

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Group 3 (100 mg/kg)

The result for the histological section obtained from rat in this group, illustrated there was a marked atrophy of glomeruli with sever necrosis and degeneration in the epithelial line of renal convoluted tubules with dilation in lumen of these tubules, figure (10, A). While, the section in figure (10, B), showed higher magnification marked atrophy of a glomeruli with necrosis in the epithelial cells with line the renal convoluted tubules, also there is hemorrhage in the renal tissues.

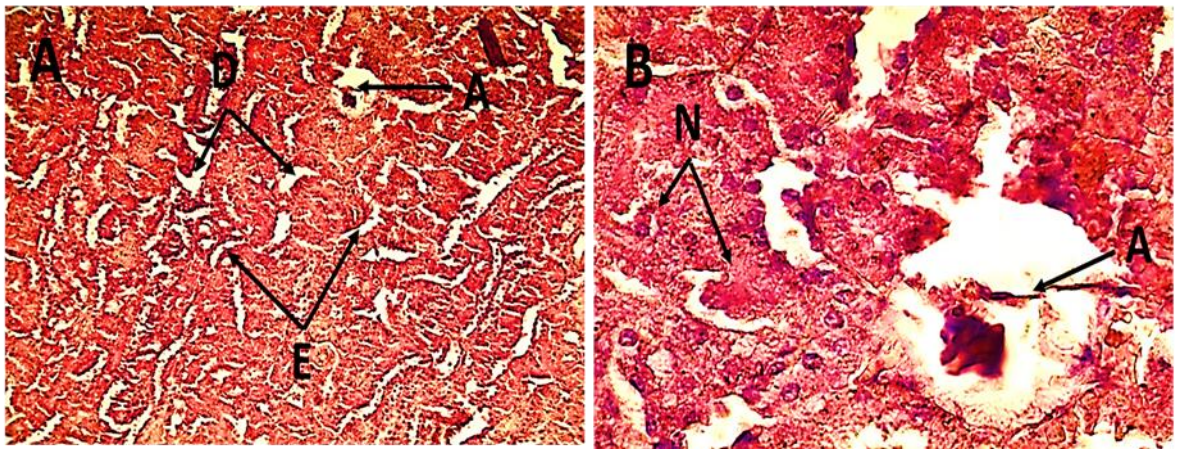

Figure 10: Section of kidney tissues rats drenched with aspirin (70 mg/kg suspended in 0.5 ml of drinking water).A: 10x and B: 40x. A: marked atrophy of glomeruli (A) with sever necrosis in epithelial cells which lining tubules € with mild dilation of renal convoluted tubules (D). 10 x H and E. B: higher magnification, with marked atrophy of glomerulus and necrosis of renal convoluted epithelium tubules (N).40 x H and E.

Group 4 (150 mg/kg)

The results demonstrated an extensive necrosis in the all renal tissues with marked atrophy of a glomeruli with cystic dilation of renal convoluted tubules, also there was hemorrhage in the renal tissues, figure (11, A). Furthermore, there was a marked atrophy of glomeruli tuft with extensive necrosis in the epithelial cells of renal convoluted tubules, figure (11, B).

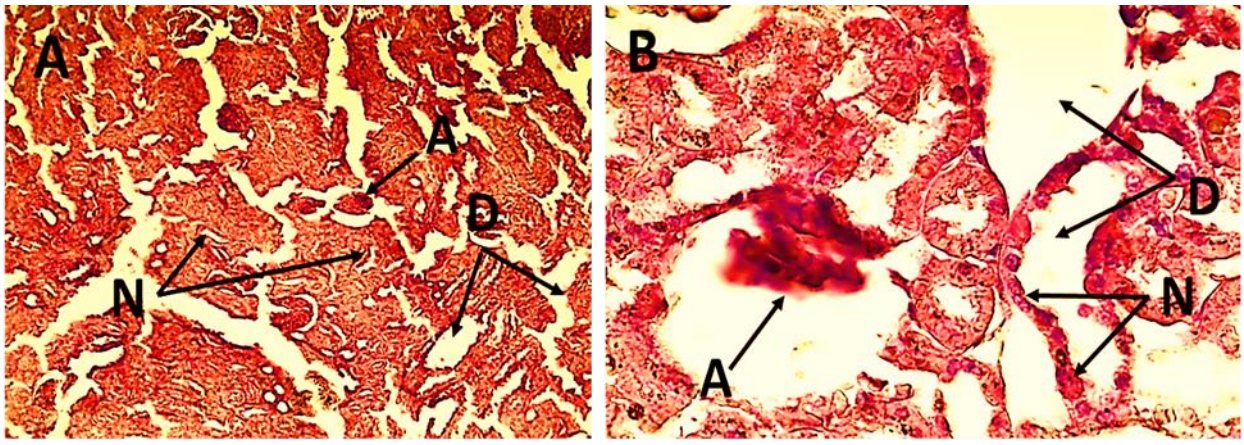

Figure 11: Section of kidney tissues rats drenched with aspirin (150 mg/kg suspended in 0.5 ml of drinking water). A: 10x and B: 40x. A: marked and severe necrosis of renal convoluted tubules with marked dilation of it. Moreover, marked atrophy glomeruli. 10x H and E. B: severe

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atrophy of glomeruli (A) and sloughing with necrosis (N) of epithelial cells which lining renal convoluted tubules (N) with dilation of these tubules. 40x H and E.

Discussion

When compared to the control group, there was a highly significant decrease between experimental groups in RBCs, Hb and PCV during the treatment period. According to the study results, RBCs, Hb and PCV values decrease in the order with rising in the aspirin dose. The results of this study in compatible with earlier study that supported the relationship between aspirin use and a reduction in PCV and Hb levels (7).

Other researchers found that low and high dose of aspirin significantly increased the total RBCs count, hemoglobin (Hb) content, and packed cell volume (PCV) relative to the control group respectively (8) . Aspirin connected to blood disorders like anemia and cytopenia in a large number of clinical investigations. Although aspirin blood problems were thought to have a modest relative risk, but there have been substantial death rates owing to aspirin-induced blood disorders (9). The cumulative effects of long-term aspirin use resulted in anemia, which consequently reduced the haemoglobin level, and lead to decrease the blood's oxygen-carrying capacity and the amount of oxygen that reached the tissues. Anemia was a side effect of aspirin that was frequently documented in older patients taking the medicine (10). Many researchers approved the relationship between the low-dose aspirin and its frequent application as a primary and secondary preventative measure for a variety of medical conditions [7-10-11]. Additionally, studies confirmed the effect of taking aspirin coupled with acute or chronic blood loss due to GI tract induced erosion. They believed that this mechanism contributed to the development of iron deficiency anemia (12).

The results showed a significant difference increase in urea and creatinine while showed decrease in Uric acid. According to several researchers, who agree with current results, urea is the most frequent waste product produced during the breakdown of proteins, and a high urea level in the blood was a reliable sign of renal disease. Elevated urea may cause by increased protein catabolism and faster amino acid deamination for gluconeogenesis (13).

However, after 2 weeks of treatment, aspirin was shown to lower the fractional excretion of uric acid, according to some other researchers who disagreed with the study's findings. A relatively significant decreased uric acid clearance and creatinine clearance was found in those aspirin therapy only. While serum creatinine and uric acid concentration, remained stable during both drug administration periods (14). The Low-doses of aspirin were associated with an increase in serum uric acid levels according to researchers who found that these doses of aspirin were could significantly decreased both creatinine and uric acid excretion within 1–2 weeks. One week after the drug was withdrawn, uric acid excretion returned to normal while creatinine clearance remained low (15). Another study found that using Mini-dose aspirin significantly changed renal function and UA management in a group of within a week in a group, mainly in those who already had hypoalbuminemia (16).

According to certain studies' findings, elevated creatinine levels are associated with decrease in renal function, particularly glomerular function. Nonsteroidal anti-inflammatory medication abuse caused damage in the kidney (17). Increased serum levels of urea, uric acid, and creatinine activities after 30 days of treatment, demonstrated the detrimental consequences of sub-chronic aspirin administration (18). The reason of increased serum levels of urea and creatinine is aspirin-induced inhibition of prostaglandin synthesis, which results in renal vasoconstriction and decreased renal perfusion (19).

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In comparison to control group, a section of kidney tissues showed mild atrophy of glomeruli with a few dilation of renal convoluted tubules in rats treated with low dosage of aspirin. Whilst with high dose of aspirin, the results showed a complete distraction and atrophy of glomeruli tubule with sever necrosis and degeneration in the epithelial line of renal convoluted tubules with dilation in lumen of these tubules finally. Different previous studies that agreed with our histological changes in kidney section and supported the results of marked atrophy of a glomerulus, severe necrosis in all renal tissues and haemorrhage in the renal tissues, accompanied with a complete distraction and sloughing of the epithelium lining of renal tubules that led to tubular necrosis and dilation, they thought that the reason may resulted from the inhibition of prostaglandin caused by aspirin treated (20). As well as lipid peroxidation increased while glutathione content, also GPx activaties decreased in liver and kidney due to aspirin (21-19-22). aspirin poisoning in fact salicylates are primarily excreted by the kidneys. therefore, renal insufficiency or failure contributed to further accumulation of salicylates which uncoupled oxidative phosphorylation at a cellular level ,producing an increased metabolic rate.in addition ,aspirin induced an oxidative stress in both liver and kidney revealed by an decrease in lipid peroxidation level associated with a decrease in enzymatic antioxidants as well as SOD, CAT, and GPx .furthermore ,the increase of lipid peroxidation ,which affected the physicochemical properties ,fluidity as well as integrity of cell membrane led to cell damage and necrosis (24 -25) .

Furthermore, atrophic alterations in the cortex of aspirin-treated rat kidneys, namely in the proximal and distal convoluted tubules, consistent with our findings. Ischemia generated by vasoconstriction of renal arterioles destroyed the medulla and tubular epithelial cells, which led to provocative alterations. This medicine suppresses the generation of prostaglandins, resulting in the unopposed constriction of arterioles, ischemia in the tubules, and cell death of the epithelial layer. Other staff members who took aspirin had a renal miscarriage; symptoms included vacuolar degradation of proximal tubules, localised tubular degeneration, and a dramatically decreased proximal tubular per unit area (23). Aspirin is anti-inflammatory (via lowering cytokines and chemokine recruitment) and antioxidant qualities were gave the drug its benefits (by scavenging free radicals, enhancing the non-enzymatic and enzymatic antioxidants, and chelating metal ions), in contrast high and prolonged aspirin use, which produced the ROS, and caused glomerular atrophy to be seen (reference). The epithelial cells that line the kidney tubules have deteriorated, and there was a haemorrhage throughout the renal tissue. Recent researches (5-16-20), demonstrated atrophic alterations in the cortex of aspirin-treated rat kidneys, namely in the proximal and distal convoluted tubules, consistent with our findings. Ischemia generated by vasoconstriction of renal arterioles destroyed the medulla and tubular epithelial cells, which led to provocative alterations. This medicine suppresses the generation of prostaglandins, resulting in the unopposed constriction of arterioles, ischemia in the tubules, and cell death of the epithelial layer. Other staff members who took aspirin had a renal miscarriage; symptoms included vacuolar degradation of proximal tubules, localised tubular degeneration, and a dramatically decreased proximal tubular per unit area (21).

Conclusion

Interestingly, the results of current research conclude that aspirin with different doses had a negative effect on the blood parameters including RBCs, Hb and PCV with demonstrated decreased their levels, whereas increased the level of urea and creatinine and decreased the level of uric acid. Furthermore, aspirin has showed further negative effect on kidney tissue from mild atrophy to extensive necrosis in the all-renal rat tissues.

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Conflict of interest

The present study has no conflict of interest.

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