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Some Hematological And Biochemical Changes Associated With Aspirin Administration In Albino Rats

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الملخص

بعض التغيرات الدموية والبيوكيميائية المصاحبة لإعطاء الأسبرين في الجرذان البيضاء

تناولت الدراسة تأثير إعطاء الأسبرين عن طريق الفم يوميا (324 ملغ/ كجم من وزن الجسم/ يوم)لمدة 10 أسابيع على صورة الدم الكاملة وبعض القياسات البيوكيميائية في ذكور الجرذان البيضاء. أظهرت النتائج بأن إعطاء الأسبرين عن طريق الفم يوميا أدى الى زيادة عامة في العدد الكلي لخلايا الدم البيضاء(WBC)، والصفائح الدموية (PLT) بلغت النسبة 9.24 و24.52٪ على التوالي. بينما ظهر انخفاض ذو دلالة إحصائية($p<0.01$) في كل من، عدد خلايا الدم الحمراء الكلي(RBC)، ومحتوى الهيموغلوبين(خضاب الدم) والهيماتوكريت و (MCV) مقارنة مع المجموعة الضابطة. كما أظهرت النتائج ارتفاع تركيزات البولينا وحمض البوليك والكرياتينين والكالسيوم والبوتاسيوم نتيجة إعطاء الأسبرين، بينما انخفضت نسبة الجلوكوز في الدم، والكوليسترول الكلي والدهون الثلاثية، و البروتينات الكلية والصوديوم وكذلك نشاط أنزيم الفوسفاتيز القلوي (ALP). بينما ازدادت مستويات الكالسيوم و البوتاسيوم.

الكلمات المفتاحية : الأسبرين، معايير الدم، أنزيم الفوسفاتيز القلوي، وظيفة الكلى، والجرذان البيضاء.

Abstract:

The present work investigated the effect of daily oral administration of aspirin (324 mg/kg body wt. /day)for 10 weeks on complete blood picture and some biochemical parameters of male albino rats. Daily oral administration of aspirin provoked a general increase in white blood cells count (WBC)and blood platelets count (PLT) with percentage changes of 9.24 & 24.52**%** compare to control respectively. Whereas, red blood cells count (RBC), hemoglobin content (Hb), hematocrit (HCT) values the mean corpuscular volume(MCV)and mean corpuscular hemoglobin(MCH)showed significantly decrease ($p<0.01$) compared to the control level.

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The concentrations of urea, uric acid, creatinine, calcium and potassium were elevated due to aspirin administration, while serum glucose, total cholesterol, triacylglycerols , total proteins, sodium and alkaline phosphatase (ALP)activity were decreased.

Key words:Aspirin, hematological parameters, Alkaline phosphatase, kidney function, albino rats.

INTRODUCTION:

Nonsteroidal anti-inflammatory drugs(NSAIDs)have been used in various forms. Their popularity is still enormous. Despite this long history and large volume, the mechanisms of how NSAIDs achieve their actions are still not completely unraveled, (Vane, 2000). NSAIDs have an analgesic and antipyretic effects. It also have, in higher doses, an anti-inflammatory effects. As analgesics, NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group of drugs are aspirin(ASA), ibuprofen, and naproxen, all of which are available over the counter in many areas (Hinz, *et al.*, 2008 and Warden, 2010).

The NSAIDs exerts their therapeutic effects through inhibition of cyclooxygenase(COX)isoform 2 (COX-2), while the inhibition of COX-1 by ASA leads to apparent side effects (Marjan, *et al.*, 2014). It probably act by inhibiting prastanoid synthesis by acetylation of fatty acid cyclooxygenase(Durand *et al.*, 2002a). Aspirin thus irreversibly blocks cyclooxygenase(COX), an effect short-lived in endothelial or smooth muscle cells due to resynthesis(Durand *et al.*, 2002b). The duration of cyclooxygenase blockade by ASA, however depends on the type of cell studied. This difference in the duration of the effect of ASA is the rationale for the use of 50 – 1500 mg/day of this drug with long inter – dose intervals in an attempt to inhibit thromboxane production in platelets (Olajide, *et al.*, 2005).

Aspirin, one of the widely used NSAIDs, is probably the most highly consumed pharmaceutical product in the world due to its low cost and high effectiveness. It is estimated that humans, around the world, consume about 120 billion aspirin tablets of 300 mg each year. Aspirin has a wide range of therapeutic uses. It is used for the treatment of inflammatory joint diseases, rheumatoid arthritis, pericarditis, Kawasaki disease, prevention of thrombosis, antiplatelets (in cardiovascular disease),analgesic,

antipyretic, rheumatic fever and many other causes (Al-Janabi, et al., 2005 and TASHSP, 2011).

Recently, aspirin has gained greater importance not only as an analgesic but also as a cardio protective drug (Mershant and Modi, 2004). Aspirin is a potentially life-saving as it is an important antiplatelet agent in the treatment of cardiovascular disease(Woessner and Simon, 2013). It has been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue(Krumholz, *et al.,* 1995 and Julian, *et al.,* 1996). Aspirin has been reported to be used in preventing heart attack and strokes (Hall and Lorenc, 2010), colorectal cancer (Manzano and Perez-Segura, 2012) as well as myocardial infarction (BNF, 2003). However, the use of Aspirin also associated with significant morbidity and mortality due to its adverse effects on multiple organ systems (Matzke,1996). Never the less, Several nonsteroidal anti-inflammatory drugs have been associated with liver damage (Lapeyre *et al.,* 2006). On the other hand, ASA is, also applied to plant fruits as preharvest treatments to increase fruit weight and ameliorate fruits quality (Giménez, *et al.,* 2014).

Numerous clinical observations have associated the use of aspirin with blood disorders like anemia and cytopenias. While the relative risk of occurrence of blood disorders with the use of aspirin is considered to be low, significant mortality rates have been reported due to blood disorders caused by the use of aspirin (Raybak, 1992). Although blood disorders with the use of aspirin have been well documented clinically, relatively few experimental studies have been conducted to clarify and confirm the association. It has been shown, by Navratil, *et al.,* (1992), that oral administration of low doses of aspirin significantly reduces circulatory erythrocyte and leukocyte counts suggesting the inhibitory action of this drug on bone marrow hemopoiesis. Merchant and Modi (2004), concluded that, aspirin in either acute or chronic doses induces anemia associated with leucocytosis in mice; the anemia does not seem to be induced due to alterations in iron metabolism. The drug appears to use multiple targets which affect red cell production and maturation processes. Results obtained by Suwalsky *et al.*,(2013)indicated that ASA interact with human erythrocytes and their molecular models in a concentration-dependent manner perturbing their bilayer structures. A large dose of salicylate stimulates corticosteroid secretion by the adrenal cortex. In connection to

this, aspirin reduces the level of corticosterone and T3 hormone that consequently declined serum cholesterol and glucose metabolism (Mohammed, *et al.,* 2010).

The present study aimed to investigate the effects of aspirin administration on blood indices and some biochemical parameters in rat's serum.

MATERIALS AND METHODS:

Twelve adult male albino rats were used in the present study weighing 120-140g. Rats were purchased from the Breeding Unit of Biology Department, Faculty of science, Islamic University of Gaza. Rats were left for one week before experimentation to adapt to laboratory conditions. Animals were housed in well-aerated cages under normal environmental conditions of temperature and humidity: temperature (25–27°C), relative humidity 40–60% and a light/dark cycle of 14 and 10 h). Animals were fed on commercial balanced diet and tap water was offered ad libitum all over the experimental period.

Animals were divided into two groups, housed six to a cage and treated as follows: The first group served as control. Animals of the second group were orally administrated with aspirin at a dose of 324mg/kg body weight/day (Abdel Gawad ,1991) all over the experimental duration of 10 weeks. Aspirin was suspended in normal saline and administered orally by gastric intubation in the dose regimes. The control animals received equivalent amounts of saline only.

Animals of both control and treated experimental groups were decapitated at the end of the experiment. Blood samples were collected in 2ml tubes containing Tri-potassium EDTA (K_3 EDTA) with first drop avoided for hematological tests and in 10ml plain tubes for serum preparation. Clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min. and then transferred into Eppendorf tubes and stored in a deep freezer (-20^{o}C) until chemical analysis. However determination of glucose, total proteins and enzyme activities were carried out on fresh serum.

A complete blood count (CBC) for albino rats were determined by the Cell-Dyn 1700 which generates the following measurements on Tri-potassium EDTA (K_3 EDTA) anticoagulant whole blood (ABBOTT laboratories, 2001) which were analyzed in Al-shifa Hospital Laboratories.

Serum samples were analyzed for glucose, total cholesterol and triacylglycerols concentrations using the methods described by Trinder

(1969), Allain *et al.*, (1974) and Fossati & Prencipe, (1982) respectively. Serum total protein was determined according to the biuret reaction as designated by Armstrong and Carr (1969). The kits were purchased from Biotech laboratories U.K. Urea determination is based upon the cleavage of urea with urease (Berthelot's reaction) according to Fawcet and Scott (1960).The kit was purchased from Boehringer Mannaheim GmbH Diagnostica. Serum uric acid was determined using the SPINREACT reagent kits and following their instruction manual described by Fossatti et al., (1980). Serum creatinine was determined without protein precipitation according to Bartels et al.,(1972)using the DiaSys reagent kits. The measurement of serum ALP activity was based on Bessey et al., (1946) method. Boehringer reagent kits were used in the previous enzyme assay. Serum electrolytes; Na^+, K^+ and Ca^{2+} levels were estimated by standard flame photometer methods (Bishop and Fody, 2005).

Data analysis:

Data were computer analyzed using SPSS version 11.0 for windows (Statistical Package from the Social Sciences Inc., Chicago, Illinois). Statistical analysis was performed by one-way analysis of variance. The differences between the means were tested by Duncan's multiple range test; $p < 0.05$ was considered as significant. Percentage change was also calculated.

RESULTS:

The result presented in table(1) showed, that oral administration of aspirin provoked a significant increase in white blood cells (WBCs) count as compared to control group at the end of the experiment. In contrast to WBCs count, the red blood cells (RBCs) count showed a highly significant decrease upon aspirin administration recording value of $5.41\pm0.11X10^6$ Cell/µL.

In general, the effect of daily oral administration of aspirin on hemoglobin (Hb)content was parallel to their action on RBC count. Mean corpuscular hemoglobin (MCH) exhibited a significant decrease in treated rats compared to control ones. The present results also showed a significant decrease in hematocrit value(HCT), and mean corpuscular volume (MCV) in treated rats compared to control ones. The oral administration of aspirin caused generally, an increase in mean corpuscular hemoglobin concentration(MCHC)and blood platelets(PLT)count with values of 2.5 and 24.5% respectively compared to control group.

The mean values of serum glucose, triacylglycerols, total cholesterol and total proteins as affected by aspirin were summarized in the table (2). Daily oral administration of aspirin for 10 weeks decreased serum glucose level by 8.9% as compared to control. Serum triglycerides, total cholesterol and total protein levels at the end of the experimental period the recorded values were 83.13, 75.11 mg/dl and 7.10 g/dl respectively for the treated rats by aspirin as compared to control.

Non protein nitrogen constituents concentration in albino rat's serum after oral administration of aspirin were tabulated in table(3), in general, oral administration of aspirin increased urea, uric acid and creatinine significantly as compared to control level. The effect of aspirin was more pronounced on creatinine. On the other hand calcium and potassium were increased as compared to control rats. However, sodium was decreased generally in treated rats. Activity of alkaline phosphatase was non significantly decreased recording percentage of change equal to 6.3% as compared to control levels table (4).

Table (1):Effect of oral administration of aspirin(324mg/kg body weight/day) on blood picture of albino rats

	Control		Treated group
RBC ($X10^6$ cell/µl)	6.66±0.19	**Mean ± S.E** **% of change** **p**	5.41±0.11 -18.77 <0.01
Hb (g/dl)	13.98±0.31	**Mean ± S.E** **% of change** **p**	9.8±0.65 -29.89 <0.01
HCT (%)	38.77±0.4	**Mean ± S.E** **% of change** **p**	26.50±0.35 -31.65 <0.01
MCV (μm^3)	58.21±0.16	**Mean ± S.E** **% of change** **p**	48.98±0.17 -15.86 <0.05
MCH (Pg)	20.99±0.17	**Mean ± S.E** **% of change** **p**	18.11±0.50 -13.72 <0.05
MCHC (%)	36.06±0.51	**Mean ± S.E** **% of change** **p**	36.98±0.69 2.55 >0.05

WBC ($X10^3$ cell/µl)	6.71±0.33	**Mean ± S.E** **% of change** **p**	7.33±0.59 9.24 <0.05
PLT ($X10^3$ /µl)	671.95±25.16	**Mean ± S.E** **% of change** **p**	836.74±31.13 24.52 <0.01

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

Table(2): Effect of oral administration of aspirin (324mg/kg body weight/day) on some chemical constituents of rat's serum

	Control		**Treated**
Glucose (mg/dl)	111.50±1.95	**Mean ± S.E** **% of change** **p**	101.59±2.13 -8.9 <0.05
triacylglycerols **(mg/dl)**	86.99±4.18	**Mean ± S.E** **% of change** **p**	83.13±4.16 -4.4 >0.05
Total Cholesterol (mg/dl)	82.19±3.13	**Mean ± S.E** **% of change** **p**	75.11±2.15 -8.6 <0.05
Total protein (g/dl)	7.91±2.10	**Mean ± S.E** **% of change** **p**	7.10±2.12 -10.2 <0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

Table (3): Effect of oral administration of aspirin (324mg/kg body weight/day) on Non- protein nitrogen constituents of rat's serum

	Control		**Treated**
Urea (mg/dl)	33.10±1.40	**Mean ± S.E** **% of change** **p**	38.67±1.70 16.8 <0.05
Uric acid (mg/dl)	2.50±1.11	**Mean ± S.E** **% of change** **p**	3.93±1.19 57.2 <0.01
Creatinine (mg/dl)	0.45±0.04	**Mean ± S.E** **% of change** **p**	1.20±0.06 166.7 <0.01

SE:Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

Table (4) Effect of oral administration of aspirin (324mg/kg body weight/day) on serum electrolytes constituents of rats

	Control		Treated
Ca^{2+} (mEq/L)	8.33±2.15	**Mean ± S.E** **% of change** **p**	8.87 6.5 >0.05
K^{+} (mEq/L)	3.10±0.13	**Mean ± S.E** **% of change** **p**	3.29 6.1 >0.05
Na^{+} (mEq/L)	134.17±5.19	**Mean ± S.E** **% of change** **p**	131.16±6.16 -2.2 >0.05
Alkaline phosphatase (U/L)	40.7±3.40	**Mean ± S.E** **% of change** **p**	38.15±4.18 -6.3 >0.05

SE: Standard error; P value P>0.05 Non-significant; P<0.05 significant; P<0.01 highly significant.

DISCUSSION:

Hematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Saliu et al., 2012). Hematological disorders as a result of aspirin ingestion are well documented clinically. The results of the present study further confirm the association, where anemia accompanied by leucocytosis was observed in rats treated with aspirin. Prolongation of bleeding time was one of the first clinically recognized hematological side effects of aspirin administration (De Geatano & Cerletti., 1988). Anemia, thrombocytopenia, agranulocytosis and leucopenia are some of the most frequently reported adverse effects of aspirin (Rayback, 1992). It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia (American Diabetes Association, 2000)

In the present study, aspirin treatment reduced hemoglobin, hematocrit (packed cell volume), and MCH, MCV. The significant reductions in HCT and Hb values caused by aspirin could indicate induction of anemia and decrease in oxygen- carrying capacity of the blood as well as the amount of oxygen delivered to the tissues respectively. Also, aspirin caused non-significant change in the MCHC value which suggest and absence of hereditary spherocytosis since MCHC values are known to be elevated in

hereditary spherocytosis . however PLT were higher than controls. These results are in agreement with that obtained by Navratil et al., (1992), Mershant and Modi, (2004) and Oyedeji, et al.,(2013). Aspirin is known to cause gastrointestinal tract erosion resulting in occult bleeding; it is also reported to reduce iron uptake from it resulting in iron deficiency (Rainsford, 1984 and Langman et al., 1994). This effect coupled with acute or chronic blood loss due to GI tract erosion induced by aspirin is believed to cause iron deficiency anemia in humans (Farrow, 1989 and Langman et al., 1994). On the other hand, the increase in WBC count in treated rats with aspirin when compared to control rats concomitant with that observed by Merchant and Modi (2004).

At the biochemical level, The obtained data showed that, aspirin decreases serum glucose levels treated with high dose of aspirin. But improved both fasting and postprandial hyperglycemia in patients with type 2 diabetes, an effect that could be attributed to decreased basal rates of hepatic glucose production ,enhanced peripheral insulin sensitivity, and decreased insulin clearance (Hundal, et al., 2002).

Concerning lipid metabolism, results demonstrate that triacylglycerols and total cholesterol level were decreased in response to aspirin oral administration to the rats. The possible explanation of these observed decrease may be attributed to an increase in Lipid peroxidation level (Gómez-Oliván,2014) and resided in direct or indirect action of aspirin on lipid metabolism (Berne and Levy, 1998).

The decreased levels of total protein in treated rats with aspirin when compared to control rats concomitant with that observed by Hundal, et al., (2002). The decrease of total protein could be attributed to an increase in amino acid deamination.

The significantly increased level of blood urea is a good indicator for kidney disordere. Urea is the principle end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is possible an acceptable to interpret the elevated levels of urea (Bishop et al., 2005). The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely, 1976).

In general uric acid content in rat's blood serum increased after aspirin administration. Uric acid is the end product of the catabolism of tissue nucleic acid, purine bases metabolism (Wolf, et al., 1972). Creatinine is the last variable of non protein blood constituents; it appears in serum in

amounts proportional to the body's muscles and is more readily exerted by the kidneys than urea and uric acid (Stryer, 1995). Elevated creatinine concentration is associated with abnormal renal function, especially as it relates to glomerular function. Chronic excess ingestion of non steroid anti inflammatory drugs causes renal disease (Bishop, et al., 2005).

Alkaline phosphatase activity exhibited general decrease in aspirin treated rats as compared to the control. The decreased activity of the alkaline phosphatase enzyme may be due to necrosis and the inflammatory reaction (Abdel Gawad, 1991). Concerning electrolytes (calcium, potassium and sodium), results demonstrate that calcium and potassium were increased but sodium was decreased in response to aspirin oral administration. Changes in kidney function and related parameters in chronic renal failure are well established in terms of elevated levels of creatinine, urea, calcium and potassium and decreased serum sodium (Kaplan and Pesce, 1989).

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