

Effect of diclofenac sodium on some biochemical parameters of male rats.

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Abstract:

The present study aimed to study the effectiveness of diclofenac sodium on some biochemical parameters of male rats. Eighteen of male rats, were utilized in this study, were divided into three groups, the first group (control group) was injected by (0.5ml /animal) of physiological saline. The second group was injected by (0.5ml/animal) of (1mg/kg) of diclofenac sodium. The third group was injected by (0.5ml /animal) of (2mg/kg) of diclofenac sodium. The animals were injected intra peritoneal for 10 days as one dose daily. The results showed no significant differences ($p < 0.05$) in the glucose level of the male rats treated with diclofenac sodium compared with the control group. The results display a significant increase ($p < 0.05$) in levels of cholesterol, triglycerides, liver enzymes (ALT) and (AST) of the male rats treated with diclofenac sodium when compared with control group, which treated with normal physiological saline. In conclusion, diclofenac sodium at high dose causes alterations in biochemical parameters in male rats. These adverse effects may be contributed to oxidative stress induced by the drug. However, the toxic effects of diclofenac sodium could be acute or reversible.

Keywords: diclofenac sodium, glucose, cholesterol, triglyceride, ALT and AST.

Introduction:

Diclofenac is a phenylacetic acid derivative that is specially formulated as a non-steroidal anti-inflammatory drug (NSAID), They compete with arachidonic acid to bind cycloxygens (COX), resulting in decreased formation of prostaglandins, This decrease effect at least partly

explains Working mechanism of the drug (Vane and Botting, 1996). Diclofenac has strength against COX-2 which is much greater than indomethacin, naproxen, or several other nonsteroidal anti-inflammatory drugs. The selectivity of diclofenac for COX-2 resembles that of celecoxib. In addition, it appears to reduce intracellular concentrations of free amino acids in leukocytes, possibly by altering release or absorption (Juni *et. al.*, 2002).

The name diclofenac derivative from its chemical name: 2-(2,6 dichloranilino) phenylacetic acid, It was first synthesized by Alfred Sallmann and Rudolf Pfister and introduced as Voltarin by Ciba-Geigy (now Novartis) in 1973 (Altman *et. al.*, 2015).

Diclofenac is absorbed efficiently from the gastrointestinal tract. Plasma concentrations peak range from 1.5 to 2.0 hours after ingestion of fasting subjects, although diclofenac has proportional short plasma life (1.5 hours), it persists in synovial fluid (Vane and Botting, 1996).

Diclofenac is analgesic, antinociceptive, antipyretic and anti-inflammatory activities, It is utilized as initial treatment for inflammatory and degenerative rheumatic diseases, as well as for painful conditions due to inflammation of non-rheumatic origin and acute attacks of gout (Vane and Botting, 1996; León-Reyes *et. al.*, 2008). It is also been shown to be efficient in treatment from Salmonella infection in mice, and is being investigated TB treatment (Dutta *et. al.*, 2007a ; Dutta *et. al.*, 2007b). Diclofenac is associated with severe gastrointestinal toxicity and several adverse effects on lung, hepatic and renal tissues (Tomic, *et. al.*, 2008).

The consumption of diclofenac has been associated with a significant increase in blood vessels and coronary artery risk in a study including coxib, diclofenac, ibuprofen and naproxen, and some complications of the upper gastrointestinal tract have been reported, Major vascular events were increased by about a third by diclofenac, mainly due to an increase in major coronary events, compared to placebo, of 1,000 patients specify to diclofenac for a year, three had major vascular events, one of which was fatal, Vascular death increased dramatically by diclofenac (Bhala and Emberson 2013).

Diclofenac may cause stomach-related side effects and nephrotoxicity, similar to other NSAIDs, by blocking prostaglandin synthesis (Yapar *et. al.*, 2008). Liver synthesized fibrinogen which is converted into fibrin and participates in coagulation, while antithrombin acts as an anticoagulant, NSAIDs inhibit thromboxane production and platelet aggregation, thus expressing anticoagulation activity (Langford and Mehta, 2006; Ganidagli *et. al.*, 2008). In addition, NSAID administration depresses the excessive production of adenosine deaminase by immune system cells during infection (Altan *et. al.*, 2010; Yazar *et. al.*, 2010).

In this study is determined the effect of diclofenac sodium (injection) at different doses on some biochemical parameters in albino rats.

Materials and Methods

Experimental animals:

The study was carried out on eighteen mature male rats (*Rattus norvegicus*), age was 10-12 weeks and weighing between 180 - 200 gm. The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. The animals were divided into three equal groups, each group consist of (6) rats:

- The first group(control group) was injected by (0.5ml/animal) from normal physiological saline (0.9%NaCl).
- The second group was injected by (0.5ml/animal) of (1mg/kg) of Diclofenac sodium.
- The third group was injected by (0.5ml/animal) of (2mg/kg) of Diclofenac sodium.

The animals were injected intra peritoneal for 10 days as one dose daily.

Biochemical parameters:

At the end of experiment, the overnight fasted animals (the control and experimental animals) were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture, 5 mL of blood samples were collected from heart and put tubes without EDTA

and centrifugation at 3000x for 15 minutes for obtained serum. The biochemical parameters included glucose, cholesterol, triglyceride, aspartate transaminase (AST), alanine transaminase (ALT) were determined by enzymatic colorimetric methods using Atlas Medical (UK).

Statistical analysis:

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ($P < 0.05$) was considered to be statistically significant. and used to calculate least significant difference (LSD) values for the comparison of means following.

The results :-

The results showed no significant differences ($p < 0.05$) in the glucose level of the male rats treated with (1mg/kg) and (2mg/kg) of diclofenac sodium groups (2 and 3) compared to control group which treated with normal saline, and the rats treated with (2mg/kg) of diclofenac sodium group (3) showed no significant differences ($p < 0.05$) in the glucose when compared with (1mg/kg) of diclofenac sodium group (2), (table 1).

Table(1): Effect of diclofenac sodium on glucose levels of male rats:

Animal groups	Glucose level (mg/dL)
Group(1) control	100.19 ± 0.48^a
Group(2) (1 mg/kg)	100.89 ± 0.74^a

Group(3) (2 mg/kg)	101.88 ± 0.47^a
LSD	2.01

Values are means ± S.E.

Different letters refer to a significant differences (p<0.05).

Similar letters refer to non-significant differences (p<0.05).

The results showed a significant increase (p<0.05) in the cholesterol and triglyceride level of the male rats treated with (1mg/kg) and (2mg/kg) of diclofenac sodium groups (2 and 3) compared with the control group which treated with normal saline, while the rats treated with (2mg/kg) of diclofenac sodium group (3) showed significant increase (p<0.05) in the cholesterol and triglyceride level when compared with (1mg/kg) of diclofenac sodium group (2), (table 2).

Table (2): Effect of diclofenac sodium on cholesterol and triglyceride levels of male rats:

Animal groups	Cholesterol level (mg/dL)	T.G level (mg/dL)
Group(1) control	70.41 ± 0.78^c	17.40 ± 0.95^c
Group(2) (1 mg/kg)	83.32 ± 0.67^b	26.52 ± 0.72^b
Group(3) (2 mg/kg)	87.16 ± 0.36^a	38.54 ± 0.96^a
LSD	2.36	4.69

Values are means ± S.E.

Different letters refer to a significant differences (p<0.05).

Similar letters refer to non significant differences (p<0.05).

The results showed a significant increase ($p < 0.05$) in level of ALT of the male rats treated with (1mg/kg) and (2mg/kg) of diclofenac sodium groups (2 and 3) compared with the control group which treated with normal saline, while the rats treated with (2mg/kg) of diclofenac sodium group (3) showed no significant differences ($p < 0.05$) in the level of ALT when compared with (1mg/kg) of diclofenac sodium group (2) (table 3).

The results showed a significant increase ($p < 0.05$) in level of AST of the male rats treated with (1mg/kg) and (2mg/kg) of diclofenac sodium groups (2 and 3) compared with control group which treated with normal saline, while the rats treated with (2mg/kg) of diclofenac sodium group (3) showed significant increase ($p < 0.05$) in the level of AST when compared with (1mg/kg) of diclofenac sodium group (2), (table 2).

Table (3): Effect of diclofenac sodium on liver enzyme levels of male rats:

Animal groups	ALT level (U/IL)	AST level (U/IL)
Group(1) control	16.68 ± 1.26^b	20.12 ± 0.79^c
Group(2) (1 mg/kg)	32.59 ± 1.43^a	34.21 ± 1.00^b
Group(3) (2 mg/kg)	33.21 ± 1.00^a	59.56 ± 0.42^a
LSD	9.30	3.59

Values are means ± S.E

Different letters refer to a significant differences ($p < 0.05$).

Similar letters refer to non significant differences ($p < 0.05$).

Discussion:

The results showed no significant differences ($p < 0.05$) in the glucose level of the male rats treated with with (1mg/kg) and (2mg/kg) of diclofenac sodium groups (2 and 3) compared with the control group which treated with normal saline.

The results showed a significant increase ($p < 0.05$) in the cholesterol and triglyceride level of the male rats treated with diclofenac sodium compared with the control group. These findings concur with earlier investigations on the elevation of lipid levels the triglycerides, total cholesterol levels in rats due to DFS (Das and Roy, 2011). Drug-induced hepatotoxicity has been shown to be associated with high cholesterol levels and triglycerides (Pejic and Lee, 2006; Iweala *et. al.*, 2011). The present study demonstrated a significant elevation in lipid profiles of rats treated with DFS. A marked increase in serum triglycerides and serum cholesterol was observed in DFS-treated rats (Baravalia *et. al.*, 2011). The results demonstrated a significant elevation ($p < 0.05$) in level of ALT and AST of the male rats treated with diclofenac sodium compared with the control group. Biochemical investigations thus suggest that administration of diclofenac sodium at various dose levels has significant effect on liver and kidney functions, there was dose dependent significant rise in the serum levels of AST, ALT indicating pathological changes in the hepatobilliary and nephric system of significant nature, This could be related to lesions in the intestine, liver, reduced food intake and absorption, this results were Similar also observed in mice, rat, rabbit (Sakr, 1996; Anonymous, 1999; Hickey, 2001; Dadhaniya, 2007).

Significant changes happen in serum level of ALT, AST after diclofenac management, these enzymes are functional indicators of liver, Functional and structural alteration in liver leads to elevation of these enzymes in circulation, AST, ALT are found in liver cells, these enzymes are intracellular and are being situated in mitochondria or cytoplasm or

both and when cell's function altered, break or destroyed, the enzyme escapes into the blood (Breen *et al.*, 1986).

Diclofenac sodium (DFS) was used to induce liver damage as it has been reported to be hepatotoxic (Gupta *et al.*, 2004). The hepatoprotective effects of DFS induced liver injury in rats were assessed. The hepatotoxic effects of DFS in both humans and experimental animals have been well-documented (Aydin *et al.*; 2003). DFS has been shown to produce liver damage leading to alterations in biomarkers of liver function, lipid profile and endogenous antioxidant status of liver tissue, One of the most sensitive and exciting indicators of hepatocyte injury is the release of intracellular enzymes such as transaminases and serum alkaline phosphatase into the circulation (Aydin *et al.*, 2003). Induction of hepatic injury with diclofenac in rats resulted in severe hepatotoxicity as reflected by an increase in the serum levels of GOT, GPT and ALP, It was observed that DFS elevated all these enzymes significantly, indicating severe hepatic cell necrosis (Ahmed and Khater, 2001). These results were agreed with Ahmad *et. al.* (2012).

In conclusion, diclofenac sodium at high dose causes alterations in biochemical parameters in male rats. These adverse effects may be contributed to oxidative stress induced by the drug. However, the toxic effects of diclofenac sodium could be acute or reversible.

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الخلاصة

هدفت الدراسة الحالية إلى دراسة فعالية ديكلوفيناك الصوديوم على بعض المعايير البايوكيميائية في ذكور الجرذان المختبرية. حيث تم استخدام ثمانية عشر حيوان من ذكور الجرذان المختبرية في هذه الدراسة، وتم تقسيمها إلى ثلاث مجموعات، حقنت المجموعة الأولى

(مجموعة السيطرة) بواسطة (0,5 مل/حيوان) من المحلول الفسيولوجي. كما تم حقن المجموعة الثانية بتركيز (0,5 مل/حيوان) من (1 ملغم/كلغم) من ديكلوفيناك الصوديوم. كما تم حقن المجموعة الثالثة بتركيز (0,5 مل/حيوان) من (2 ملغم/كلغم) من ديكلوفيناك الصوديوم. حقنت الحيوانات داخل الغشاء البريتوني ولمدة 10 أيام وبواقع جرعة واحدة يوميا. اظهرت النتائج عدم وجود فروق معنوية في مستوى الجلوكوز لدى ذكور الجرذان المختبرية المعاملة بالديكلوفيناك الصوديوم مقارنة مع مجموعة السيطرة. كما اظهرت النتائج وجود زيادة معنوية في مستويات الكوليسترول والدهون الثلاثية وانزيمات الكبد في ذكور الجرذان المختبرية المعاملة بالديكلوفيناك الصوديوم عند مقارنتها مع مجموعة السيطرة التي عوملت بالمحلول الفسيولوجي. نستنتج ان الجرعة العالية من ديكلوفيناك الصوديوم قد يسبب تغيرات في بعض المعايير البايوكيميائية في ذكور الجرذان المختبرية ويمكن أن تسهم هذه الآثار السلبية في الإجهاد التأكسدي الناجم عن المخدرات ومع ذلك، فإن الآثار السمية لديكلوفيناك للصوديوم يمكن أن تكون حادة.