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Rabbits' biochemical and hematological validation after the traditional use of diclofenac sodium and dexamethasone combination

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التحقق من صحة الأرانب الكيموحيوية والدموية بعد الاستخدام التقليدي لمزيج ديكلوفيناك الصوديوم و ديكساميثازون

الملخص

أجريت هذه الدراسة لتقييم تأثير مزيج من الأدوية شائعة الاستعمال في قطاع غزة (ديكلوفيناكالصوديوم(SD) وديكساميثازون(DM))على بعض القياسات الدموية والكيموحيوية للأرانب عند جرعات مختلفة. تم تقسيم إثنان وعشرون من الأرانب عشوائيا إلى3 مجموعات، أعطيت المجموعة الأولى(10أرانب)1مل محلول ملحي في العضل وأبقى كمجموعة ضابطة، أما المجموعة الثانية(6أرانب) فلقد تلقت31 جرعة من أدوية البحث، بينما أعطيت المجموعة الثالثة)6أرانب(40جرعة،(علماً بأن الجرعة تتكون من SD بجرعة 3ملجم/كجم وزن الجسم مرة واحدة في اليوم بالاضافة ل DM بجرعة 1ملجم/كجم وزن الجسم مرة واحدة في اليوم لمدة 31 يوما).إن إعطاء SD وDM لهذه الفترة أوضح انخفاض ملحوظ في هيموجلوبين الدم، كرات الدم الحمراء، الهيماتوكريت، وقيمMCHC والصفائح الدموية وتغييرات واضحة في الزيادة من WBC، MCV وMCH. وعلاوة على ذلك كان هناك انخفاض كبير في مستويات البروتين الكلي والألبومين والجلوبيولين، وزيادة في أنشطة إنزيمات ALT وALP وAST. بالإضافة لذلك، كانت هناك زيادة كبيرة في الكرياتينين واليوريا ومستويات حمض اليوريك، ونقص واضح في نسبة الجلوكوز والكوليسترول ومستويات الدهون الثلاثية(التراي اسيل جليسيرول). يمكن الاستنتاج من هذه الدراسة أن إعطاءSD وDM لفترة طويلة ينتج عنها بعض الآثار السلبية على القياسات الدموية والكيموحيوية الخاصة بوظائف الكبد والكلى ، ويمكن أن يعزى ذلك إلى جهد الاكسدة التي تسببها هذه الأدوية.

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Abstract

This study was carried out to evaluate the effect of a widely used combination in Gaza Strip, diclofenac sodium (SD)and dexamethasone (DM), at different doses on some rabbits' hematological and biochemical parameters. Twenty two rabbits were randomly divided into 3 groups, the first one (10 rabbits) was given 1ml saline intramuscularly and kept as control. The second group (6 rabbits) received 31doses while the third group (6 rabbits) was given 40 doses of the combination SD / DM (SD at a dose of 3 mg / kg b.wt. i. m. once/day and DM at a dose of 1 mg / kg b.wt. i. m. once/day for 31 days. Administration of SD and DM for this period induced a significant decrease in Hb, RBCs, hematocrit, MCHC and platelets values and an obvious changes in the increment of WBC, MCV and MCH Values. Moreover, there were a significant decrease in serum total protein, albumin and globulin levels, and an increase in the activities of amino transeferases (ALT), alkaline phosphatase (ALP), and serum aspartate amino transferase (AST). In addition there were significant increment in creatinine, urea and uric acid levels and an obvious decrement in glucose, cholesterol and triacylglycerol levels. It could be concluded that administration of SD and DM at long interval dose induced some adverse effects on hematological and biochemical parameters of liver and kidney. That could be attributed to oxidative stress induced by the combined drugs.

Key words: Dexamethasone, Diclofenac sodium, rabbits, Biochemical, Hematological , Gaza Strip.

Introduction:

Dexamethasone(DM)is a potent synthetic member of the glucocorticoid class of steroid hormones. It acts as an anti-inflammatory and immunosuppressant (Jeklova et al.,2008), its potency is about 20-30 times that of hydrocortisone and 4-5 times of prednisone. DM can be used for a whole lot of purposes therapeutically, some of its therapeutic use include; Anti-inflammatory, oncologic uses, endocrine and obstetric purposes. It can also be used for diagnostic purposes(Cheville, 2006). A rapid cardiovascular effects of dexamethasone in rabbits with meconium-induced acute lung injury was detected by(Daniela et al.,2008). DM is contraindicated in: existing gastrointestinal ulceration, Cushing syndrome, severe forms of heart insufficiency, severe hypertension, uncontrolled diabetes mellitus, system tuberculosis, severe systemic viral, bacterial and fungal infections, it was hypothesized that it delayed wound healing by inhibiting mitotic cell

division (Adams, 2001). Peers and Flower in 1991, was reported a site of anti-inflammatory action of dexamethasone in rabbit skin.

Diclofenac sodium(SD)is a non-steroidal anti-inflammatory drug (NSAID) that works by reducing hormones that cause inflammation and pain in the body (Kumar et al., 2003). It is a phenyl-active derivative with different pharmacological features. It has anti-inflammatory, anti-pyretic and analgesic activities. SD was developed specifically as an anti-inflammatory agent and has been used in the treatment of canine osteoarthritis(Aliu, 2007). It is well absorbed orally but undergoes subsequent first pass elimination, such that only 50% is available systematically. The drug is 99% protein bound, metabolized in the liver and excreted both in urine and bile. The plasma half-life is about 2hours. SD has good tissue penetrability and accumulates in synovial fluid. The concentration in synovial fluid is maintained for 3 times longer period than in plasma, thus exerting extended therapeutic action in joints. The urine is the primary route of excretion for the drug and its metabolite (Aliu, 2007). There are many side effects observed with the use of this drug. It is not recommended for pediatric uses, while its overdosing is potentially toxic. SD causes a rare but potentially fetal hepatotoxicity that may be associated with the formation of reactive metabolites and subsequent adverse hepatitis effects may arise in certain individuals (Bhogaraju et al., 1999) SD provokes proliferation of bile duct, hepatocellular degeneration, non-specific hepatitis with portal and lobular activity. It also elevates markedly transaminases levels, decreases the glycogen content of the hepatocytes and impaires ATP synthesis by the mitochondria with futile consumption of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)(Bort et al., 1991). Some studies have shown that SD accounts for the thickening of the glomerular basement membranes together with mild focal tubular necrosis and intraluminal secretions in the proximal convoluted tubules (Farag et al., 1996). Other less frequent effects on the urinary tissues include haematuria, proteinuria, interstitial nephritis, lipid peroxidation and papillary necrosis (Farag et al., 1996). SD has been also reported to damage moderately the seminiferous tubules and to impair spermatogenesis (Steitiea et al., 1994). Although data about the SD/DM combination is rare, the effects of dexamethasone, diclofenac, or placebo on the inflammatory response after cataract surgery was investigated by Laurell and Zetterström (2002). Other

study regarding this combination was investigated the therapy in experimental corneal injury (Laria et al., 1997).

Finally, the present study aims to investigate the hematological and biochemical effects of a widely used combination in Gaza Strip, diclofenac sodium (SD) and dexamethasone (DM), at different doses on some rabbits' hematological and biochemical parameters.

Materials and methods:

Experimental animals and dosing:

The used adult rabbits in the present study were weighing 1000-1500g. They were purchased from local markets. Rabbits were left in the animals house for 1 week before experimentation to adapt to laboratory condition. They were kept in plastic cages with wire mesh covers and maintained under the following conditions: temperature (20ºC– 21ºC), relative humidity (40% - 60%) and a light /dark cycle of 14 and 10 hours. The cages were freshly spread by wood saw to absorb urine of animals. Rabbits were given free access to commercial balanced diet and water ad libitum all over the experimental period. Animals were divided into three groups, as follows : the first group was comprised 10 rabbits and given 1 ml saline intramuscularly and kept as a control. The second group (6 rabbits) received 31doses and the third (6 Rabbits) group was given 40 doses of SD at a dose of 3 mg / kg b.wt. i. m. once/day and DM at a dose of 1 mg / kg b.wt. i. m. once/day for 31 days and for 40 days (Sebnem et al., 2005). All chemicals used were of analytical grade and were procured from Sigma Chemical Company Germany. Diclofenac sodium amp. and Dexamethasone amp. were purchased from Gaza local pharmacies.

Blood sampling and processing:

Control and treated rabbits were decapitated at the end of 31 and 40 days. Blood was collected in dry centrifuge tubes. Sera were separated and kept at -20ºC until analysis. However, determinations of enzyme activities were carried out on fresh serum samples, On the other hand, about 2 mL of blood samples were collected in a tube containing dipotasium ethylene diamine tetra acetate (EDTA) for the hematological tests.

Measurement of biochemical and blood indices:

Serum glucose, triacylglycerol and total cholesterol were determined using the method described by Trinder (1969); Fossati et al.,1980 and Allain et al., 1974. Serum urea measurement was based on the cleavage of urea

with urease (Berthelot's reaction) as described by Faweet and Scott (1960), serum uric acid was determined following the method described by Fossati and Prencip(1982).Serum creatinine was measured without protein precipitation according to Bartels and Bohner (1972), serum total protein was described by Biuret reaction as designed by Armstrong and Carr, 1964. The kits were purchased from Biotech laboratories, UK. Serum albumin was determined using RANDOX reagent kits, following their instruction manual according to the method described by Doumas et al., 1971. The concentrations of globulins (g/dL) were equal to total protein – albumin. The activities of serum AST and ALT were determined according to the method described by Gloiser and Mager, 1972. The measurement of serum ALP activity was based on the method of Bessey et al., 1946 and Perry et al., 1983.

Hematological parameters:

Determination of hematological parameters were carried out using an 18 automated parameter hematology analyzer. ABX Micros 60 from Horiba ABX. France.

Data analysis:

Data were computer analyzed using SPSS version 13.0 for windows (Statistical Package for the Social Sciences lnc. Chicago, Illinois, USA). Means were compared by independent-samples test followed by Duncan's multiple range test(DMRT), $p<0.05$ were considered as significant. Percentage change was also calculated.

Results:

Serum glucose and lipid profile mean values of rabbits affected by the combination of SD (3mg/kg/day) and DM (1mg/kg day) administration are summarized in table1. The data revealed that administration of this combination doses for 31 days decrease significantly serum glucose level by -11.08% at 31 doses and -5.63% at 40 doses as compared to the control level. Triacylglycerol and cholesterol were decreased by treatment by drugs to be – 19.09 % at 31 doses , -9.87% at 40 doses and -5.99 % at 31 doses , -0.55 % at 40 doses, respectively.

Table (1): Effect of diclofenac sodium (3mg/kg/day)and dexamethasone (1mg/kg day) administration on glucose and lipid profile of rabbits

Parameters	Experimental period		
	Control n=10	Treated (31 dose) n=6	Treated (40 dose) n=6
Glucose (mg/dl) **% change** **P value**	90.2±0.25	80.21 ± 0.22 -11.08 P < 0.05	85.12 ± 0.20 -5.63 P > 0.05
Cholesterol (mg/dl) **% change** **P value**	200.10±0.36	188.11± 0.33 -5.99 P > 0.05	199.0± 0.31 - 0.55 P > 0.05
Triacylglycerol (mg/dl) **% change** **P value**	100.10±0.20	80.99±0.25 -19.09 P < 0.01	90.22± 0.26 - 9.87 P > 0.05

All values were expressed as mean ± S.E;P<0.05 significant; P<0.01 highly significant.

Data in Table(2)showed that administration of the combination of SD (3mg/kg/day) and DM (1mg/kg day) for 31 days increased urea by 8.35% at 31 doses , but at 40 doses it has a significant increase by 13.58%. Uric acid was increased by 6.28% at 31 doses and by 7.25% at 40 doses, while the effect of the combination was highly significant on creatinine concentration by 23.71% at 31 doses and 28.87% at 40 doses, as compared to control level

Table (2):Effect of diclofenac sodium(3mg/kg/day)and dexamethasone (1mg/kg/day) administration on urea; uric acid and creatinine of rabbits

parameters	Experimental period		
	Control n=10	Treated (31 dose) n=6	Treated (40 dose) n=6
Urea (mg/dl) **% change** **P value**	34.25±0.60	37.11 ± 0.69 8.35 P > 0.05	38.90 ± 0.40 13.58 P < 0.05
Uric acid (mg/dl) **% change** **P value**	4.14±0.21	4.40± 0.26 6.28 P > 0.05	4.44± 0.25 7.25 P > 0.05
Creatinine (mg/dl) **% change** **P value**	97.10±0.04	1.20±0.03 23.71 P < 0.01	1.25± 0.02 28.87 P < 0.01

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant

Results presented in Table(3)indicated that administration of SD (3mg/kg/day)and DM (1mg/kg/day)increased the activities of ALP, ALT and AST at 4.86%, 3.32% and 4.25% respectively at 31 doses , but at 40 doses the increase were 7.52%, 6.23% and 6.18%, respectively. On the other hand, it was found that administration of the combination was highly significant decreased ($p< 0.01$) for serum total protein concentration by -16.99 % at 31 doses and by -33.72 % at 40 doses as compared to control level. Also, serum albumin concentration under the influence of the combination for 31 days was insignificantly decreased by -5.74 % at 31 doses and highly significant decrease by -21.45 % at 40 doses. Results in table (3) showed a significant decrease in globulin concentration after SD and DM treatment by -12.12 % at 31 doses and a highly significant decrease by -23.94 % at 40 doses as compared to control level .

The data in (table 4) represent blood indices in rabbits after administration of SD (3mg/kg/day) and DM (1mg/kg/day) for 31 days. The more obvious changes resulted from the administration of the combination were the highly significant increase in WBC count, MCV and MCH by 59.00% , 93.75% and 82.37%, respectively at 31 doses , and by 72.54% , 91.94% and 78.18%, respectively at 40 doses. Data also reveal that there were highly significant decreases in RBC count, hemoglobin(Hb), hematocrit, PLT count, and significant decrease in MCHC by -61.95% , -30.61% , -26.24% , -48.45% and -5.93%, respectively at 31 doses , and by -62.66% , -33.47% , -26.24%, -49.71% and -7.19% , respectively at 40 doses compared to control levels.

Table(3):Effect of diclofenac sodium(3mg/kg/day)and dexamethasone (1mg/kg/day)administration on enzymes activities and protein profile of rabbits

parameters	Experimental period		
	Control n=10	Treated (31 dose) n=6	Treated (40 dose) n=6
ALP (U/L) **% change** **P value**	40.15±0.15	42.10 ± 0.39 4.86 P > 0.05	43.17 ± 0.11 7.52 P > 0.05
ALT (U/L) **% change** **P value**	34.00±0.16	35.13± 0.16 3.32 P > 0.05	36.10± 0.14 6.23 P > 0.05

AST (U/L) **% change** **P value**	30.11±0.22	31.39±0.20 4.25 P > 0.05	32.15± 0.19 6.18 P > 0.05
Total Protein (gm/dl) **% change** **P value**	7.71±0.26	6.40±0.21 -16.99 P < 0.01	5.11± 0.19 -33.72 P < 0.01
Albumin (gm/dl) **% change** **P value**	3.91±0.15	3.5±0.14 -5.74 P > 0.05	2.6± 0.17 -21.45 P < 0.01
Globulin (gm/dl) **% change** **P value**	3.80±0.10	2.90±0.12 -12.12 P < 0.05	2.51± 0.11 -23.94 P < 0.01

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant

Table (4): Effect of diclofenac sodium (3mg/kg/day) and dexamethasone (1mg/kg/day) administration on hematological parameters of rabbits

Parameters	Experimental period		
	Control n=10	**Treated (31 dose) n=6**	**Treated (40 dose) n=6**
WBC count ($x10^3$cell/ul) **% change** **P value**	7.51±0.18	12.10±0.30 59.00 P <0.01	13.13±0.24 72.54 P <0.01
RBC count ($x10^6$ cell/ul) **% change** **P value**	5.65±0.21	2.15±0.24 -61.95 P <0.01	2.11±0.19 -62.66 P <0.01
Hb (g/dl) **% change** **P value**	11.89±0.15	8.25±0.19 -30.61 P <0.01	7.91±0.16 -33.47 P <0.01
Hematocrit (%) **% change** **P value**	37.50±0.22	27.66±0.26 -26.24 P <0.01	26.88±0.24 -31.27 P <0.01
MCV (fi) **% change** **P value**	66.37±0.32	128.65±0.40 93.75 P <0.01	127.39±0.31 91.94 P <0.01
MCH (pg)	21.04±0.23	38.37±0.29	37.49±0.26

% change **P value**		82.37 P <0.01	78.18 P <0.01
MCHC (g/dl) **% change** **P value**	31.71±0.27	29.83±0.22 -5.93 P >0.05	29.43±0.24 -7.19 P >0.05
Platelets(x10^3 cell/ul) **% change** **P value**	388.0±29.11	200.0±36.30 -48.45 P <0.01	195.11±25.5 -49.71 P <0.01

[All values were expressed as mean±S.E; P<0.05 significant; P<0.01 highly significan

Discussion:

Although diclofenac sodium(SD)and dexamethasone(DM) combination is one of the most frequently prescribed non-steroidal anti-inflammatory drugs (NSAIDs) worldwide for the treatment of inflammation and pain; data on the biochemical and hematological alterations on rabbits are limited, therefore, the present study was designed to identify the effects of these combination, at different doses, on some rabbits' hematological and biochemical parameters.

In comparison with the respective control rabbits, there was a general decrease in serum glucose levels in rabbits in response to number of SD/DM administration. It was found that SD/DM may indirectly, decreases the glycogen content of the hepatocytes and impaires ATP synthesis by the mitochondria(Bort et al., 1991). The decrement observed in serum triacylglycerol and cholesterol content in response to number of treatment by SD/DM administration drugs; take place in the liver due to imbalance between the normal rabbits of lipid synthesis, utilization and secretion. The possible explanation of these observed decrement may be reside on the action of SD/DM combination on lipid metabolism or lipid peroxidation (Farag et al., 1996).

Exposure to therapeutic increasing doses of SD/DM produced a significant increase in urea, the principal end product of protein catabolism an accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated level of urea. The increment in blood urea, might be also due to the destruction of RBCs during the treatment. The presence of some toxic compounds might increase blood urea and decrease plasma protein , on the other hand the elevation of blood urea might suggest that animals experienced hemo-concentration due to

mild dehydration (Bhogaraju et al., 1999). Moreover, the serum uric acid levels exhibited an increment in the treated rabbits by increasing the experimental duration. This may be due to high degradation of purines or an increase of uric acid level by inability of its excretion by urinary system (Aliu, 2007). In comparison with the respective control rabbits, there was a highly significant increase in creatinine levels in response to number of SD/DM doses administrated (Bort et al., 1991).

The obtained results indicate that long exposure to SD/DM combination, produces a clear increment in serum AST, ALT and ALP in treated rabbits as compared to control. The liver enzymes are normally found in circulation in small amounts because of hepatic growth and repair. As a liver specific enzyme ALT only significantly elevated in hepatobiliary disease. Increase in AST level, however can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma (Aliu, 2007) .

The liver and kidney are responsible for SD and DM metabolism and excretion. It may cause hepatotoxicity and nephrotoxicity during its metabolism (Bhogaraju et al., 1999). Consequently, elevated ALT and AST activities observed in the current study in response to SD/DM administration could be a common sign of impaired liver function. On the other hand, alkaline phosphatase belongs to a group of enzymes catalyze the hydrolysis of phosphomonoesters at alkaline pH. ALP present in cell surface in most human tissues. The highest concentration are found in the intestine, liver, bone, spleen and kidney (Bhogaraju et al., 1999). The specific location of the enzyme with both sinusoidal and bile canalicular membranes accounts for the more predominant elevations in certain disorders as observed in the present study with SD/DM administration. Impaired secretion of hepatic ALP of liver cell origin (Aliu, 2007).

Acute cell necrosis liberate ALP in the circulation and serum enzyme level is elevated. However, the activities of these enzymes were reduced after the recovery period. SD/DM induced oxidative stress has lowered, however, the cellular injury may still persist as indicated by increased AST, ALT and ALP activities. Exposure to therapeutic increasing doses of SD/DM produced a highly significant decrease of total protein, albumin and globulin levels in rabbits. This decrease in serum total protein may be due to lowered synthesis of albumen and globulin in the liver in response to SD/DM intake. It was reported that albumin levels are decreased in liver disease (Aliu, 2007).The decrease in these blood proteins of the rabbit may be due to

usage of different amino acids in the production of antibodies in response to SD/DM administration (Cheville, 2006).

The obtained results indicate that long exposure to SD/DM combination, produces a clear increment of WBCs, MCV and MCH values. This Highly significant increase in WBCs count indicated the activation of defense mechanism and immune system of rabbit. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals (El-Maddawy and El-Ashmawy, 2013). Leukocytosis was manifested by lymphocytosis, which was the main features of the differential leukocytic count.

It was found that Exposure to therapeutic increasing doses of SD/DM produced a highly significant decrease in the red blood cells (RBCs), Hb, Hematocrit and platelets count . This finding may be explained on the basis of inhibitory effect of SD/DM on histogenesis. The decreased in RBC count and hemoglobin (Hb) lowered the oxygen supply to different tissues thus resulting in low energy production. Decrease in Hb contained MCHC can be explained due to decreased in size of RBCs or impaired biosynthesis of heme in bone marrow (El-Maddawy and El-Ashmawy, 2013).

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