The effect of subacute exposure of prednisolone in liver and kidney functions in males albino rats

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Abstract
This study is dealing in animal house in college of Medicine / Kufa University. Which is conducted to study the effect of subacute exposure of prednisolone drug on some liver enzymes (ALT,AST and AP), and Blood Urea (BU) by administration of different doses of prednisolone in (18) of Albino Swiss males rats by stomach tube for duration (21) days.

The results showed significantly increase in serum level of (ALT) for (T2) group of animals at level (p ≤ 0.01) in comparison to (T1)&(C) groups of animals, while the serum level of (AST) revealed significantly increase in both (T1&T2) group of animals at level (p ≤ 0.01) in comparison with (C), furthermore, the levels of (BU) showed significantly increase at level (p ≤0.01) in (T2) group of animals in comparison with (T1)&(C), but
there is no significantly changes in serum levels of (AP) at level (p ≤0.05) between all groups of treatment and control.
The histopathological examination for livers & kidneys revealed mild degeneration with cytoplasmic vacuolation in livers of (T₁) group animals, while there are multinecrosis areas in livers of (T₂) group animals.
In contrast with control group which had no histopathological changes, while the biopsy or (histopathological) changes in kidneys of (T₂) group animals appeared extensive tubular necrosis with areas of hemorrhage.

Introduction:
Prednisolone is a synthetic corticosteroid drug with predominantly glucocorticoid and low mineralocorticoid activity (1). It is a white, crystalline powder, odorless; slightly soluble in water, alcohol, chloroform and dioxane. Its chemical designation (C₂₁H₂₈O₅) and defined as: 11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione (Fig. 1). (1, 2)

Figure 1. Structure of Prednisolone (C₂₁H₂₈O₅)

Prednisolone is used to treat a wide variety of acute and chronic disorders, including arthritis, asthma, allergic diseases, hepatitis, congenital adrenal hyperplasia, systemic lupus erythematosus and certain haematological, infectious, cardiac, dermal, neurological, metabolic, gastrointestinal (GI) diseases, replacement therapy, graft (kidney transplantation as immunosuppressive), as well as malignant diseases and many inflammatory states. Furthermore, it is used in treatment of severe shock. (3, 4)
However prednisolone is thought to act by the induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. (5, 6) Following oral administration prednisolone is rapidly absorbed from the gastrointestinal tract, the systemic availability is almost complete and reported to range from 75% to 98% .(6, 7, 8) and a volume of distribution (Vd) 0.22 – 0.70 L/kg . (9, 10, 11)
Prednisolone is pharmacologically active and may be metabolized in a variety of tissues, including liver, lung, kidney, and skin. (12, 13) the serum half-life of it is known to be (2-4) hours, (3, 6) and with clearance has been reported to be 111 mL/min/1.73 m². (10). The possible side effects of prednisolone are fluid retention of the face (moon face, Cushing’s Syndrome), acne, constipation and mood swings. A lengthy course of prednisolone can cause bloody or black tarry stools, muscle weakness, pain in back, hips, ribs, arms, shoulders, or legs, thin, shiny skin and urinating at night. (6, 10)
Prednisolone is used intravenously at very high doses for the treatment of severe diseases like shock, and it is used over a wide dose range, low dose therapy is considered to include doses up to 10 mg prednisolone per day, being most commonly prescribed at approximately 40 - 80 mg/day. (1, 2)
In such study (15) patients administrated oral prednisolone (25 mg twice daily) found that it had a relative anti-inflammatory potency four times to induce remission in immune thrombocytopenia of hydrocortisone.
Prednisolone was administrated with dose 50 mg/day for 6 days after that, they found increased amino nitrogen concentrations 3.8±0.1 mmol/l (patients received prednisolone) vs 3.5±0.1 mmol/l (Control patients) at level of significance (p ≤ 0.05). (16) also basal Urea Nitrogen Synthesis Rates (UNSR) values were significantly increased following prednisolone administration 51.2±6.3 vs 23.3±6.5 (Control patients)
at level \( p \leq 0.05 \), prednisolone administration led to increase levels of amino acids in blood and loss of nitrogen as urea, and the functional hepatic nitrogen clearance (FHNC) is denoted to hepatic kinetics of conversion amino acids to urea nitrogen, that mean prednisolone increased the ability of amino nitrogen conversion into urea nitrogen.\(^{16, 17}\)

Prednisolone administration increased (FHNC) from 24.6±4.7 l/h (Control patients) to 47.3±5.9 l/h (patients received prednisolone).\(^{16}\) long standing treatment with prednisolone at 30 mg/day in a group of patients showed increases in Aspartate amino-transferase (AST) levels at 129 IU/L, Alanine amino-transferase (ALT) levels 183 IU/L ,\(^{17, 18}\)

In such study \(^{19}\) that found chronic non- supprative destructive cholangitis involving epitheloid granuloma formation with plasma cell infiltration in biopsy were performed from patients received prednisolone for a long duration of treatment with 30 mg/day .

There is no absolute maximum dosage, however, intensity and frequency of adverse events are observed to rise with increasing dose. Prednisolone is not considered to be a narrow therapeutic index drug.\(^{1}\)

The aim of the present investigation was to study the effects of subacute exposure of prednisolone on liver enzymes [Alanine amino-transferase (ALT), Aspartate amino-transferase (AST), and Alkaline Phosphatase], and Blood Urea. Furthermore, study the histopathological changes in Livers and Kidneys of animal groups.

**Material and method :-**

Eighteen Albino Swiss rats weighing (150 ± 15) gm were subjected and maintained under uniform environmental condition at temperature (22-25) c\( ^\circ \) with a dark / light cycle 14 / 10 hours and supplied with feed and water ad-libitum.

The animals were divided into three equal groups as T\(_1\), \(T_2\), and Control (C), that received prednisolone\(^*\) for twenty one (21) days orally through stomach tube subsequent doses as (1.2) mg / kg B.W. as therapeutic dose \(T_1\) and (3.6) mg / kg B.W. as three folded dose (as high therapeutic dose) \(T_2\) while, the animals of Control (C) group dosed with distilled water for the same period . At the end of dosing period , blood collected from the heart of animals and centrifuged with 5000 round per minute (rpm) for five minutes to obtain serum for determination liver enzymes and serum urea . Certain biochemical tests for evaluation liver functions through estimation of liver enzymes such as Alanine amino-transferase (ALT)**, Aspartate amino- transferase (AST)**, the pyruvate which produces by transaminase of (ALT) react with 2,4-Dinitrophenyl hydrazine (NAPH) to give colored hydrozones, while oxaloacetate which produces by (AST) decarboxylates spontaneously to pyruvate, in both reaction measured colorimeter at 510 nm\(^{20}\), then apply the following equations :-
\[(T-C / S-B) \times 0.4 \times 1 / 30 \times 1000 / 0.1 \quad \text{(ALT)}\]
\[(T-C / S-B) \times 0.4 \times 1 / 60 \times 1000 / 0.1 \quad \text{(AST)}\]

\(T = \text{test}\)
\(C = \text{control}\)
\(S = \text{standard}\)
\(B = \text{blank}\)
\(0.4 = \text{normality of NaOH}\)
\(30, 60 = \text{time of pyruvate formation}\)
\(0.1 = \text{amount of serum}\)
\(1000 = \text{pyruvate formed per litter}\)

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and Alkaline Phosphatase (AP)* estimation through colorimeter determination of librates phenol in the presence of 4-amino antipyrine and potassium ferricyanide (21), then the following equation applied:

\[\frac{\text{Optical density of Sample}}{\text{Optical density of Standard}} \times n\]
\(n = 20\)

also blood urea by urea – kit** enables end point enzymatic determination of urea concentration (Conc.) (urease - modified Benhelot reaction) in serum, urease hydrolyzes urea by producing ammonium which is formed green color indophenol in an alkaline medium when reacts with salicylate and hydrochloride, the color intensity is proportional to the urea Conc. in sample (22), then the following equation applied:

\[\text{Sample Conc.} = \frac{[\text{Absorption of Sample}]}{[\text{Absorption of Standard}]} \times n\]
\(n = \text{Conc. of Standard}\)

Biopsy of livers and kidneys from all treated and Control animals sent to histopathological study presented in 10 % formalin.

**Statistical Analysis:**

Analysis of variance (ANOVA) one way and least significant differences (LSD) under significance (p ≤ 0.05) for Alkaline phosphatase while other parameters are under significance (p ≤ 0.01) to compare data of different groups throughout the period of experiment (23).

**Results:**

There are no signs of toxicity i.e. (no muscular tremor, no lacrimation, no salivation and no convulsions) appeared in all treated and Control animals and they were normal posture and normal food and water intake throughout the period of experiment.

Statistical analysis showed significant increased at level (p ≤ 0.01) in Alanine aminotransferase (ALT) of (T2) group animals in comparison with both (T1 & Control animals), while there is no significant changes in

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serum level (ALT) of both (T1) group of animals and Control group (Table 1),
while the serum level of Aspartate amino-transferase (AST) achieved significant
increases in both T1 & T2 group of animals in comparison to Control
group under the significance (p ≤0.01) (Table 1).

But, there are no significant changes in serum level of Alkaline Phosphatase (AP) at
level (p ≤0.05) between all animal groups (T1, T2 and Control)(Table 1).

The Blood Urea (BU) level showed significant increase in animals of (T2) group at
level (p ≤0.01) in comparison of T1 and C groups, while there is no significant
changes between T1& C groups (Table 1).

Table (1) : The effects of subacute exposure of prednisolone on the liver enzymes and
blood urea in males rats.

- T1 dose = 1.2 mg / kg B.W. (therapeutic dose).
- T2 dose = 3.6 mg / kg B.W. (three folded dose).
- Different small letters mean there are significant differences between groups at level
(p ≤0.01) for all parameters except Alkaline Phosphatase (AP) at level (p ≤0.05)
- n = number of animals .

Histopathological Examination :-

Revealed mild degeneration with cytoplasmic vacuolation in livers of animals that
received therapeutic dose (T1), (Fig. 2) , while there are multinecrosis areas in livers of
animals received three folded dose of prednisolone (T2), (Fig. 3). In contrast with
control animals which reveal no pathological changes. The histopathological changes of
kidney appeared only in the animals received three folded dose (T2) of prednisolone
which include extensive tubular necrosis with area of hemorrhage (Fig. 4).

Figure 2. Liver of male rat received therapeutic dose of prednisolone (T1) (X400).
Figure 3. Liver of male rat received three folded dose of prednisolone (T2) (X400).
Figure 4. Kidney of male rat received three folded dose of prednisolone (T2) (X400).

Discussion :-

For the last three decades prednisolone have been widely used in a variety of disease
states. The basis of glucocorticoid therapy, for example in patients undergoing kidney
transplantation, is largely empirical, and at a clinically acceptable dosage balanced
between graft maintenance and adverse effects, then the differences in the kinetic
behaviour of prednisolone may be one reason for disturbance of this balance, but the
intra-individual consistency
of their kinetic behaviour has been poorly investigated. (1, 2)

There are no signs of toxicity i.e. (no muscular tremor, no lacrimation, no salivation
and no convulsions ) appeared in all treated and Control animals and they were normal
posture and normal food and water intake throughout the period of experiment, because
of prednisolone acting within cells to prevent the release of certain chemicals that are
important in the immune system, These chemicals are normally involved in producing
immune and allergic by decreasing the release of these chemicals in a particular area
(inflammation is reduced). This can help control a wide number of disease states. (1)
The increasing in Alanine amino-transferase (ALT) in T₂ animals group (which received three folded dose of prednisolone) at 174.3±10 at level p ≤0.01 is agreed with study of (Ito et al., 2003) (18) who found increase in ALT at 183 IU/L and may be due to release of enzyme (ALT) which is found in high concentration in cytoplasm of hepatocytes which were found mutinecrosis areas of liver (24) (Fig. 3), also the increasing of Aspartate amino-transferase (AST) in both T₁ and T₂ animals group at 91.3±5.1, 151.1±12.2 respectively is agreed with study of (Ito et al., 2003) (18) and may be due to the release of the small amounts of enzymes from the damaged hepatocytes which are detected in the liver biopsy of both group of animals (T₁ and T₂), the levels of these enzymes may be used clinically to provide an index of the extent of liver damage, and may be due to another effect of the drug on cardiac muscles (24, 25), while the Blood Urea (BU) level was increased only in T₂ group of animals at 29.3±1.2 may be due to up – regulate hepatic urea synthesis (16, 17) and basal Urea Nitrogen Synthesis Rates (UNSR) and increased the ability of amino nitrogen conversion into urea nitrogen (15, 16, 17) and also due to extensive tubular necrosis with areas of hemorrhage in kidney that appeared in biopsy of kidney of (T₂) group of animals (Fig. 4) which may be led to renal insufficiency which (consequently) resulted an increase (BU) levels.

References :-