

## ORIGINAL ARTICLE

# Protective Role of Olive Oil and Polyherbal Drug on Renal Dysfunction Induced by Dexamethasone in Albino Rats

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## Abstract

*This study was plan to evaluate the biochemical effects of olive oil and polyherbal drug against dexamethasone-induced renal dysfunction in a male albino rat. Male rats (20) were divided into four equal groups: Group 1: rats were injected subcutaneously with normal saline and consider as normal control. Group 2: rats were injected subcutaneously with dexamethasone (0.1 mg/kg body weight). Group 3: rats were injected dexamethasone (0.1 mg/kg body weight) subcutaneously, and then treated with olive oil (200 mg/kg body weight) by oral gavage. Group 4: rats were injected subcutaneously with dexamethasone (0.1 mg/kg body weight), and then treated with the polyherbal drug in a dose of 200 mg/kg body weight by oral gavage. After 3 weeks, serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) activities, blood urea nitrogen (BUN) and creatinine levels were estimated. Administration of dexamethasone caused elevation of serum levels of Creatinine, BUN, ALT, AST, Glucose, Hb1Ac and Albumin activities. Treatment with Olive oil and polyherbal drug showed a significant increase in the body weight of rats in the group treated with olive seed extract orally compared with the dexamethasone control group. Olive oil and polyherbal drug positively affect dexamethasone-induced renal alteration in albino rats.*

**Keywords:** Renal alteration, hepatic, dexamethasone, Olive oil, polyherbal drug

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## Introduction

Increasing rates of obesity, diabetes mellitus and arterial hypertension suggest that the total number of patients affected by chronic kidney disease (CKD) will further increase shortly. Chronic kidney disease (CKD) means that kidneys are damaged, and it cannot filter blood as they suppose to do. This damage can cause wastes material to increase in the body. This may also cause other problems that can harm other organs. High blood pressure and diabetes are the most common causes of CKD. Whereas life expectancy and quality in CKD patients decrease significantly during the progression to end-stage renal disease (ESRD), the incurred

costs due to renal replacement therapy (RRT) increase dramatically after that <sup>[1]</sup>. Therefore, prevention and/or delay of RRT and reduction of CKD complications, mainly CVD, remain compelling goals to be achieved <sup>[2]</sup>. Twenty-six million people in America have chronic kidney disease (CKD). Anaemia, cardiovascular diseases, secondary hyperparathyroidism, renal osteodystrophy and other complications are common in CKD.

Olive oil, extracted from the fruit of *Olea europaea* L. (Oleaceae family), is composed of a glycerol fraction, constituting approximately 90–99 %, and of a non-glycerol or unsaponifiable fraction (0–4–5 %). The pulp of olives contains these compounds, which are

hydrophilic, but they are also found in the oil. The class of phenols includes numerous substances, such as simple phenolic compounds like vanillic, gallic, coumaric and caffeic acids, tyrosol and hydroxytyrosol and more complex compounds like the secoiridoids (oleuropein and ligstroside), and the lignans (1-acetoxypinoresinol and pinoresinol). The major phenolic compounds in olive oil are hydroxytyrosol tyrosol and oleuropein,. The phenolic compounds present in olive oil are radical scavengers and strong antioxidants.<sup>[3]</sup> Methods for the evaluation of the olive oil polyphenols content Hydroxytyrosol, tyrosol, vanillic, caffeic, syringic, p-coumaric, ferulic, cinnamic and elenolic acids olive oil can provide many health benefits for patient's suffering with cardiovascular diseases and CKD. e.g., high blood pressure is a dangerous complication for patients with kidney disease. Olive has the function of decreasing patient's blood pressure. Besides, because there are abundant substances in olive oil, such as squalene, nitric oxide, monounsaturated fatty acid. This affects of reducing the risk that can lead to cardiovascular diseases. Olive oil also has an obvious effect in promoting patient's blood circulation, and some substances in it also can prevent the formation of thrombus.

The dexamethasone is a corticosteroid that is used in the treatment of many cases of inflammatory diseases, and in certain bowel disorders, and certain cancers, and also to prevent chronic lung disease in immature newborns. Previous studies on the rats showed that the use of prenatal dexamethasone in gestation lead to a reduction in glomerular number and glomerulosclerosis, and hypertension<sup>[4]</sup> and in other research showed that the dexamethasone in toxic dose leads to renal failure<sup>[5]</sup>. In previous research, we found that the olive oil has a protection against the effect of oxidative stress induced by oxidant agent as mercuric chloride and protects from HgCl<sub>2</sub> induced renal dysfunction<sup>[6]</sup>. In this research, we studied the effect of olive oil and polyherbal drug on the renal disease induced by dexamethasone.

## Materials and Methods

### *Olive Oil Preparation*

Olive oil was purchased commercially from a local market in Hail City, KSA. The olive oil

was filtered (0.22 µm sterile filters) using a vacuum pump, then stored in a brown bottle at 4 °C until use.

### *Polyherbal drug Preparation*

For producing the polyherbal drug, eight types of the herbs were purchased from local market of Hail (KSA) following confirmation of the complete morphology under microscopy. The polyherbal drug was prepared by mixing and grinding of the eight herb (Table I). The herbs (Total 270 g) were boiled in 1 litre distilled water for three hours, at 80°C and subsequently filtered. The resultant filtrate was stored at 4°C in the dark until required.

### *Drugs and Chemicals*

Dexamethasone (4 mg/mL) was used in this research work. Dexamethasone was diluted with normal saline and administered subcutaneously to the selected group of animals. All chemicals used in our study were of analytical grade.

### *Animals*

The twenty male albino rats of the Wistar Strain weighing 180–200 g used for this study were got from the animal house of the College of Pharmacy, University of Hail, Hail KSA. They were housed at 25 ± 2 °C, 12 h:12 h dark and light cycle. Animals were provided with a standard food and water ad libitum. Rats were acclimated to laboratory conditions for ten days before dosing. The experimental work on male rats was carried out in accordance with the Institutional Scientific and Research Ethics Committee, College of medicine, Hail University.

Twenty adult male rats were divided randomly into four equal groups: Group 1: animals were injected subcutaneously with normal saline daily for threeweeks and consider as normal control. Group 2: rats were injected subcutaneously with dexamethasone daily for threeweeks in a dose of 0.1 mg/kg body weight. Group 3: animals were injected subcutaneously (0.1 mg/kg body weight) dexamethasone, and then treated with olive oil in a dose of 200 mg/kg body weight by oral gavage daily for 3weeks. Group 4: animals were injected subcutaneously (0.1 mg/kg body weight) dexamethasone, and then treated with the polyherbal drug in a dose of 200 mg/kg body weight by oral gavage daily for 3week.

**Table 1:** Herbs used for the preparation of polyherbal drugs

| Herbs              | Scientific names            | Amount in gms |
|--------------------|-----------------------------|---------------|
| Cumin              | <i>Cuminum cyminum,</i>     | 50            |
| Black seed         | <i>Nigella sativa,</i>      | 50            |
| Fenugreek          | <i>Trigonella foenum</i>    | 50            |
| Flax seeds         | <i>Linum usitatissimum,</i> | 50            |
| Cinnamon           | <i>Cinnamomum verum,</i>    | 25            |
| Funnel seed, saunf | <i>Foeniculum vulgare,</i>  | 25            |
| Ajwain             | <i>Trachyspermum ammi,</i>  | 10            |
| Turmeric           | <i>Curcuma longa</i>        | 10            |

**Sample collection and tissue preparation**

The blood samples were collected before sacrifice and were used for various tests by using a standard protocol. The rats were sacrificed by cervical dislocation under light ether anaesthesia. Plasma and serum were separated and stored at -20°C for the further biochemical test. The relevant body parts such as kidney were removed rapidly for homogenate preparation of tissue. The homogenate were centrifuged at 4°C to separate the nuclear debris and were used for estimation.

**Biochemical Examination**

The glucose level in serum was assayed using the glucose oxidase method<sup>[4]</sup>. Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were assayed according to Reitman and Frankel<sup>[6]</sup> using reagent kits purchased from UDI diagnostic company (Jeddah, Saudi Arabia).

**Biochemical analysis**

Measurement of serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), blood urea nitrogen (BUN) Glucose, Album, Hb1Ac and creatinine: Blood samples were centrifuged at 3000 rpm for 5 min. Sera were collected, and the levels of ALT, AST, BUN, Albumin and creatinine were measured using a Genesys 10S US Vis. Chemistry Analyzer.

The following analytes were studied:

- Metabolites: Glucose, Albumin, Creatinine and BUN.
- Enzymes: ALT and AST.

1. Quantitative determination of aspartate aminotransferase (AST) in serum using a UV kinetic method<sup>[7]</sup>.
2. Quantitative determination of glutamate pyruvate transferase (ALT) in serum using a UV rate method<sup>[7]</sup>.
3. The Creatinine concentration was estimated by the Jaffe reaction method which measures the Creatinine concentration in the serum<sup>[8]</sup>.
4. The Urea concentration was estimated by the colorimetric enzymatic assay of urease method which measures the urea concentration in the serum as described by Berthelot<sup>[9]</sup>.

**Kidney and Body weight changes**

Body weight of rats will be measured weekly. Kidney weights were measured after scarifying the rats.

**Evaluation of renal function**

Glomerular filtration rates (GFR) were estimated through the creatinine and blood urea nitrogen. Proteinuria which is a sensitive indicator of renal damage at the glomerulus or tubular epithelium was measured as total protein in serum and glomerular filtration as implicated in several studies.

**Histopathological analysis of tissues**

Kidney and pancreas were stored for future histopathological examinations.

**Statistical analysis**

All analyses were done using SPSS version 18.0 (Chicago, IL). Data were given as mean values ± SEM. Two groups were compared using Student's t-test. The Kolmogorov-Smirnov test was used to assess the normality of numeric variables. The results are defined as Mean ± Standard Deviation (SD). Differences between groups were obtained by using one-way analysis of variance (ANOVA) tests with Tukey-Kramer HSD as post-ANOVA tests. A p-value of ≤ 0.05 was considered statistically significant; Significance was expressed at p ≤ 0.05.

**Results**

The results showed significant (P<0.05) increase in the serum glucose concentration in

Dexamethasone group compared to the control group (Table 2)

But no significant increase observed in the serum glucose concentration of the other experimental groups compared to the control group. There was significant increase observed in the serum BUN concentration of the experimental groups compared to the control group.

The effect of inclusion of *Dexamethasone* on rat serum AST and ALT activity is presented in Table [2]. The results showed significant (P<0.05) increase in the serum AST and ALT activity in Dexamethasone group compared to the control group (Table 2). But no significant increase observed in the serum AST and ALT activity of the other experimental groups compared to the control group.

With the exception of *Dexamethasone* group there was no significant (P<0.05) changes in serum Hb1Ac% between the experimental groups and the control group.

The effect of inclusion of Olive oil; Olive + Dexamethasone; polyherbal + dexamethasone and only dexamethasone on rat serum is presented in Table [2].

The results showed significant (P<0.05) increase in the serum albumin concentration in olive and *Olive + Dexamethasone* groups compared to the control group (Table 2). But no significant increase observed in the serum albumin concentration of the other experimental groups compared to the control group. polyherbal + dexamethasone group resist the change in serum parameter as compare to dexamethasone group.

**Table 2:** The effect of inclusion of Olive oil and polyherbal drugs on rat serum parameters.

| Group                               |    | Glucose | Creatinine | BUN  | AST   | ALT  | Hb1Ac | Albumin |
|-------------------------------------|----|---------|------------|------|-------|------|-------|---------|
| Control                             |    | 81.67   | 0.7        | 45   | 6     | 8.1  | 2     | 3.1     |
| Group 1 (Olive)                     | A1 | 78      | 0.8        | 53.9 | 6.8   | 8.6  | 2.7   | 3.8     |
|                                     | A2 | 79.8    | 0.7        | 53.4 | 5.5   | 7.1  | 3.9   | 4.5     |
|                                     | A3 | 76.2    | 0.6        | 53.7 | 5.7   | 6.0  | 3.3   | 5.1     |
| Group 2 (Olive + Dexamethasone)     | B1 | 136.1   | 1.1        | 53.2 | 5.9   | 6.6  | 3.7   | 6.4     |
|                                     | B2 | 148.2   | 1.8        | 53.5 | 3.5   | 7.9  | 2.7   | 3.8     |
|                                     | B3 | 142.6   | 1.1        | 53.6 | 3.5   | 6.7  | 3.4   | 5.3     |
| Group 3 (polyherbal+ Dexamethasone) | C1 | 62.9    | 1.4        | 53.8 | 8.84  | 7.8  | 4.2   | 3.3     |
|                                     | C2 | 63.0    | 1.8        | 53.4 | 7.0   | 6.4  | 5.4   | 3.3     |
|                                     | C3 | 73.4    | 1.2        | 53.7 | 10.6  | 6.3  | 5.6   | 3.3     |
| Group 4 Dexamethasone               | D1 | 184.8   | 2.9        | 64.1 | 281.3 | 26.3 | 8.7   | 3.2     |
|                                     | D2 | 173.3   | 3.4        | 73.1 | 243.9 | 30.5 | 8.0   | 3.4     |
|                                     | D1 | 166.3   | 3.2        | 64.0 | 270.5 | 32.8 | 9.4   | 3.9     |

**Table 3:** Effect of inclusion of Olive, Olive + Dexamethason, polyherbal+ Dexamethasone , and Dexamethasone on serum glucose, Creatinine, BUN, AST, ALT, Hb1Ac, and Albumin for 3 weeks. Mean ± SE

| Parameter          | Control        | Olive         | Olive+ Dexamethasone | Herbal+ Dexamethasone | Dexamethasone    |
|--------------------|----------------|---------------|----------------------|-----------------------|------------------|
| Glucose (mg/dl)    | 86.023ab± 3.90 | 99.33a± 31.67 | 119.00a± 62.08       | 129.63a ± 76.12       | 254.80ac± 179.73 |
| Creatinine (mg/dl) | 0.67± 0.14     | 0.70± 0.09    | 1.33a± 0.36          | 2.13a± 0.27           | 3.17a± 0.23      |
| BUN (mg/dl)        | 42.67± 2.88    | 53.67 a± 0.23 | 53.43a± 0.19         | 53.63a± 0.19          | 67.07ac± 4.67    |
| AST ratio(U/L)     | 6.17± 0.34     | 4.67± 3.30    | 7.63± 6.40           | 8.81± 1.61            | 231.90a± 40.96   |
| ALT (U/L)          | 7.97± 0.37     | 4.57± 3.38    | 14.73± 14.85         | 6.83± 0.75            | 29.87 a± 2.95    |
| Hb1Ac%             | 2.00± 0.27     | 4.97± 1.76    | 2.93± 0.96           | 5.07± 0.68            | 8.37a± 1.10      |
| Albumin (g/dl)     | 3.03± 0.27     | 4.47 a± 0.58  | 5.17a± 1.17          | 3.30± 0.00            | 2.83± 0.54       |

a,b,c: Row means with no common superscript differ significantly at (P<0.05)

## Discussion

The present study was conducted to find out therenoprotective effects of polyherbal drug and olive oil on dexamethasone-induced renal disease in male albino rats. Polyherbal formulations are abundantly used in developed countries as compared to allopathic medicine for the treatment of different types of ailments. We formulated Polyherbal drug which contains a variety of herbs including *Cuminum cyminum*, *Nigella sativa*, *Trigonella foenum*, *Linum usitatissimum*, *Cinnamomum verum* etc. This Polyherbal formulation includes various herbs such as *Nigella sativa*, *Trigonella foenum* that has been reported to have a protective effect on renal diseases<sup>[10,11]</sup>. Each of these herbs has been reported to possess different types of flavonoids, therefore exhibiting nephron-protective actions when given in combination.

Fenugreek (*Trigonella foenum*), responsible for antioxidant anti-inflammatory action that hinders in many signalling pathways and thus protecting body from many diseases including renal diseases.

Cinnamon (*Cinnamomum verum*) has strong ability on dyslipidemia associated with hypercholesterolemia. It reduces cholesterol and TG levels<sup>[12]</sup>. *Cumin* (*Cuminum cyminum*) has been reported as anti-obesity. Therefore, it is responsible for decrease body weight and decreases serum cholesterol, triglycerides, uric acid and creatinine<sup>[13,14]</sup> that was also observed during sub-acute toxicity.

During the sub-acute toxicity study non-significant changes in haematological and biochemical parameters of lipids and renal markers were observed depicting that these polyherbal drugs could be safely employed for the treatment of renal disorders.

The current study reveals the protection and the dose-response effect conferred by olive oil and polyherbal drug against dexamethasone-induced oxidative stress in experimental male albino rats.

The administration of dexamethasone significantly decreased the body weight and relative kidney and liver weights at the end of the experimental period. These results were in accordance with the findings of Eason et al; 2000 and Prashant 2012.<sup>[15,16]</sup>

Olive oil and polyherbal drug treatment repressed dexamethasone-induced weight loss and showed a conversely marginal increase in body weight. The improvement of body weight gain after olive oil and polyherbal drug treatment.

For assessment of the effect of olive oil and polyherbal drug, we have studied ALT, AST, BUN, Albumin and creatinine, which are usually elevated in the manifestation of the renal disease. In our study, administration of dexamethasone led to a significant elevation in AST and ALT activities, and conversely to a decrease in albumin level. These results are in accordance with other researches<sup>[17,18]</sup>.

Elevation of ALT and AST enzyme is attributed to the damaged structural integrity of the kidney.<sup>[19]</sup> Additionally, the decreased level of albumin after dexamethasone administration may be due to its damaging effect on RNA and DNA. Treatment with olive oil and polyherbal drug to dexamethasone-exposed rats produced significant improvement in kidney functions, indicating the beneficial role of olive oil and polyherbal drug to counteract the dexamethasone-induced renal dysfunctions. These results are in accordance with other scientist<sup>[20,21]</sup>.

In our study, the dexamethasone-administered male rats showed a significant elevation in blood glucose level, this agrees with previous studies.<sup>[16]</sup> A dose-dependent decrease in glucose levels was observed in olive oil and polyherbal drug treated rats compared to the dexamethasone control.

It was also found that the rats treated with dexamethasone showed a significant elevation in serum BUN and creatinine levels. Treatment of rats with olive oil and polyherbal drug resulted in a significant improvement in BUN and creatinine levels. These findings agree with<sup>[22]</sup>, who showed the ameliorative effects of grape seed on serum kidney functions of paracetamol-induced hepatotoxicity in rats.

Accordingly, this is because olive oil and polyherbal drug have free radical scavenging activities and antioxidant, therefore protecting against replenishing the reduced glutathione content and oxidative stress. These findings agree with results reported by various researchers<sup>[23-25]</sup>, who reported that oral administration of olive oil and polyherbal drug

ameliorated and enhanced the antioxidant defence against Ehrlich solidtumour-induced oxidative stress in mice.

## Conclusion

Our findings suggested that dexamethasone is capable of triggering marked oxidative stress. Supplementation with polyherbal drug and olive oil is undeniably an ameliorative and it has definite therapeutic actions against dexamethasone-induced oxidative stress.

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**Conflict of Interest:** None declared

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**Ethical permission:** Obtained

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