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***PROTECTIVE EFFECT OF ALFALFA (MEDICAGO SATIVA L.)  
LEAVES AGAINST HYPERURICEMIA ON EXPERIMENTAL RATS***

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## **PROTECTIVE EFFECT OF ALFALFA (*MEDICAGO SATIVA L.*) LEAVES AGAINST HYPERURICEMIA ON EXPERIMENTAL RATS**

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### **Abstract**

**Objective:** The aim of this study was to investigate the possible antihyperuricemic effect of alfalfa leaves powder and extract as a natural antioxidant on aspirin-induced hyperuricemia rats.

**Methods:** Thirty five of white albino male rats (Sprague-Dawley strain), weighing  $120 \pm 5$ g, Rats were randomly distributed into 5 groups each containing 7 rats: negative control, hyperuricemic (positive untreated), hyperuricemic + allopurinol (5 mg/100g b.w/rats), hyperuricemic + alfalfa powder (10 g/kg/diet/day), hyperuricemic + alfalfa extract (1 ml/ kg/ b.w/ rats /day) were given to the rats for 4 weeks. At the end of the study, rats were anaesthetized by diethyl ether and sacrificed and blood samples were collected for biochemical examinations.

**Results:** Uric acid, creatinine, urea levels were increased in positive control group (hyperuricemic) compared with the negative control group and decreased in all the treatment groups. In addition increased levels of MDA and decrease activity of SOD and total antioxidant in positive group (10.17, 17.96 and 1.70, respectively) were reversed with alfalfa powder and extract therapy. An increase in of AST, ALT, and ALP activities corrected by treatment with alfalfa powder and extract were observed in hyperuricemic rats.

**Conclusion:** The findings of this study suggest that treatment with alfalfa powder (10g/kg/diet/day) and extract (1ml/kg/b.w/rats /day) ameliorates metabolic derangement by enhancing uric acid levels and the antioxidant defense against aspirin-induced hyperuricemia in rats, the antioxidant activity may be related to their phenolic compounds.

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**Key words:** Hyperuricemia; *Medicago sativa*; phenolic compound; Uric acid; Total antioxidant capacity; Superoxide dismutase

## **INTRODUCTION**

Hyperuricemia is a common metabolic disorder and frequently accompanied by oxidative stress and elevation of serum uric acid level in the blood. Hyperuricemia considered as a major risk factor for development of gout, which has been associated with many diseases such as kidneys dysfunction, hypertension, cardiovascular diseases, diabetes, hyperlipidemia and metabolic syndrome (**Gibson, 2012 and Simão *et al.* 2012**). Hyperuricemia may manifest as articular pain or tumefaction or it may be with no symptoms. Impaired renal excretion of uric acid rather than uric acid overproduction, considered to be the main cause of hyperuricemia (**Hikita *et al.* 2011**). The excessive serum uric acid crystallizes and deposits in the joints and leads to recurrent inflammatory arthritis (**Eggebeen, 2007**). The incidence rates of hyperuricemia and gout have increased rapidly over the past decade, which is believed to be associated with the change in life styles and diet structure, influence from environmental factors and the use of certain medications, mainly including acetylsalicylic acid, diuretics, and ciclosporin, the latter of which is known to reduce the kidney clearance of urate (**Terkeltaub, 2010 & Kielstein and Tausche, 2016**).

Non-steroidal anti-inflammatory drugs (NSAID) are commonly used as medication with an expensive pecuniary encumbrance worldwide. Aspirin (acetylsalicylic acid) is one of the widely used nonsteroidal anti-inflammatory drug, 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. It is used for various pathological conditions such as inflammatory joint diseases, rheumatoid arthritis, pericarditis, Kawasaki disease, prevention of thrombosis, coronary artery disease, analgesic, antipyretic, rheumatic fever and many other causes (**Al - Janabi *et al.* 2005 and Tashsp, 2011**). However, the use of Aspirin also associated with significant morbidity and mortality due to its adverse effects on

multiple organ systems, including renal dysfunction, renal cell cancers and hepatotoxicity (**Li et al. 2012 and Vyas et al. 2016**).

Since any injury in human body is associated with an imbalance of oxidative stress and antioxidant defense system, theoretically it would be possible to limit oxidative damage and ameliorate disease progression by natural antioxidants supplementation (**Zincă and Vizireanu, 2013**). Indeed, in recent years, appreciable interest has been generated on the plant of alfalfa which is one of the most reputable herbal medicinal plant that are used in traditional medicine for over 1,500 years due to the high content of amino acids, bioactive components such as phenols and flavonoids, enzymes, vitamins and minerals (**Finkler, 1985 and Furgał and Milik, 2008**). In addition, traditional medicinal use of alfalfa leaves in treatment of arthritis, kidney dysfunction, demulcent, cardiogenic, cancer, depurative, antirheumatic pains, lactagogue, fever, antiscorbutic , furuncles and other unrests (**Inamul, 2004; Khaleel et al. 2005; Asgary et al. 2008; Rana et al. 2010 ;Bora and Sharma 2011 and Gawel, 2012**). Furthermore, the plant extract has been shown to have anti-tumor activity against certain types of leukemia cells and selective toxicity of cancer cells in experimental animals through enhancing estradiol and lipid profile levels (**Amraie et al. 2015 and Nabatchian et al. 2015**).

Phytochemical reports on alfalfa manifested that the plant contains flavonoids, phenols acids, alkaloids, coumarins, phytoestrogens, digestive enzymes, saponins and phytosterols (**Zhu et al. 2009; Sun et al. 2013; Wang et al. 2014 and Jing et al. 2015**). In several studies, different amounts of flavonoids have been found in alfalfa, which indicated that it may be utilized as a potential natural source of antioxidant compound that can be used for pharmaceutical, food and feed industries (**Caunii et al .2012; Wang et al. 2012 and Karimi et al. 2013**).

The purpose of this study was to evaluate the effect of consumption of alfalfa (*Medicago sativa* L.) leaves powder and extract as a potential source of natural antioxidants against aspirin induced hyperuricemia in rats.

## **MATERIALS AND METHODS**

**Plant material:** Fresh alfalfa leaves (*Medicago sativa* L.) were obtained from Agriculture Research Center, Giza, Egypt.

**Chemicals and reagents:** Aspirin (*Acetylsalicylic acid*)<sup>®</sup> was obtained from Ameriya Company for Pharmaceutical and Chemical industries, Cairo, Egypt. Zyloric drug (*Allopurinol*)<sup>®</sup> Tablets 100 mg from Glaxo Smithkline Pharmaceuticals Ltd. Diagnostic kits were manufactured by Ranbaxy Diagnostics Ltd., were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

**Experimental animals:** Thirty five of white albino rats (Sprague-Dawley strain), weighing  $120 \pm 5$ g, provided from National Research Center, Cairo, Egypt. Rats were housed as groups in wire cages under the normal laboratory conditions, clean-air room with temperature of  $24 \pm 5^\circ\text{C}$ , a relative humidity of  $60 \pm 4\%$ , illumination (12 h light/12 h dark cycles) water and food were available ad-libitum throughout the period of 6 weeks of the investigation. The basal diet was prepared according to (NRC, 1995) and composed of casein (200 g/kg), corn starch (497 g/kg), sucrose (100 g/kg), cellulose (30 g/kg), corn oil (50 g/kg), mineral mixture (100g /kg), vitamin mixture (20 g/kg) and DL-methionine (3 g/kg). Rats were allowed to acclimatize for 2 week before the experiment.

**Preparation of the plant:** Alfalfa leaves were washed with tap water to remove remaining soil and other impurities. The leaves were cut into small pieces, homogenized and used for analysis or in food applications. All determinations were carried out in triplicates. Twenty five grams of alfalfa powder leaves were extracted with 500 ml boiling distilled water for 5 min. The heated decoction was taken and allowed to cool for 30 min., at room temperature and filtrated twice. The filtrate was lyophilized and stored in refrigerator. The rat dose of aqueous extract was (1ml/kg/b.w/rats/day) body weight orally by stomach tube.

### *Analytical Methods*

**Proximate composition:** Moisture content, total solids, crude fiber, ash, protein and crude ether extract were determined according to (AOAC, 1995). Total carbohydrates were calculated by difference.

**Determination of total phenolics and phenolic compounds:** The methanolic extracts yield of plant for leaves was determined according to the method described by Zeyada *et al.* (2008). Total phenols content were assayed calorimetrically using the folin – ciocalteau method Gamez – Meza *et al.* (1999) in methanolic extract. The content of phenolics was expressed as gallic acid equivalent in mg/g of extract (GAE g-1). HPLC system was used for analysis of methanolic extracts to identify the phenolic compound exactly according to Merfort *et al.* (1997). A C18 – Clc – ODS Hypersil reversed phase column 4.6 × 250 mm particle size 5um, Col No 0923002, N was used. A variable wavelength UV detector was used to detect phenolic compounds constituents at 300 nm. Elution was performed at a flow rate at 1.0 ml/min with mobile phase of acetic acid (A) and acetonitrile (B), starting with 100 % A at 30 min, then 90 % A and 10 % B for 10 min, 40 % A and 60 % B over the next 10 min and finally 100 % B over the final 10 min to purge column. Identifications of the phenolic compounds were based on the comparison of the retention times of peaks obtained by Torres *et al.* (1987).

### *Experimental design for rats:*

Rats were randomly divided into two main groups with 7 rats each. The groups were as follows:

**First main group:** Negative group control (fed in basal diet)

**Second Main Group:** Hyperuricemic groups (28 rats) induced with single high dosages of aspirin (3.5 g/Kg) by oral gavage according to Caspi *et al.* (2000). Blood was extracted from tail vein for uric acid, urea nitrogen and creatinine analysis from each rat to make sure the induction of hyperuricemia in aspirin group as hyperuricemic rats. Then, the second main group divided into three subgroups as follows:

- **Subgroup I:** Hyperuricemic + allopurinol (5 mg/100g /b.w./rats/day/orally) according to **Meira Fields *et al.* (1996)**
- **Subgroup II:** Hyperuricemic + alfalfa powder (10 g /100 g basal diet).
- **Subgroup III:** Hyperuricemic + alfalfa extract (1 ml /kg b.w./rats/day/orally).

During the experiment period, the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine food intake and body weight gain according to **Chapman *et al.* (1959)**.

#### ***Sample preparation***

At the end of the experiment period, the rats were fasted overnight then the rats were anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till required biochemical analysis at -20°C.

#### ***Biochemical analysis of serum***

Uric acid was determined according to the method described by **Fossati *et al.* (1980)**. Urea nitrogen was determined according to **Patton and Crouch (1977)**. Creatinine was determined according to **Bartels *et al.* (1972)**. Serum alanine and aspartate aminotransferase (ALT, AST), alkaline phosphates (ALP) enzymes, were estimated according to **Reitman and Frankel (1957)**, **Kind and King (1954)** and **Weichselbaum (1946)** respectively.

#### ***Determination of serum antioxidant parameters***

Malondialdehyde (MDA), Superoxide dismutase (SOD) activity, total antioxidants capacity (TAC), were determined according to **Ohkawa *et al.* (1979)**; **Nishikimi *et al.* (1972)** and **Cao *et al.* (1993)**, respectively.

#### ***Statistical analysis:***

All data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary,



NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and  $p < 0.05$  was used to indicate significance between different groups (**Snedecor and Cochran, 1967**).

### **RESULTS:**

The present study aimed to clear the possible effect of alfalfa leaves powder and extract as a natural antioxidant on aspirin- induced hyperuricemia.

#### **1 - Proximate composition of alfalfa leaves (dry basis %).**

Table 1 summarizes the results of proximate analysis of alfalfa leaves. Alfalfa leaves contains (4.65, 1.69, 4.25, and 5.12 %) for protein, ether extract, ash and crude fiber contents respectively on dry weight basis. The obtained results indicate that alfalfa leaves relatively contain remarkable amounts of protein, ash and fiber which consider a major cause of their quality and functionality.

**Table 1: Proximate composition of alfalfa leaves \***

Parameters	Content %
Crude protein	4.65 ± 0.25
Crude ether extract	1.69 ± 0.71
Crude fiber	5.12 ± 1.55
Ash	4.25 ± 0.11
Total carbohydrates	84.41 ± 1.07 7

\*On dry weight basis

#### **2- HPLC chromatography analysis of phenolic compounds of alfalfa extracts (%)**

In this study, the HPLC chromatography was used to determine the phenolic compounds in alfalfa leaves extracts. The HPLC analyses indicated the presence of gallic acid, protocatechuic acid, chlorogenic acid, p.hydroxybenzoic acid and caffeic acid as phenolics, (The most abundant phenolic compound in alfalfa leaves were caffeic acid, chlorogenic acid and p.hydroxybenzoic acid comprising 67.96 % of phenolic compound, while

the total phenolic value was 27.95 mg GAE/g dry matter (Tables 2). The HPLC chromatogram shows the presence of remarkable amount of phenolic compounds in the leaves of alfalfa extract.

**Table 2: Methanolic extract yield, total phenolic content and phenolic compounds (%) in alfalfa extracts as identified by HPLC.**

Phenolic compound	(%)
Gallic acid	2.89
Protocatechuic acid	3.10
Chlorogenic acid.	18.90
P.hydroxybenzoic acid	12.04
Caffeic acid	37.02
*Total phenolic content	27.95
Methanolic extracted yield	24.10

Values expressed as mean of three replicates  $\pm$  standard deviation, \* as mg GAE g-1 dry extract

### ***3- Effect of alfalfa powder and extract on body weight gain, food intake and FER of control and hyperuricemic rats***

The effect of continuously consuming alfalfa powder and extract on health of hyperuricemic rats was examined by measuring body weights each week (Table 3). There was a gradual decrease in body weight gain and FER of hyperuricemic group (positive control), when comparing with negative control group (-ve). However the consumption of alfalfa powder and extract for 4 weeks reversed the effect of aspirin as it showed significant increase in body weight gain and FER comparing with hyperuricemic (untreated) group ( $p < 0.05$ ).

**Table 3: Mean values ± SD of body weight gain, food intake and FER of control and hyperuricemic rats**

Groups	Control	Hyperuricemic	Hyperuricemic	Hyperuricemic	Hyperuricemic
Parameters	(-ve)	untreated (+ve)	+ Allopurinol	+ Alfalfa powder	+ alfalfa extract
<b>Initial weight (g)</b>	124.57± 5.50a	124.55± 5.41a	125.71± 6.71a	123.81± 6.24a	123.51± 5.43a
<b>Weight gain (g)</b>	149.20± 13.21a	111.21± 9.41c***	139.51± 10.2b**	140.81± 11.25b**	145.11± 10.22b**
<b>Food intake (g)</b>	16.31± 1.71a	15.39± 1.39a	15.87± 1.47a	15.35± 1.30a	15.99± 1.27a
<b>FER</b>	0.109± 0.003a	0.152± 0.004c***	0.113± 0.001b**	0.109± 0.005b**	0.104± 0.002b**

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Values with the same letters indicate non- significant difference (P<0.05) and vice versa  
FER: Food efficiency ratio.

#### **4- Effect of alfalfa powder and extract on renal function of control and hyperuricemic rats.**

The hypouricemic effects of both alfalfa powder and extract on aspirin-induced hyperuricemic rats are shown in Table 4. In negative control rats, serum uric acid levels were  $1.83 \pm 0.26$  mg/dl. However in aspirin-induced hyperuricemic rats (positive group), serum uric acid levels were elevated significantly to  $4.41 \pm 1.01$  mg/dl ( $p < 0.05$ ). This data indicates that aspirin successfully induced hyperuricemia. Serum uric acid levels of the hyperuricemic group treated with allopurinol (5 mg/100g b.w) for 4 weeks was lowered significantly by 54.87%. The serum uric acid levels of hyperuricemic rats treated with alfalfa powder (10 g/100g basil diet) and alfalfa extract (1 mg/kg/ b.w./rats/day/) were lowered significantly by 45.35% and 52.38% compared with positive control group.

During the 4 week post treatment period with both alfalfa powder and extract, renal function gradually improved, but not uniformly (Table 4). Mean serum urea and creatinine levels improved, which reached to  $41.90 \mu$  /mg and 1.02 mg/dl in treatment group with alfalfa extract versus  $56.60 \mu$

/mg and 1.87 mg/dl in hyperuricemic rats (untreated). Creatinine, produced from creatine, is filtered out of the blood by the glomerulus of kidneys and excreted in urine, and its level in the blood is an indicator for renal function. Treatment with alfalfa powder and extract and either allopurinol affect serum creatinine levels (Table 4), which suggests that renal function was improved by alfalfa consumption.

**Table 4: The Mean values ± SD of serum uric acid, creatinine and urea of control and hyperuricemic rats**

Groups	Control	Hyperuricemic	Hyperuricemic	Hyperuricemic	Hyperuricemic
Parameters	(-ve)	untreated (+ve)	+ Allopurinol	+ Alfalfa powder	+ alfalfaextract
Uric acid (mg/dl)	1.83 ±0.26c	4.41 ±1.01a***	1.99 ±0.61b*	2.11 ±0.77 b*	2.20 ±0.67 b*
Urea (µ /mg)	38.55± 4.31c	56.60± 5.11a***	40.77± 5.06b*	44.96± 5.81b*	41.90± 6.19b*
Creatinine (mg/dl)	0.76± 0.10c	1.87± 0.32a***	0.95± 0.20b*	1.13± 0.19b*	1.02± 0.12b*

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

#### **5- Effect of alfalfa powder and extract on some liver function parameters of control and hyperuricemic rats.**

As illustrated in Table 5, aspirin led to a remarkable increase in serum AST, ALT and ALP levels in positive control group as comparing with negative control group (p < 0.05). Treating with both alfalfa powder and extract significantly decreased (p < 0.05) activities of AST, ALT and ALP levels of hyperuricemic rats near to the level of the control (-ve). However hyperuricemic rats that treated with alfalfa extract showed significant reduction more than alfalfa powder in liver activities enzymes (p < 0.05) which reached to a level similar to the allopurinol treated hyperuricemic animals. Though, the significant elevation (p < 0.05) witnessed in the AST, ALT and ALP levels of hyperuricemic untreated rats

was reduced by the treatment with both alfalfa powder and extract but not comparable to the negative control rats.

**Table 5: The Mean values  $\pm$  SD of serum ALT, AST, and ALP of control and hyperuricemic rats**

Groups	Control	Hyperuricemic	Hyperuricemic	Hyperuricemic	Hyperuricemic
Parameters	(-ve)	untreated (+ve)	+ Allopurinol	+ Alfalfa powder	+ alfalfaextract
AST ( $\mu$ /ml)	49.67 $\pm$ 5.31b	60.75 $\pm$ 7.11a***	51.21 $\pm$ 6.41b**	53.74 $\pm$ 7.45b**	52.91 $\pm$ 8.01b**
ALT ( $\mu$ /ml)	37.33 $\pm$ 3.45c	50.95 $\pm$ 5.77a***	39.11 $\pm$ 6.81b**	41.83 $\pm$ 5.75 b**	39.23 $\pm$ 5.61b**
ALP ( $\mu$ /ml)	47.10 $\pm$ 4.95c	61.20 $\pm$ 7.81a***	54.91 $\pm$ 6.51b**	56.38 $\pm$ 6.91b**	51.14 $\pm$ 8.11b**

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Values with the same letters indicate non-significant difference (P<0.05) and vice versa.

AST: Aspartate aminotransferase , ALT :Alanine aminotransferase, ALP: Alkaline phosphatase

#### **6- Effect of alfalfa powder and extract on MDA, SOD and total antioxidant capacity levels of control and hyperuricemic rats.**

As shown in Table 6 , induction hyperuricemia by aspirin caused an elevation on MDA levels, and a decline in SOD and total antioxidant capacity levels compared with negative control group (-ve). However, the antioxidants biochemical parameters SOD and TAC showed a significant increase in the consumed alfalfa groups versus the corresponding positive group. After treatment with alfalfa powder and extract for 4 weeks markedly reversed the alterations in biochemical parameters brought by aspirin. The level of MDA was reduced by 58% while SOD and the TOC levels increased by 34 and 91% in alfalfa extract treated group. Similarly, MDA levels decreased by 55% and SOD and total antioxidant levels increased by 33 and 83% in alfalfa powder treated group. Compared to hyperuricemia (untreated) group, the group that treated with alfalfa extract was more effective in elevating the content of SOD (24.16 vs 17.96 U/mL, P < 0.05).

Thus treating with alfalfa powder and extract were almost restored values to near normal and standard drug (Allopurinol) levels with no significant differences versus the positive group (untreated).

**Table 6: The Mean values ± SD of serum malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidants of control and hyperuricemic rats**

Groups	Control	Hyperuricemic	Hyperuricemic	Hyperuricemic	Hyperuricemic
Parameters	(-ve)	untreated (+ve)	+ Allopurinol	+ Alfalfa powder	+ alfalfaextract
Malondialdehyde (MDA) mmol/L	3.97 ±0.33 <sup>c</sup>	10.17 ±0.55 <sup>a</sup>	4.08 ±0.65 <sup>b**</sup>	4.56 ±0.77 <sup>b***</sup>	4.22± 0.69 <sup>b***</sup>
Superoxide dismutase (SOD)U/mL	25.26± 7.01 <sup>a</sup>	17.96± 1.99 <sup>bc***</sup>	24.41± 2.21 <sup>ab***</sup>	23.91± 2.41 <sup>b***</sup>	24.16± 2.19 <sup>ab***</sup>
Total antioxidants mmol/L	3.52 ±0.25 <sup>a***</sup>	1.70± 1.14 <sup>c</sup>	3.32± 0.77 <sup>b**</sup>	3.11± 0.83 <sup>b**</sup>	3.25± 0.94 <sup>ab***</sup>

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Values with the same letters indicate non- significant difference (P<0.05) and vice versa

## DISCUSSION

Hyperuricemia is the most important risk factor for gout which, caused by the imbalance between production and excretion of uric acid. Many researchers have directed their attention to herbal medicines and natural products, making efforts to identify their active compounds and develop new hypouricemic agents with higher clinical effectiveness and safety (Wang *et al.* 2016). With the increasing inclination towards the herbal and organic medicine in the present generation, Alfalfa entire plant is a kind of wonder herb used in traditional medicine with many utility values include phytochemicals such as phenols and flavonoids may be considered as perfect choice to treat hyperuricemia and gout (Kalimuddin *et al.* 2016).

The present study demonstrated that both alfalfa extract and powder effectively reduced serum uric acid level near to the normal value in the

hyperuricemic rats. Moreover, in vitro study, elevated level of uric acid was able to stimulate oxidant generation and reduction in antioxidant levels in cultured cells (**Sautin et al. 2007**). Similar findings that oxonic acid induced hyperuricemia and renal injury triggered intrarenal oxidative stress have also been reported by **Sanchez-Lozada et al. (2008)**. Furthermore with the finding of **Elyazji et al. (2015)** since they reported that aspirin administration caused changes in kidney function and related parameters in chronic renal failure are well established in terms of elevated levels of creatinine and urea.

More importantly, recent epidemiologic reports have suggested the prospective direct role of chronic hyperuricemia in development of inflamed kidney tissues and progressive renal failure (**Mok et al. 2012**). So, it should be emphasized that the presently therapeutic medications that used for treating hyperuricemia and gout, such as allopurinol which long has been used as first-line therapy, are frequently correlating with undesirable adverse effects which including hepatic injury, severe allergic and hypersensitivity syndromes. Thus, allopurinol is not suggested in patients with kidney or heart disease, so these adverse effect profiles limit its use (**Ramasamy et al. 2013 and Wang et al. 2016**). The use of these agents is promoted in the treatment of gout, the treatment of uric acid kidney stones, and the prevention of tumor lysis syndrome (**Juraschek et al. 2011**). Recommendations to use natural dietary approaches to lower uric acid have been suggested (**Gelber, 2008**). Flavonoids and other phytochemicals have been found to inhibit aspirin activity, which reduces uric acid levels (**Tung et al. 2010**). Phenolic compounds contribute to the overall antioxidant properties of plants mainly due to their redox activities. Generally, the mechanism functionality of phenolic compounds for antioxidant activity are equalizing lipid free radicals and protecting decomposition of hydroperoxides into free radicals (**Li et al. 2009**). In this aspect, supplementation with such rich photochemical components which, found in alfalfa leaves has newly attracted a great deal of solicitude as an alternative dietary antihyperuricemic approach (**Gao et al. 2008 and Lagowska-Lenard et al. 2010**).

Our results showed that aspirin induced hyperuricemia caused to elevate serum levels of AST, ALT and ALP in untreated hyperuricemic rats (positive group). However, the significant decrease in the serum levels of the ALT, AST and ALP in consumed alfalfa groups might be due to decreased leakage from the liver cells. This suggests that the leaves of alfalfa were able to repair the probable liver dysfunction. It seems that, the effect of alfalfa leaves on reducing liver enzymes is known to be due to its powerful antioxidant activities (**Sun *et al.* 2013; Wang *et al.* 2014 and Jing *et al.* 2015**).

The present study demonstrates the attenuating effects of alfalfa supplementation on experimental rats induced with hyperuricemia resulting to remarkable intrarenal oxidative damage and renal damage. These data support the concept that an elevation in circulating uric acid, rather than being a surrogate biomarker of kidney dysfunction, is actually an active indicator in the pathogenesis of renal disorder (**Inaba *et al.* 2013**). It also confirmed the hypothesis that alfalfa might be an effective dietary approach in the protection and management of hyperuricemia and other associated medical conditions (**Wang *et al.* 2014 and Jing *et al.* 2015**).

In current work, consumption of alfalfa powder and extract did not appear to have severe toxicity or cause undesired adverse effect. The body weight gain of all treated animals was carefully monitored weekly through 4 weeks period of treatment, there is high significant difference ( $P < 0.05$ ) between consumed alfalfa groups and hyperuricemic rats (untreated). Interestingly alfalfa extract and powder resulted in significant anti-hyperuricemic and renal protective effects that were associated with a more pronounced decrease in serum levels of uric acid, creatinine and urea. The findings of the present study are in concord with these collective facts, since SOD is one of the paramount antioxidant enzymes that remove the excessive free radicals from body to reduce their unwholesome effects and assist tissue repair, also MDA is a product of lipid peroxidation and its accumulation in the body reflects the status of tissue injury (**Marnett, 1999**). Whereby aspirin induced hyperuricemia and renal damage that was significantly linked with a significant decrease in SOD and total antioxidant



capacity levels and increase in MDA levels (Table 6), suggesting that aspirin-induced hyperuricemia in rats was associated with oxidative damage of their renal tissues. By contrast, simultaneous treatment with both alfalfa extract and powder significantly restored the renal content of SOD and total antioxidant activities as well as MDA levels towards almost their normal values (Table 6). So treatment with alfalfa powder and extract functioned to attenuate the aspirin induced oxidative stress in rats by enhancing the antioxidant activity of SOD and reducing lipid peroxidation. These results confirmed with **Adeyi et al. (2015)** indicated that treatment with alfalfa extract were normalized levels of lipid peroxidation, renal and hepatic markers dysfunction.

### **CONCLUSION**

Our findings show that alfalfa powder and extract at the doses used in this study exhibits antihyperuricemic and antioxidants activities in experimental animals of hyperuricemia-induced by aspirin. This study may provide an evidence to support therapeutic value of alfalfa leaves in treatment of hyperuricemia and its complications, due to containing remarkable amount of phenolic compound. Further studies are necessitating to explore the effect of other components in alfalfa to utilize the full therapeutic potential of alfalfa in kidney diseases.

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## التأثير الوقائي لأوراق البرسيم الحجازى ضد فرط حمض اليوريك بالدم

### فى فئران التجارب

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### الملخص العربي

يهدف هذه البحث الى دراسة التأثير المحتمل لمسحوق ومستخلص اوراق البرسيم الحجازى كمضاد لفرط حمض اليوريك بالدم وذلك باستخدامه كمضاد للاكسدة طبيعى فى الفئران المصابة بفرط حمض اليوريك المستحدث باستخدام الاسبرين.

أستخدم ٣٥ من ذكور فئران اللابينو البيضاء ذات اوزان  $120 \pm 5$  جم ، قد تم تقسيمهم عشوائيا الى خمس مجموعات كلا منها ٧ فئران ، المجموعة الاولى (الضابطة السالبة) ، المجموعة المصابة بفرط حمض اليوريك بالدم (الموجبة الغير معالجة) ، المجموعة المصابة بفرط حمض اليوريك والمعالجة بعقار الوبوريينول (٥ ملجم / ١٠٠ جم من وزن الجسم / الفئران) ، المجموعة المصابة بفرط حمض اليوريك والمعالجة بمسحوق اوراق البرسيم الحجازى بنسبة (١٠ جم / كجم / الوجبة / يوميا) والمجموعة المصابة بفرط حمض اليوريك والمعالجة بمستخلص اوراق البرسيم الحجازى بنسبة (١ مل / كجم من وزن الجسم / الفئران / يوميا) وذلك لمدة ٤ اسابيع ، وبنهاية الدراسة تم تخدير الفئران بثنائي ايثر وذبحها وقد تم اخذ عينات الدم لاجراء الفحوصات البيوكيميائية.

وقد أظهرت النتائج زيادة مستويات حمض اليوريك والكرياتينين واليوريا فى مجموعة الفئران المصابة بفرط حمض اليوريك بالدم ( الموجبة الغير معالجة) بالمقارنة بالمجموعة الضابطة السالبة والتي قد انخفضت فى جميع المجموعات المعالجة ، وبالإضافة الى ذلك زيادة مستويات المألون داي الديهايد وانخفاض فى نشاط انزيم السوبر اوكسيد ديسموتيز ومضادات الاكسدة الكلية فى المجموعة الضابطة الموجبة التى كانت كالتالى (١٠.١٧ و ١٧.٩٦ و ١.٧٠ على التوالي) والتي قد تحسنت من خلال المعالجة بمسحوق ومستخلص البرسيم. وكذلك لوحظ زيادة فى نشاط انزيمات الكبد التى تغيرت بالمعالجة بمسحوق ومستخلص اوراق البرسيم الحجازى فى الفئران المصابة بفرط حمض اليوريك بالدم.

نستخلص من نتائج الدراسة ان المعالجة بمسحوق ومستخلص اوراق البرسيم الحجازى بالنسب المختارة ادت الي تقليل اضطرابات التمثيل الغذائي من خلال تحسن مستويات حمض اليوريك والنشاط الوقائى لمضادات الاكسدة ضد فرط حمض اليوريك بالدم المستحدث باستخدام الاسبرين ، ويرجع النشاط المضاد للاكسدة لمركبات الفينول الموجودة بالاوراق.

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