



## **Effect Of Mustard On Experimental Animals Inflicted With High Level Of Uric Salts**

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**Abstract :** This study aims to determine the chemical composition of mustard seeds. Also mustard seeds content of mineral and effect of mustard seeds with different levels on kidney function in rats with high level of uric salts was study the obtained results revealed that moisture, protein, fat, fiber, ash and carbohydrates content in mustard seeds were 6.42%, 25.90%, 30.85%, 8.50%, 4.02% and 24.31% respectively. Also mustard content of minerals mainly (Mg, Na, Z, Mn, Fe, Ca, K and Cu were 470.14mg, 175.35 mg, 4.036mg, 1.865mg, 21.67mg, 214.90mg, 857.87mg and 0.524mg ) respectively. Also the obtained results revealed that, rats with high level of uric salts was high level in serum and urine in both uric acid, urea and creatinine in comparison to control group, while rats fed on mustard 10 % was observable low level of serum creatinine, urine area and uric acid, however rats fed on mustard 5 % was low level of urine uric acid, area and creatinine but in lower. percentage than group fed on 10 % mustard .

**Key words:** Mustard, Kidney function, uric acid, urea, creatinine

### **Introduction :**

Mustard belongs to the Brassicaceae family which includes more than 3200 species and 375 genera and more specifically to the genera *Sinapis* and *brassica*. White mustard (*sinapis alba*), brown or yellow mustard (*Brossica Juncea*) and block mustard (*Brassica nigra*) are world wild cultivated and used as sources of vegetable oil, as flavour enhancer or as green manure. These plants have their origin in their region around

the Mediterranean Sea and the middle east and can now be found worldwide as cultivated species and as weed. Mustard seeds, further processed and/or mixed in food products. *Brassica Juncea* more used in the USA and in Japan. **(Monsalve et al., 1993)**

This fact is importance considering that present in many foods. Food products which mustard can be found in Babies and toddlers commercial foods, Barbecue Sauce, Curry Sauce, Cumberland Sauce, flavour for flavouring fried fish or meat, ketchup (tomato sauce), Lubricant, Marinades, Mayonnaise, Meat (processed), Meat (Sausages), Mustard powder (additive of foods), salad dressing, salad oil vinaigrettes, spices for flavouring. **(Panconesi et al., 1980, Andre et al., 1994, Kanny et al., 1995, Rance et al., 2000).**

Reported to be anodyne, aperitif, diuretic and stimulant. Indian mustard is a folk remedy for lumbago, foot ache, arthritis and renal stone. **(Duke and Wain 1981).**

Kidney stone are called as renal calculi. Kidney stone normally leave the body by the route of urine stream and many stones are produced and conceded without (silent) causing symptoms. **(Laquatra and Gerlach, 1990).**

The main symptoms of kidney stone is severe pain, there is usually general weakness and sometimes fever. The large fluid intake as primary therapy and deity control of the stone constituents may be reduce the stone formation **(Williams,1995).**

The uric acid (urate) crystals occur 5-10%.symptoms the uric acid kidney stone-hematuria: blood in the urine due to minor damage to inside wall of kidney ureter and/or urethra. The prevalence of kidney stones is increasing in the united states for too rapidly to be caused by changes in the genome. It appears likely the in stone prevalence is attributable to changes in our diets and lifestyles. the increase in calories leading to obesity and an elevated body mass index and the increase in consumption of components of the solid phases of kidney stone are likely factors increasing stone formation especially in woman. **(Marvin and David 2013).**

**Shashi et al., (2013)** reported that mechanism of stone formation.

- Age, profession, nutrition, Climate, inheritance, sex, constitutions.

- Abnormal renal morphology, disturbed urine flow, urinary tract infection, metabolic.
- Increased excretion stone forming constituents, decreased excretion of inhibitors of crystallization.
- Physico-chemical change in the state of super saturation.
- Abnormal crystalluria.
- Formation of stone.

#### **Materials and methods :**

##### **Materials**

The materials used in the investigation and their sources were as follows:

1. Mustard seeds (*Brassica Juncea*) was purchased from local market (Haraz. Company) , at Cairo, Egypt.
2. Casein as main source of protein obtained from Morgan Company, Cairo, Egypt.
3. Cellulose, Vitamin mixture and salt were purchased from El Gomhoria company Cairo Egypt.
4. Sixteen adult male albino rats weighting(130-140g) obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt ,were used in this study.

##### **Methods :**

##### **Preparation of mustard seeds:**

Mustard seeds were ground into fine powdered by using electric grinder and kept in polyethylene bags until analysis.

##### **Biological experiment:**

##### **1-Basal diet composition:**

The basal diet (casein-based diet was composed of 12.3g casein(10%protein),10g corn oil (10%fat),49 minerals (4%minerals),1g vitamin mixture (1%vitamin).5g cellulose (5%fiber),choline chloride (0.2%),methionine (0.3%),and the remained is starch(69.5%)according to (AIN.1993) .

##### **Preparation of hyperuricemia rats:**

Hyperuricemia was induced in normal male rats by feeding on basal diet containing 30% fructose,12% protein,10% fat (corn oil) ,4% minerals,1% vitamin mixture,4% wheat bran and 40% starch for 10 days according to method described by **Clifford and story (1976)**.

Rats were housed in individual stainless steel cages under controlled environmental conditions, in the animal house of Research

Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt, and fed on standard diet for on standard diet for a week as an adaptation period. Diet was offered to rats in special feed cups to avoid loss conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-libitum and checked daily. The rats were divided into 4 groups (4 rats in each group) as the following :

Group(1) This group was fed on standard diet (healthy rats).

Group(2): This group was fed on standard diet only as a control positive.

Group(3): This group was fed on basal diet containing 5 % mustard seeds

Powder.

Group(4): This group was fed on basal diet containing 10% mustard seeds powder

#### **Chemical analysis:**

Moisture, fat, protein, ash, and fiber were determined according to the method recommended by **A.O.A.C. (2005)**. The carbohydrates was calculated by the differences as follows:

$\% \text{ carbohydrates} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ fiber} + \% \text{ ash})$

#### **Determination of minerals**

Minerals (Mg-Na-Zn-Mn-Fe-Ca-K-Cu) were determined using atomic absorption and flame photometer analysis in the Agricultural Research Center, Cairo, Egypt using the methods published in the **A.O.A.C. (2005)**.

#### **Analytical methods:**

- Urea was determined according to the method of **Fawcett and soctt(1960)**.
- Uric acid was determined according to the method of **Barham and trinder (1972)**.
- Creatinine was determined according to the method of **Bartles et al., (1972) and Larsen (1972)**.
- Protein was measured according to the method of **Henry (1964)**.
- Sodium was measured according to the method of **Henry (1974)**.
- Potassium was measured according to **Henry (1964)**.
- Phosphorus was measured using kits according to **A.O.A.C (1990)**

#### **Histopathological examination**

Specimens from kidney was collected from studies rats by the end of experimental period, Fixed in 15% neutral buffered formalin (pH 7.0), dehydrated in ethyl alcohol in paraffin 4.6 mn sections were prepared and stained with sections hematoxylin and eosin (**Carleton, 1976**).

**Statistical analysis:**

Statistical analysis were performed by using computer program statistical package for social science (SPSS), and compared with each other using the suitable test. All obtained results were tabulated. Statistical analysis has been achieved using IMB-P-C computer by SPSS, program **SPSS (1998)**.

**Results and Discussion**

Results in table (1) illustrates the chemical composition of mustard seed (*Brassica juncea*) and show that the mustard contained 25.90% protein. This result agreed with that obtained by (**Abul-Fadi et al., 2011**) then found that mustard contained 30 % protein and( **USDA ,2011**) who found that 24.9%. Data also indicated that value of lipids content was 30.85% this result agreement with that obtained by( **USDA, 2011**) who found that mustard contained 28.76%. The obtained results also showed that the value of fiber content and ash content 6.5% and 4.02 this result agreement with that obtained by (**Abu-Fadi et al., 2011**).

Also results in table (2) illustrates the minerals composition of mustard is shown in table (2). The minerals Mg, Na, Zn, Mn, Fe, Ca, K and Cu were 470.19, 175.35, 4.036, 1.865, 21.87, 214.90, 857.87 and 0.534 respectively.

**Table (1): Chemical composition of mustard seeds**

<b>Chemical Composition(%)</b>	<b>Mustard seeds</b>
<b>Protein</b>	25.90%
<b>Fat</b>	30.85%
<b>Ash</b>	4.02%
<b>Moisture</b>	6.42%
<b>Fiber</b>	8.50%
<b>Carbohydrate</b>	24.31%

**Table (2): Minerals content (mg/100g)**

Minerals of mustard seeds (mg/100g)	Mustard seeds
<b>Mg</b>	470.19mg
<b>Na</b>	175.35mg
<b>Zn</b>	4.036mg
<b>Mn</b>	1.865mg
<b>Fe</b>	21.87mg
<b>Ca</b>	214.90mg
<b>K</b>	857.87mg
<b>Cu</b>	0.534mg

**Table (3): Mean±SD values of serum and urine concentration of urea, uric acid and creatinine for control positive, control negative, and different groups of rats fed on basal diet containing 5%, 10% mustard.**

Parameters	Control -ve M + SD	Control +ve M + SD	5% mustard M + SD	10% mustard M + SD	LSD
Serum uric acid	1.800 <sup>c</sup> ±0.1732	2.767 <sup>a</sup> ±0.152	1.933 <sup>bc</sup> ±0.1528	2.233 <sup>bc</sup> ±0.2517	0.4398
Serum urea	33.67 <sup>c</sup> ±4.041	50.67 <sup>a</sup> ±2.082	43.33 <sup>b</sup> ±2.517	44.33 <sup>b</sup> ±3.786	5.0666
Serum creatinine	0.867 <sup>b</sup> ±0.05	1.133 <sup>a</sup> ±0.115	0.977 <sup>ab</sup> ±0.025	0.943 <sup>ab</sup> ±0.177	0.236
Urine uric acid	3.80 <sup>d</sup> ±0.265	6.90 <sup>a</sup> ±0.400	5.53 <sup>b</sup> ±0.208	5.37 <sup>b</sup> ±0.379	0.6387
Urine urea	178.00 <sup>d</sup> ±9.165	349.00 <sup>a</sup> ±13.45	304.00 <sup>b</sup> ±5.292	281.67 <sup>c</sup> ±10.408	17.849
Urine creatinine	40.67 <sup>d</sup> ±2.082	83.17 <sup>a</sup> ±4.672	69.73 <sup>b</sup> ±2.06	60.03 <sup>c</sup> ±2.281	7.9047

**Means under the same line bearing different superscript letters are different significantly (P < 0.05).**

Table (3) Illustrate the effect of mustard 5%, 10% on serum and urine (uric acid, urea - creatinine) It could be observed that adding 10% of mustard to the diet of rats with high level uric salts has shown a reduction in serum uric when compared with control positive.

The addition of mustard in 10% and 5% values have a decrease effect on serum urea when compared with control positive. This reduction was also observed in serum creatinine, urine uric acid, urea in urine and urine creatinine. These results was reported in (Grover, *et al.*, 2003).

**Table (4): Mean  $\pm$  SD values of serum and urine concentration of Na, K, P and protein for control positive, control negative, and different groups of rats fed on basal diet containing 5%, 10% mustard.**

Parameters	Control -ve M + SD	Control +ve M + SD	5% mustard M + SD	10% mustard M + SD	LSD
Serum Na	74.67 <sup>c</sup> $\pm 5.132$	99.33 <sup>a</sup> $\pm 5.132$	91.67 <sup>ab</sup> $\pm 1.528$	90.67 <sup>b</sup> $\pm 6.658$	8.1416
Serum K	4.06 <sup>bc</sup> $\pm 0.118$	4.53 <sup>a</sup> $\pm 0.503$	4.23 <sup>ab</sup> $\pm 0.115$	3.97 <sup>bc</sup> $\pm 0.115$	0.4048
Serum P	5.867 <sup>d</sup> $\pm 0.1528$	7.133 <sup>a</sup> $\pm 0.3215$	6.567 <sup>bc</sup> $\pm 0.3055$	6.367 <sup>cd</sup> $\pm 0.7234$	0.6735
Serum protein	4.867 <sup>b</sup> $\pm 0.0577$	5.333 <sup>a</sup> $\pm 0.2517$	4.967 <sup>b</sup> $\pm 0.0577$	4.900 <sup>b</sup> $\pm 0.1000$	0.2372
Urine Na	30.67 <sup>c</sup> $\pm 4.041$	62.67 <sup>a</sup> $\pm 3.512$	55.23 <sup>ab</sup> $\pm 8.476$	49.57 <sup>b</sup> $\pm 8.195$	11.807
Urine K	35.00 <sup>d</sup> $\pm 4.583$	82.33 <sup>a</sup> $\pm 3.055$	71.67 <sup>b</sup> $\pm 3.786$	67.00 <sup>b</sup> $\pm 2.000$	10.02
Urine P	43.00 <sup>d</sup> $\pm 2.646$	90.67 <sup>a</sup> $\pm 1.155$	81.33 <sup>ab</sup> $\pm 10.071$	75.67 <sup>bc</sup> $\pm 4.933$	10.159
Urine protein	20.33 <sup>c</sup> $\pm 1.528$	31.33 <sup>a</sup> $\pm 5.686$	27.67 <sup>ab</sup> $\pm 2.309$	27.33 <sup>ab</sup> $\pm 1.528$	6.1197

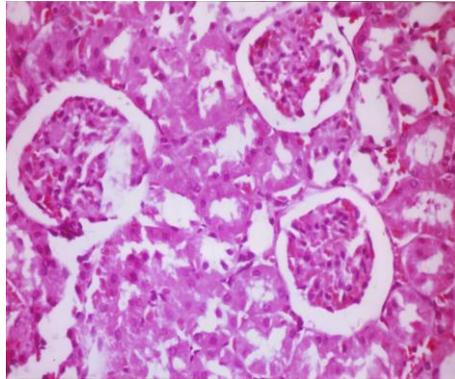
**Means under the same line bearing different superscript letters are different significantly (P < 0.05)**

Table (4): shows the effect of addition 5%, 10% mustard seeds on values of serum and urine concentration of Na, K, P and protein. It could noticed that addition of mustard seeds with 10% decrease the concentration of Na, K, P and protein in urine and serum.

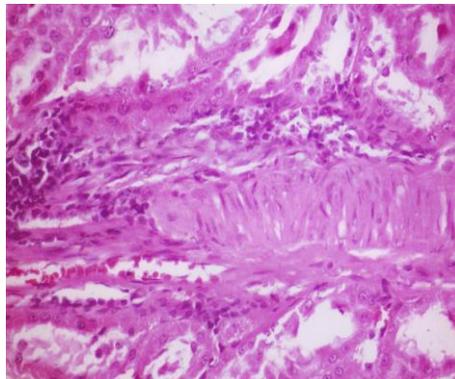
From these results it was illustrated that mustard seeds has an effect to reduce the Na, K, P and protein when added to the diet in 10% when compared to the group that 5% mustard added to the diet.

**Histopathological examination :**

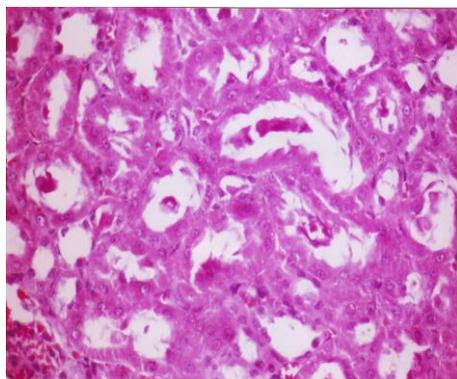
Microscopically, kidneys of rats from group 1 (control negative) non treated rats revealed the normal histology structure of renal parenchyma (H and EX 400). However, kidneys of control positive group 2 revealed thickening in the wall of renal blood vessel associated with prevasculitis. Meanwhile, kidney of rats from group 3 showed protein cast in the lumen of some renal tubules while kidneys of rats from group 4 showed vacuolations of epithelial lining renal tubules.



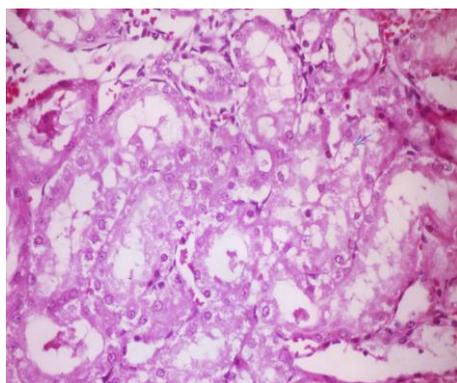
**Photo. (1):** Kidney of rat from group 1 showing the normal histological structure of renal parenchyma.(H and E x 400)



**Photo. (2):** Kidney of rat from group 2 showing thickening in the wall of renal blood vessel associated with prevasculitis.(H and E x 400)



**Photo. (3):** Kidney of rat from group 3 showing protein cast in the lumen of some renal tubules.(H and E x 400)



**Photo. (4):** Kidney of rat from group 4 showing vacuations of epithelial lining renal tubules.(H and E x 400)

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## تأثير الخردل على حيوانات التجارب المصابة بارتفاع مستوى أملاح اليوريك

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تهدف هذه الدراسة إلى تقدير التركيب الكيماوى لبذور الخردل وأيضاً محتوى بذور الخردل من الأملاح المعدنية وتأثير بذور الخردل بنسب مختلفة على وظائف الكلى فى الفئران المصابة بارتفاع مستوى أملاح اليوريك والنتائج المتحصل عليها توضح أن محتوى بذور الخردل من الرطوبة والبروتين والدهون والألياف والرماد والكربوهيدرات كانت كالاتى 6.42 % ، 25.9 % ، 30.85 % ، 8.50 % ، 4.02 % ، 24.31 % على التوالى وأيضاً محتوى بذور الخردل من الأملاح المعدنية (الماغنسيوم والصوديوم والزنك والمنجنيز والحديد والكالسيوم والبوتاسيوم والنحاس) كانت كالاتى 470.14 ملجم، 175.35 ملجم، 4.026 ملجم، 1.865 ملجم، 21.67 ملجم، 214.90 ملجم، 857.87 ملجم، 0.534 ملجم على اليوريك لديها أيضاً قيم مرتفعة عند تحليل السيرم والبول فى كلاً من حمض اليوريك واليوريا الكرياتين بالمقارنة بمجموعة الكنترول السالبة بينها الفئران التى تناولت بذور الخردل بنسبة 10 % فى غذائها المتناول وجد أن لديها إنخفاض ملحوظ فى نسب سيرم فى البول أما الفئران التى تناولت بذور الخردل بنسبة 5 % لوحظ أن لديها انخفاض فى نسب حمض اليوريك واليوريا الكرياتين فى البول ولكن هذا الانخفاض ليس بنفس الدرجة التى لوحظت فى المجموعة التى تغذت على بذور الخردل بنسبة 10 %.

**الكلمات المفتاحية:** بذور الخردل - وظائف الكلى - حمض اليوريك - اليوريا - الكرياتين.