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Biochemical and Molecular Studies on the Protective Effect of Some Natural Antioxidants Supplementation on Experimentally-Induced Hyperuricemia and Renal Injury In Rats.

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ABSTRACT

Hyperuricemia is a level of uric acid in the blood that is abnormally high. In humans, the upper end of the normal range is 360 µmol/L (6 mg/dL) for women and 400 µmol/L (6.8 mg/dL) for men. Many factors contribute to hyperuricemia, including: genetics, insulin resistance, hypertension, renal insufficiency, obesity, diet, use of diuretics, and consumption of alcoholic beverages .Causes of hyperuricemia can be classified into three functional types: increased production of uric acid, decreased excretion of uric acid, and mixed type. Some plants widely used as dietary flavours such as ginger, radish and onion can be used as treatment against development of hyperuricaemia and that is the aim of this project.

Keywords: Herbal plants – antioxidants- Biochemical effects- molecular effects

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INTRODUCTION

Hyperuricemia is abnormal high level of uric acid, it is a common metabolic disorder with a worldwide distribution(1).It has been considered as an important risk factor for gout(2).

Gout is a metabolic disorder associated with an excess of circulating uric acid resulting in the deposition of monosodium urate crystals (MSU) in tissues. A number of reversible factors contribute to increased urate production, including a high- purine diet, obesity and regular alcohol consumption (3). MSU crystals may be deposited in joints, usually in the big toe or ankle, causing neutrophil infiltration, swelling and excruciating pain (4).Estimations from the Third National Health and Nutrition Examination Survey (NHANES III) indicate that 0.5% of the total population has suffered from a gout attack. In addition, gout is currently considered to be the most common form of inflammatory arthritis in men over 40 years old, exceeding rheumatoidarthritis (5). The options for the treatment of chronic gout are allopurinol, which is an inhibitor of the xanthine oxidase enzyme, probenecide, stimulates the renal excretion of uric acid, and non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, that inhibit COX enzyme activity (6). Another drug that has been used to treat gout attacks is colchicine, which is an alkaloid derived from the autumn crocus Colchicum autumnale. However, approximately 50% of patients are non compliant with the prescribed medication, especially if they are having recurring gout flares (7) Moreover, each of these agents is associated with risks, potentially severe adverse effects and drug–drug interactions. Thus, many gout patients end up opting for treatments based on folk medicine (6).Elevated oxidative stress has been reported in gouty patient (8) Avoidance of purine-rich foods is important for gout management (9). The most important approach in the treatment of hyperuricemia is the development of xanthine oxidase inhibitors, which are effective in reducing plasma and urinary urate levels and reverses the development of tophaceous deposits (10), So food components which inhibit xanthine oxidase activity can reduce the formation of uric acid and alleviate inflammation. This is because xanthine oxidase is a key enzyme playing a role in hyperuricemia, catalyzing the oxidation of hypoxanthine to xanthine and then to uric acid, Also inhibition of renal urate reabsorption and oxidative stress has an important impact in goutmanagement. (11).

Free radicals defined as molecular species that contains unpaired electron in an atomic orbital that trigger chain reactions which can damage different cell constituents as superoxide and hydroxyl radical (12). In order to check free radicals formation to avoid oxidative stress, body has different anti-oxidant defense systems, Superoxide dismutase believed to be first line for defense against toxicity of superoxide radicals (13).

The presence of biologically-active ingredients in food can provide us with new components of beneficial effects towards diseases. The present research is a trial for management of gout through functional food components. Ginger (Zingiberofficinale Roscoe, Zingiberaceae) has been an important plant for the traditional Chinese and Indian pharmacopeia. In Asian traditional medicine, ginger has been used to relieve muscular aches, rheumatism, pains, coughs, sinusitis, sore throats, diarrhea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills and infectious diseases. It is being used worldwide as a spice and a flavoring agent. It has multifarious pharmacological activities including anti-oxidant, anti-inflammatory, anti-cancer, analgesic and anti-platelet effects (14).It also has an inhibitory effect on xanthine oxidase responsible for generation of ROS like superoxide anion (15). Black radish (Raphanussativus L. var. niger) root has been used in folk medicine since antiquity as a natural drug against flatulence, indigestion and the formation of gallstones. Moreover, it is also an effective natural drug for the stimulation of bile function and has a weak hepatoprotective activity. According to in vitro studies the squeezed juice from black radish root exhibited significant antioxidant properties (16). Red onion (Allium cepa L.) is among the important parts of diet in many world populations, and it is revered to possess anti-bacterial and anti-fungal activities, and contains the powerful antioxidants, sulphur and other numerous phenolic compounds(17). The aim of the present research is finding out functional food components of anti-gout activity. This is accomplished through testing different plant food extracts as uric acid lowering or treatment, antioxidant and anti-inflammatory in experimental gout model in rats.

MATERIAL AND METHODS

Materials:

Beef extract powder from sigma Aldrich - ginger , onion and radish extracted in the lab.

Animals and experimental design:

Preparation of extracted materials:

Extraction solution prepared by cutting material small piecesand Crushing it, the juice is taken and filtered and kept in a cooler place until use.

The beef extract powder using as protein dissolved in water and the concentration is 0.5:200g weight % of animals to goat infection.

Animals:

This study include 60 male Wistar albino rats weighing 180 to 200 g. The animals were kept and maintained under laboratory condition of temperature, humidity and light. The animals were housed five animals per cage. They were fed on standard commercial feeds with water *ad libitum*.

Experimental Design:

In the present study, a total 60 Wistar rats were used. They divided as following

Group I(control): include 10 rats they administrated only water.
Group II: include 10 rats they intraperitoneally administrated Beaf extract(to induce the experimental rat model with Hyperuricemia).
Group III:include 10 rats they intraperitoneally administrated Beaf extract (to induce the experimental rat model with Hyperuricemia) and then in second day orally administrated ginger extraction for 60 days.
Group V: include 10 rats they intraperitoneallyadministratedBeaf extract (to induce the experimental rat model with Hyperuricemia) and then in second day orally administrated radish extraction for 60 days.
Group IV: include 10 rats they intraperitoneally administratedBeaf extract (to induce the experimental rat model with Hyperuricemia) and then in second day orallyadministrated onion extraction for 60 days.
Group VI: include 10 rats they administrated Beaf extract (to induce the experimental rat model with Hyperuricemia) and then in second day orally administrated (ginger-radish and onion) extraction for 60 days.

Sample preparation

At the end of experiment, rats were weighed, and then sacrificed under anesthetized. Blood samples was taken from aorta and allowed to clot for 1-h and then centrifuged at 6000g for 10 min. Serum samples were separated and stored till used. Both kidneys of each rat onewas immediately excised and fixed immediately in 10% neutral formaldehyde for histological studies with Haematoxylin and Eosin and photographs taken and other was cut into small pieces, homogenized in 5 volume of ice-cold Tris HCL buffer (50mM, PH 7.4), and centrifuged at 10000g for 10 min. The volume of supernatant stored till used.

Determination of kidney function:

Serum levels of uric acid (UA), Creatinine (Cr) and blood urea were measured using commercial assay kits (Bioassay Systems, Hayward, CA)

Histological examination:

Isolated kidney was fixed in 10% neutral formalin, paraffinized for histological examination. Paraffin sections were prepared for staining with hematoxylin and eosin and then microscopically examined.

Determination of antioxidant:

Levels of glutathione (GSH) , superoxide dismutase (SOD) and glutathione peroxidase (GPx) as indicators for tissue antioxidant mechanisms were measured by using supernatants of kidney using commercial enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich, St. Louis, Mo., USA).

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Determination of inflammatory markers:

Serum Levels of interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 10 (IL-10) were measured by enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich, St. Louis, Mo., USA).

Statistical analysis:

Statistical analysis was performed using (SPSS) version 20, all data are presented as means± SE. Differences between groups were considered significant at $p<0.05$.

RESULTS

Table 1: Effect of Beaf extract on serum levels of uric acid, creatinine and urea.

Parameter	Control (n=10)	Beaf extractgroup (n=10)	Beaf extract& Ginger group (n=10)	Beaf extract& Radish group (n=10)	Beaf extract& Onion group (n=10)	Beaf extract& (Ginger, Radish&Onion) group (n=10)
Uric acids(mg/dl)	1.8±0.03	3.5±0.06	1.5±0.07	3.1±0.03	2.4±0.04	1.9±0.04
Creatinine(mg/dl)	0.59±0.01	1.7±0.07	0.51±0.01	1.4±0.1	0.98±0.007	0.55±0.01
Urea(mg/dl)	0.34±0.007	0.65±0.007	0.36±0.007	0.41±0.007	0.45±0.01	0.38±0.004

Table shows results as mean ± SE ,significance $p<0.0001$.

Table 2: Effects of Beaf extract on antioxidant defense systems in experimental animals groups compared with control group

Parameter	Control (n=10)	Beaf extract group (n=10)	Beaf extract& Ginger group (n=10)	Beaf extract& Radish group (n=10)	Beaf extract& Onion group (n=10)	Beaf extract& (Ginger, Radish &Onion) group (n=10)
Glutathione content (µ mol/ml)	5.1±0.03	2.5±0.11	6.2±0.07	3.5±0.07	4.3±0.09	5.3±0.07
Glutathione peroxidas activity (Units/ml)	32.5±0.13	18.4±0.07	39.5±0.1	20.8±0.04	25.7±0.07	30.6±0.07
Superoxide Dismutase activity (Units/ml)	0.31±0.004	0.07±0.007	0.29±0.007	0.21±0.004	0.27±0.007	0.3±0.04

Table shows results as mean ± SE ,significance $p<0.0001$.

Table 3: Effects of Beaf extracton inflammatory parameters in experimental animals groups compared with control group

Parameter	Control (n=10)	Beaf extractgroup (n=10)	Beaf extract& Ginger group (n=10)	Beaf extract& Radish group (n=10)	Beaf extract& Onion group (n=10)	Beaf extract& (Ginger, Radish &Onion) group (n=10)
IL-6(pg/ml)	1±0.06	1.5±0.07	1±0.08	1.3±0.07	1.1±0.07	0.98±0.007
IL-8(pg/ml)	3.5±0.07	6.8±0.07	4.6±0.1	5.9±0.05	4.9±0.08	4.1±0.04
IL-10(pg/ml)	0.84±0.007	1.6±0.1	0.88±0.008	1.2±0.09	0.91±0.01	0.86±0.01

Table shows results as mean ± SE ,significance $p<0.0001$.

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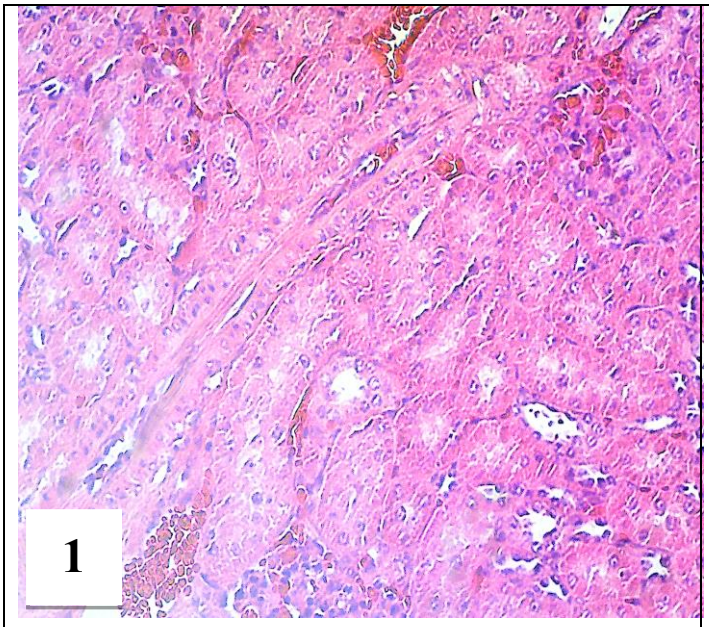

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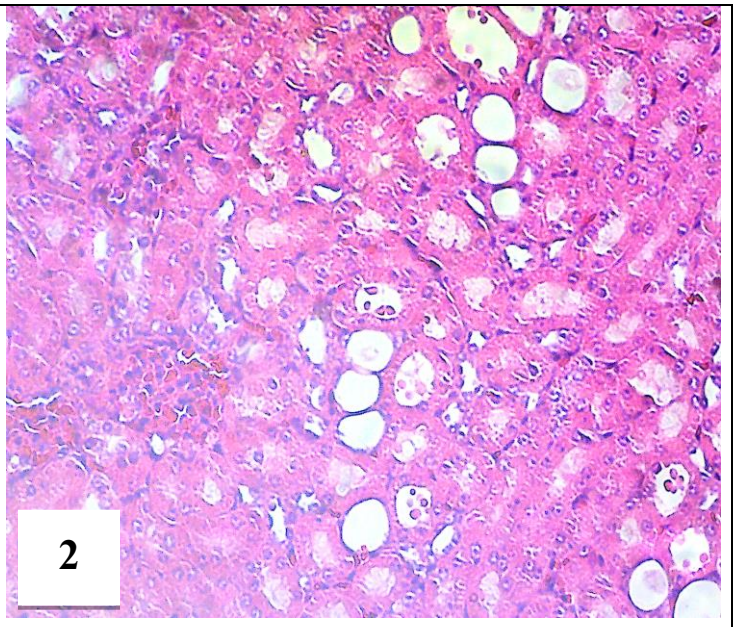

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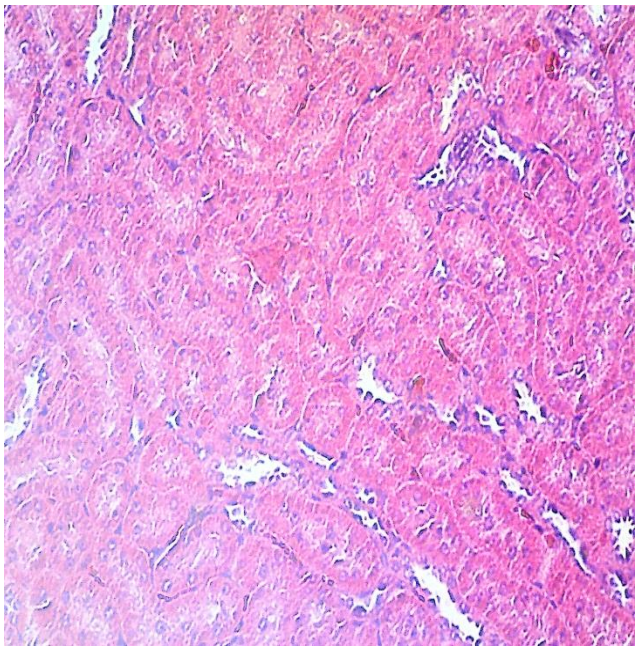

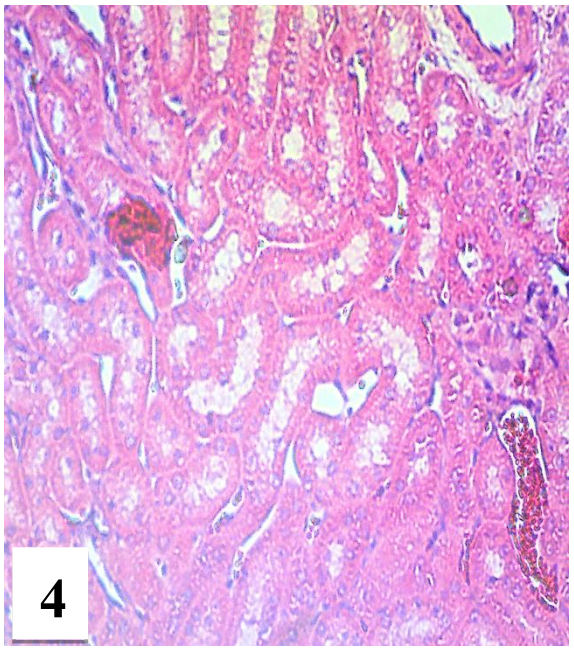

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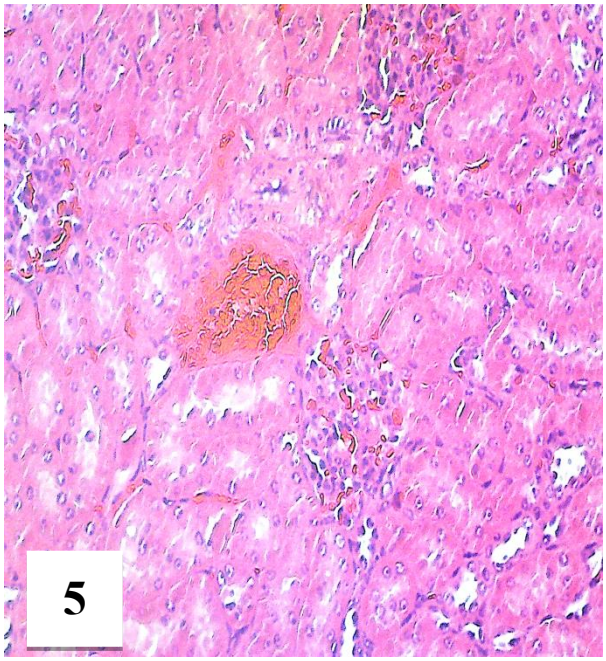

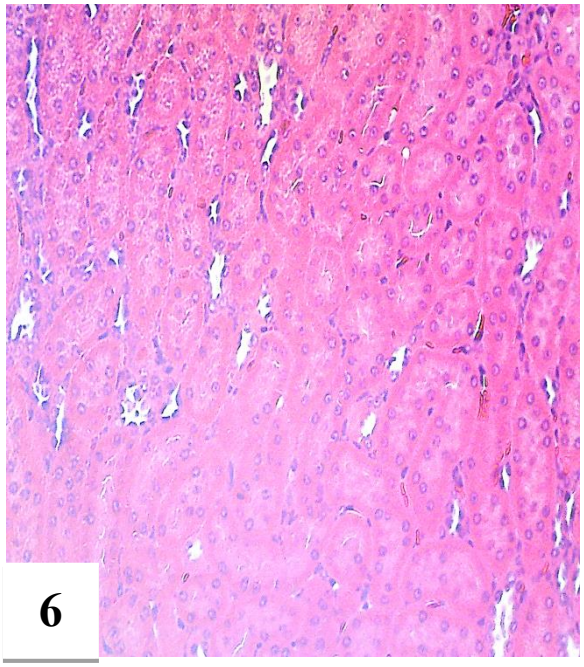

Figure 1: Kidney of normal rats showing normal glomerular and tubular structurein. Stained in H&E X250.
Figure 2: Kidney of rats that recievedBeaf extractshowed degenerationtubulointerstitial nephrites with excessive number of interstitial cellular infiltration. Stained in H&E X250.
Figure 3: Kidney of rats that received Beaf extractwith Ginger showed significant improvements in renal structure. Stained in H&E X250.
Figure 4: Kidney of rats that received Beaf extractwith onion showed moderately improved renal structure. Stained in H&E X250.
Figure 5: Kidney of rats that received Beaf extractwith Radish showed degeneration tubulointerstitial nephrites with excessive interstitial infiltration. Stained in H&E X250.
Figure 6: Kidney of rats that received Beaf extractwith (Ginger,onion and Radsh) showed improvements in renal structure. Stained in H&E X250.

DISCUSSION

Serum uric acid may have a direct role in the development of renal disease, in the present study it was tested by development of a rat model of Hyperuricemia induced by beaf extract. After eight weaks of beaf extract administration, there was a (1.9 fold) increase in serum uric acid level, serum levels of creatinine and serum levels of urea (table 1) that approved with histopathological finding that showed interstitial cellular infiltration (figure 1).Sanchez-LOzada et al.,say the same and support that Hyperuricemia role in induction of renal disease(18).

In this study, administration of (beaf extract with Ginger), (beaf extract with onion) and mix group (beaf extract , Ginger, Radish and Onion) showed anti-hyperuricemic that reflected by reducing the elevation in serum levels of uric acids, creatinine and urea and approved also by normal histological characters of the kidney (table 1 and figure 3,4 and 6). Previously, it has been reported that Ginger has been used to relieve muscular aches, rheumatism, pains, motion sickness and infectious disease. It has multifarious pharmacological activities including anti-oxidant, anti-inflammatory and anti-cancer effects (14).This in agreement withAjith et al., who said that ginger extract renderd significant protection against induced nephrotoxicity, which was evident from lowered serum urea and creatinine levels in the mice(19). Anumber of studies indicated that ginger exhibit antioxidant activity and anti free radicals abilitiesthat stimulate urea synthesis (20). The reduction of blood urea, uric acid, and creatinine in animals receiving ginger extract suggested that ginger may contains some effective comounds that influence removing certain waste products from plasma that interfered with mechanism of reabsorption inhibition of urea in the nephrons (21).

The ginger extract could prevent the depletion of antioxidant concentration and antioxidant enzymes activity in the kidneys by instructive the effect of urine flow rate on the renal eliminating of a substance such as creatinine and uric acid to the effects of flow on urea (22). The presence of polyphenols and flavinods in ginger extract may responsible for the antioxidant nephroprotective activities and reduction of serum urea, creatinine, and uric acid levels (23).

Onion as a flavonoid-rich food can reduce the elevated uric acid levels in hyperuricemicrats.Flavonoids are a group of polyphenolic compounds that are distributed in various foods and beverages of plant origin (24). The most therapeutic properties of flavonoids are their antioxidant and enzyme inhibitory activities (25,26).

The antioxidant action of Ginger may have a protective effect against Hyperuricemia. There is a strong evidence that increased oxidative damage dut to excess free radicals or generation of reactive oxygen species (ROS), in one of the most important pathogenic mechanisms in the development of many diseases including renal disease (27). Generation of ROS can lead to various forms of cellular injury as inflammation, cell death, necrosis and DNA damage (28).

Kidney tissues are rich in nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase-derived ROS, that under pathological condition can contribute to renal dysfunction (29). ROS can be detoxified by antioxidant cellular defences, including Scavenging action of glutathione (GSH), Superoxide dismutase (SOD) and glutathione peroxidase (GPx) and other antioxidant enzymatic systems. The present study agreed with these facts, as beaf extract induced Hyperuricemia and renal injury was associated with decrease in GSH and activities of SOD and GPx (table 2) but induction of beaf extract with Ginger and mix group (beaf extract, Ginger, Radish and Onion) restored the renal content of GSH, SOD and GPx activities become almost their normal values (table 2), this results in agreement with that reported by (18).

In this study , administration of (beaf extract with Radish) have no effect in decreasing serum levels of uric acids, creatinine or urea that approved in (table 1, figure 5) or in antioxidant activity that approved in (table 2).

Hyperuricemia has proinflammatory effects and causes glomerular hyper-trophy in rat kidneys. Soluble uric acid has been found to stimulate monocyte chemotaxis and release of proinflammatory mediators from vascular cells.Monocytes also become activated, resulting in expression of a number of proinflammatory genes, including IL-1, TNF-α, IL-6, IL-8 and cyclooxygenase-2 (30).In experimental models of inflammatory arthritis, IL-10 is protective and mice deficient in IL-10 show exacerbated joint inflammation (31) this approved in (table 3).Proinflammatory effect has role in the development of renal injury and progressive renal diseases (32).

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