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Effect of Gingko Biloba and Onion Juice on xanthine oxidase enzyme and renal function in induced hyperuricemic rats

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ABSTRACT

The present study was conducted to evaluate the role of some natural safe source plants on the hyperuricemia in ratsusing Ginkgo biloba leaf extract and onion juice against hyperuricemia in induced hyperuricemic rats using potassium oxonate as an inducing agent. 40 Sprague Dawleystrain, mean weighting 80-110 g were used, albino rats were categorized into 4 equal groups as follows:

Group GI: Control group: Only given basal diet, Group GII: Given basal diet plus 6.6 mg/kg/day plus potassium Oxonate. GIII: Co-treated group in which, rats were received basal diet, 6.6 mg/kg/day potassium oxonate plus gingko biloba leaf extract 80 mg/kg body weight.Group GIV: Co-treated group in which, rats were received basal diet, 6.6 mg/kg/day potassium Oxonate plus 10 mg /kg of onion

At the end of 4 weeks, blood samples were collected and sera were separated and analyzed for determination of serum XO activities, UA , urea and creatinineconcentrations also the kidney specimens were removal for histopathological examination.

The obtained results were statistically analyzed and represented in 1 table, 1 figure and 4 photos. The general findings can be summarized as follows:

PO caused hyperuricemia and increment in serum XO ,uric acid urea and creatinine, caused various degrees of pathological damages in kidneys.

There is a high significant difference of uric acid, urea and creatinine from potassium oxonate (GII) compared with control group (GI), co treated potassium oxonate with onion group(GIV) and co treated potassium oxonate with ginkgo biloba (GIII) at $p<0.00$. kidneys of rat received potassium oxonate showing inflammatory cells infiltration inside and in between destructed renal tubules. kidneys of rat received potassium oxonate and gingko biloba showing minimal tissue reaction. kidneys of rat received potassium oxonate and onion showing degeneration and necrosis in some of tubular epithelium.

Key words:hyperuricemia, potassium oxonate, ginkgo biloba leaf extract, onion juice, XO enzyme. uric acid.

INTRODUCTION

Uric acid is the end product of purine metabolism, and its concentration is mainly controlled by endogenous metabolism (synthesis and cell turnover), and the rate of excretion and reabsorption in the kidney [1].

Hyperuricemia is defined as a serum urate concentration exceeding the limit of solubility [2]. Hyperuricemia is a key risk factor for the development of gout, and has been linked to renal dysfunction, cardiovascular diseases, hypertension, hyperlipidemia, cancer, diabetes and metabolic syndrome [3].

Xanthine oxidase(XO) catalyses the two last steps in purine catabolism in man, forming the end product uric acid from hypoxanthine and xanthine. The mammalian enzyme exists mainly as a dehydrogenase, which utilizes NAD+ as the electron acceptor, but can be converted into an oxidase both in vivo and in vitro. The oxidase form utilizes molecular oxygen as the electron acceptor and releases substantial amounts of reactive oxygen metabolites under certain conditions, e.g. during tissue reoxygenation after hypoxia [4, 5].

Xanthine oxidase is involved in the medical condition known as gout, which is characterized by hyperuricemia that leads to uric acid deposition in the joints resulting in painful inflammation. Hyperuricemia,

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which is present in 5–30% of the general population, seems to be increasing worldwide and is considered an important risk factor in serious disorders, e.g. renal failure [6]

Potassium oxonate, a selectively competitive uricase inhibitor, produced hyperuricemia in rats. Renal function seriously impaired the in potassium oxonate induced hyperuricemia in rats [7, 8].

Onion is a species of the Alliaceae family and is the second most important vegetable crop in the world. Besides making a significant nutritional contribution to the human diet, onions also have medicinal and functional properties [9].

Nile & Park (2013) showed that onion has significant antioxidant activities [10]. Previous researches reported that onion contains various fiber, flavonoids and phenolic and in skin and external layers of onions are relatively high. In addition, onion bulbs also contain sulfurous compounds and fructans (a type of oligosaccharide) that could be beneficial for human health [11, 12]

Biopolymers of various biological materials such as Ginkgo biloba, grape seed, lycopene, royal jelly, and have been used to decrease of the toxicity created by chemicals [13].

Oral administration of onion at 3.5 and 7.0 mg kg-1 day-1 for 7 days was able to reduce serum uric acid levels in hypouricemic rats with no significant effects on the level of this compound in the normal animals. This property can be considered as an advantage for the onion. As onion is a common component of the usual diets in almost throughout the world, this natural food could be served as a possible alternative for allopurinol, or at least in combination therapy to minimize the side-effects of allopurinol, in particular in long-term application. Although the mechanism of the hypouricemic action of onion is not completely understood, this effect could be attributed in part to its inhibitory effects on xanthine oxidase activity [14].

Zhang et al (2017) demonstrated that ginkgo folium could alleviate the abnormal metabolic status of hyperuricemia[7].

Gingko biloba is considered the oldest tree species to survive on earth, with a history dating back over 200 million years. Some Ginkgo trees have been known to live well over an average of 1,000 or more years. Gingko.biloba leaf extract is the most widely sold phytomedicine in Europe where it is used to treat the symptoms of vascular dementia, tinnitus of vascular origin, peripheral claudication, and early stage Alzheimer's disease [15]. It is a popular herb used in traditional Chinese medicine [16].

Although there are many published clinical studies on onion and ginkgo biloba in the literature, unfortunately, the protective role of them on potassium induced toxicity in human and animals is still poorly understood. The aim of the present research was to evaluate the protective role of onion and gingko biloba on potassium oxonate induced nephrotoxicity in rats.

Aim of the study: To evaluate the effect of onion and Ginkgo biloba leaf extract against hyperuricemia induced by potassium oxonate in rats.

MATERIAL AND METHODS

Animals

A total of 40 male albino rats, Sprague Dawley strain, mean weighting 80-110 g were used, the animals were randomly divided into 4 equal groups and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25-30 °C with about 25% relative humidity. The room was lighted on a daily Photoperiod of 12 hr. light and dark.

Diet

The diet used in the present study was the balanced basal formulated following the protocols of Harlan –teklad Corporation and national institute of health[17]

Chemicals:

• Potassium oxonate

Preparation of Potassium oxonate solution: potassium oxonate freshly prepared daily by dissolving 6.6 mg of potassium oxonate in 40 ml of 0.9% saline,

•Ginkgo biloba

Preparation of Ginkgo biloba solution: 80 mg of Ginkgo biloba extraction (powder form inside capsule tablets) were dissolved in 10 ml of distilled water

•Onion

Preparation of Onion Juice:

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The outer dry skins and any inedible outer portions of onion were removed and the remaining edible portion was weighed and completely blended in distilled water (1:1 w/v). The freshly prepared juicy sample was administrated to each animal by gastric gavage.

Experimental design

The animals were divided into five equal groups (10 rats each):

- Group I : Control group which was unsupplemented, only given basal diet.
- Group II :Hyperuricemic group: given basal diet and injected i.p with 1ml of potassium oxonate sol by dose of 6.6 mg/kg/ b wt. daily.
- Group III : : Gingko biloba leaves extraction hyperuricemic treated group: given basal diet and injected i.p with 1ml of potassium oxonate sol (6.6 mg/kg/ b wt.), after two hours it was injected i.p with 1 ml of gingko biloba sol (80 mg/kg/ b wt.) daily.
- Group IV : Onion juice hyperuricemic treated group : given basal diet and injected i.p with 1ml of potassium oxonate sol (6.6 mg/kg/ b wt.), after two hours it was administered 1.5 ml of onion juice (50 %) by gastric gavage daily.
- The experiment continued for four weeks. Diet was given to rats for a period of four weeks.

At the end of the therapeutic period, under light ether anesthesia, blood samples were collected from the orbital sinus and centrifuged at 1500 × g for 15 min. Serum samples were then separated and collected in clean tubes and stored at -20°C.

After that, mice were sacrificed by decapitation. Kidney fixed in 10% formalin Sections were then prepared for histopathological staining with hematoxylin and eosin (H&E).

Methods

Chemistry

Measurement of uric acid by the uricase enzymatic reaction, creatinine measured by Jaffe's reaction and urea measured by the enzymatic urease method.

Determination of xanthine oxidase activity (XO, EC 1.17.3.2)spectrophotometrically by the enzymatic assays measuringand monitoring the production of uric acid from xanthine according to **Prajda and Weber's**method

The assay mixture consisted of phosphate buffer (pH 7.4), molecular oxygen and enzyme solution.

After preincubation at 37°C for 15 min, the reaction was initiated by the addition of the substrate solution.

After 30 min, the reaction was terminated by adding 0.5 mL HCl (0.6 M), and the absorbance was measured at 290 nm spectrophotometer using kinetic method[18].

Statistical analysis

The statistical analysis was carried out using SPSS for Windows version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistically significant differences between the groups were compared using one-wayanalysis ofvariance (ANOVA) and Dunnet test for multiple comparison (compare all vs. control).

The data are displayed as mean ± SD values, and values of P<.05 are considered statistically significant.

RESULTS

Table (1): Changes in xanthine oxidase, uric acid, urea and creatinine in difference groups.

Group	Xanthine oxidase U/mg protein	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
GI	2.58 ± 0.39^{b}	2.2 ± 0.2^{c}	7.86 ± 0.43^{b}	$0.62 \pm .86^{b}$
GII	3.27 ± 0.43^{a}	2.85 ± 0.27^{a}	9.35 ± 0.5^{a}	0.85 ± 0.63^{a}
GIII	2.59 ± 0.45^{b}	2.39 ± 0.15^{bc}	7.92 ± 0.37^{b}	0.66 ± 0.07^{b}
GIV	2.91 ± 0.33^{b}	2.51 ± 0.2^{b}	9.1 ± 0.53^{a}	0.69 ± 0.11^{b}
p-value	<0.001	<0.001	<0.001	<0.001

N.B. Different letter in the same column means presence of significant variations

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Biochemical results

Table 1: Data expressed as mean ± SD of 10 rats. There is a high significant difference of uric acid, urea and creatinine frompotassium oxonate (GII) comparedwith control group (GI), co treated potassium oxonate with onion group(GIV) and co treated potassium oxonate with GLE (GIII) at p<0.00. The difference checked by one-way ANOVA and Dunnet test for multiple comparison (compare all vs. control).

Regarding xanthine oxidaseenzyme Also using one-way ANOVA and Dunnet test for multiple comparison (compare all vs. control), there is a high significance difference from potassium oxonate (GII) comparedwith control group (GI), co treated potassium oxonate with GBLE (GIII) at p<0.01.andco treated potassium oxonate with onion group(GIV)

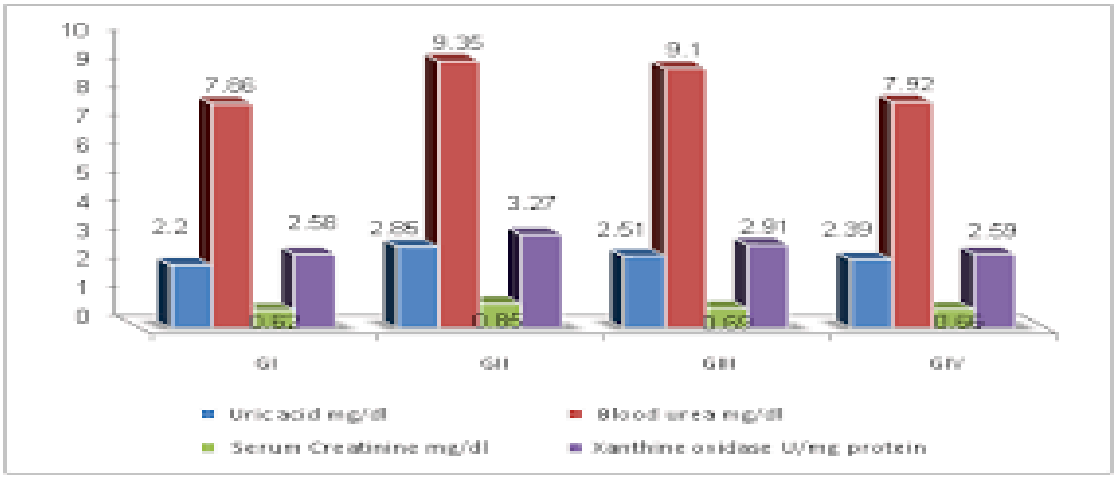

Fig. 1: Changes in urea, creatinine, uric acid and xanthine oxidase in different groups

Histopathological results

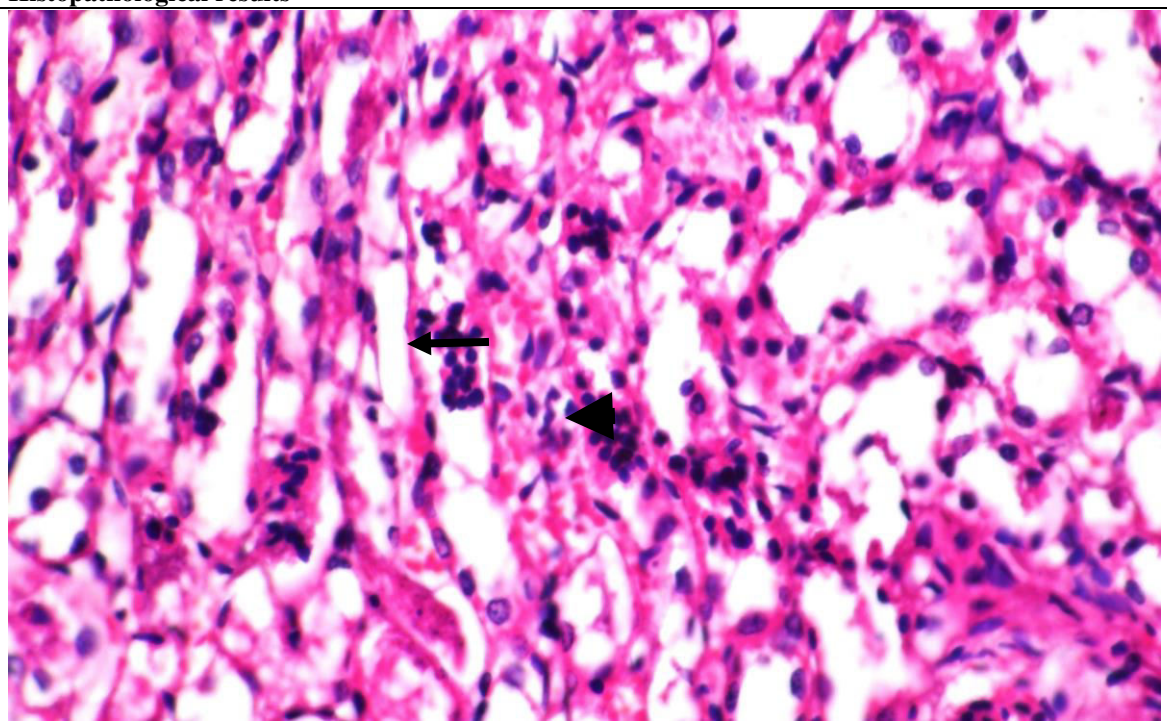

Photo.1: kidneys of rat received potassium oxonate showing inflammatory cells infiltration inside and in between destructed renal tubules.

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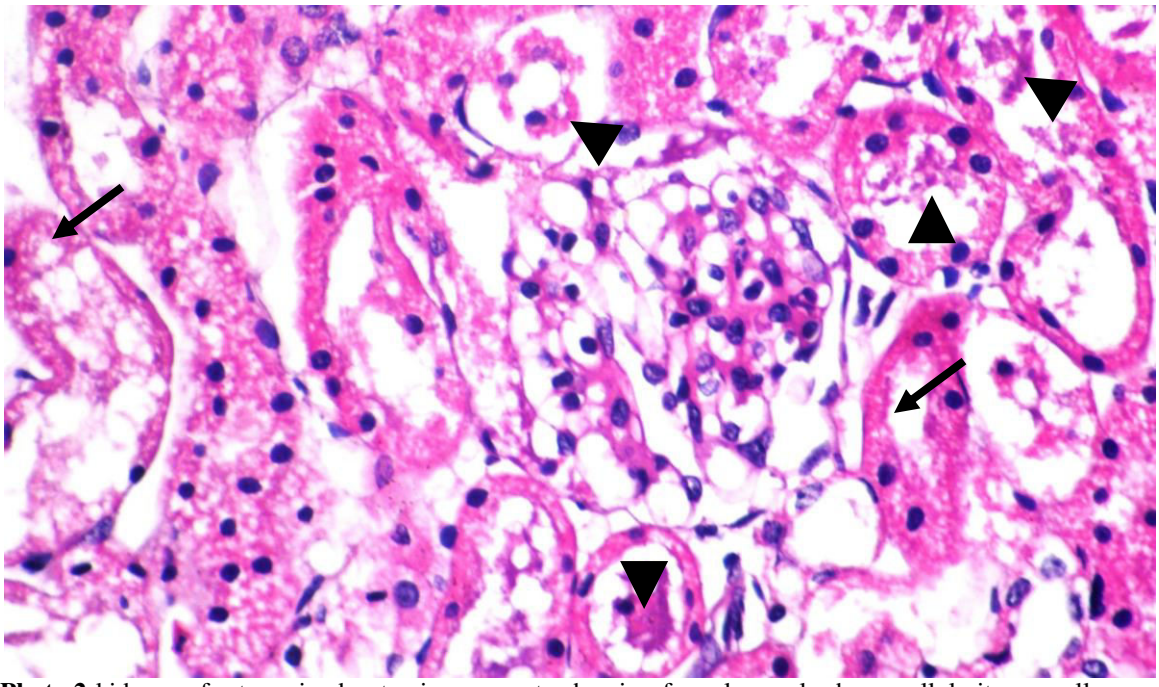

Photo.2:kidneys of rat received potassium oxonate showing few glomerular hypercellularity as well as necrosis in some renal tubular epithelium (arrow) with presence of necrotic cell debris in tubular lumen (arrow head) (H&E X 400).

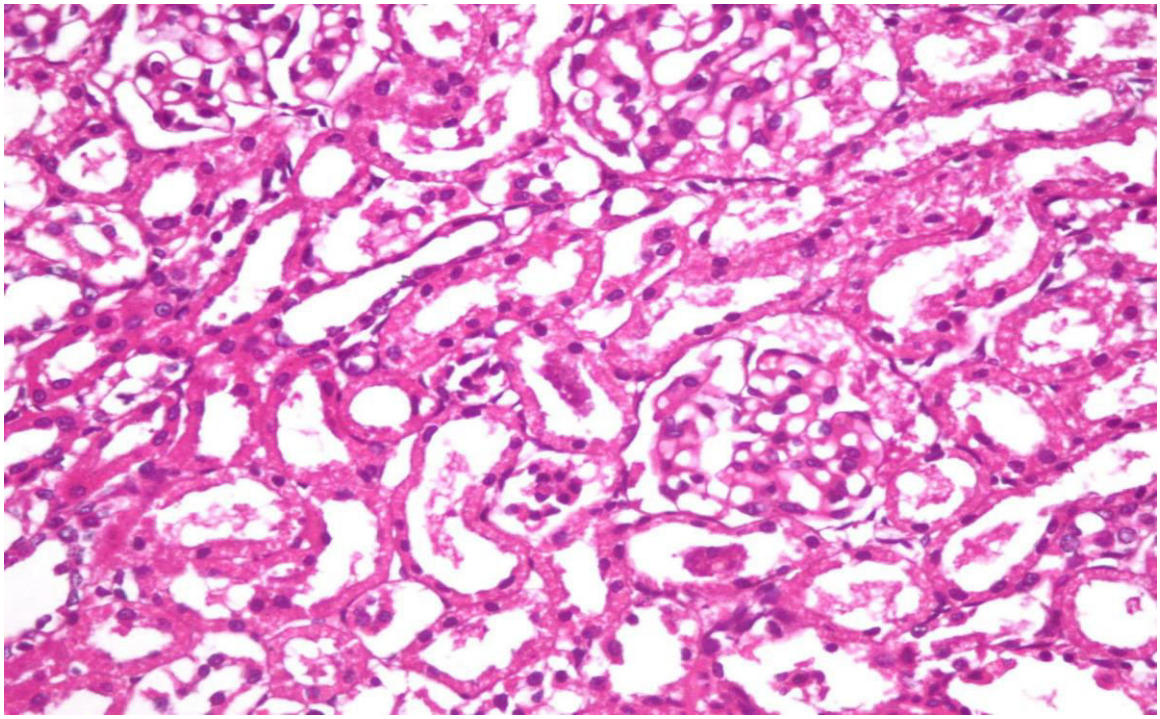

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Photo.3: Kidneys of rat received potassium oxonate and gingko biloba showing minimal tissue reaction in the form of necrosis in some tubular epithelium with presence of necrotic cell debris in tubular lumen.

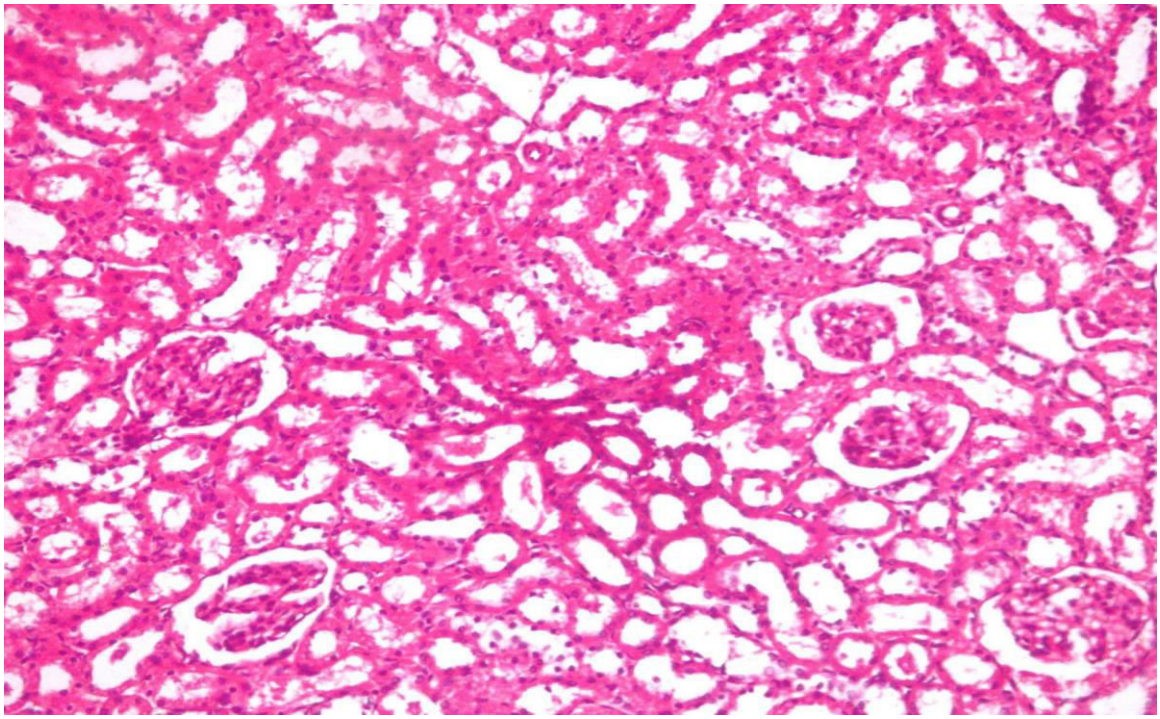

Photo.4: kidneys of rat received potassium oxonate and onion showing degeneration and necrosis in some of tubular epithelium.

DISCUSSION

Hyperuricemia is a metabolic disorder which may play an important role in the development of, nephrolithiasis, gout, hyperlipidemia and hypertension [3, 19].

The research conducts biochemical and histopathological investigation into whither onion and GLE has a protective and ameliorated effect on hyperuricemia induced by potassiumoxonate.

There is an elevation of urea, creatinine and uric acids in potassium oxonate group when compared with control group. This elevation decreased in co treated groups with onion and GLE. In agreement with **Elatrash and Abd El-Haleim (2017)** who found that urea, creatinine and uric acids levels decreased with GLE treated group compared with monosodium glutamate group[20]. **Haidari et al. (2008)** revealed that fresh onion juice has significant hypouricemic effects on serum uric acid levels in normal and hypouricemicrats[13].Abd-Allh SO(2014) in here study significant reduction ($P < 0.05$) in serum creatinine, uric acid in co treated Gingko bilobacompared to mercury treated group[21]. Yapar k et al(2010) found that serum blood urea and creatinine levels significantly decreased in co treated Gingko biloba plus uranium rats compared to those treated with uranium[16].

Regarding xanthine oxidase activity, the study revealed that co treated potassium oxonate group with onion (GIV) and co treated potassium oxonate group with GLE(GIII) has significant inhibitory effect compared to hypouricemicpotassium oxonate group (GII). In harmony with Nile S H & Park S W (2013) who concluded that all onion extracts showed a good to excellent activity profile for inhibition of xanthine oxidase, compared to that of the standard, allopurinol[10].

CONCLUSION

It can be concluded from the present findings that onion and gingko biloba studied can be considered as potential antihypeuricemic drugs and xanthine oxidase inhibitors. These GLE and onions should be subjected to more

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extensive studies, including optimization, extraction and isolation of active phytochemicals, to develop an antioxidant and xanthine oxidase inhibitor drug candidate with an improved potency.

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