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Hypouricemic and xanthine oxidase inhibitory activities of methanolic extract of *Ocimum basilium* leaves on yeast extract/potassium oxonate-induced hyperuricemic rats

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Abstract: Xanthine oxidase (EC 1.17.3.2) is a rate-limiting enzyme in the biosynthesis of uric acid. Over activity of this enzyme and increased intake of dietary food rich in nucleic acids result in hyperuricemia. During this reaction molecular oxygen acts as electron acceptor, producing superoxide radicals and hydrogen peroxide. Allopurinol, a commonly used XO inhibitor, has various adverse effects such as renal toxicity and fatal liver necrosis. The use of plant-based drugs is considered much safer compared to synthetic drugs. *Ocimum basilicum* extract effectively reverting the effects of high oxidizing agents such as hydrogen peroxide. In addition, basil extract presents anti-inflammatory properties. The present study was designed to investigate *invitro* antioxidant and anti-inflammatory activites and the possible hypouricemic and xanthine oxidase inhibitory activites of methanolic extract of *O basilicum* leaves (OBLE) on yeast extract(YE)/potassium oxonate(PO) induced hyperuricemic albino rats at two dose level (200mg/kg and 400mg/kg) using allpurinol(5mg/kg) as standard drug. OBLE showed strong antioxidant activity (80%) *invitro*, however lower than the standard pyrogallate(92%). *Invivo* results showed significant reduction on uric acid at the two doses with higher level ($P<0.01$) caused by 200mg/kg OBLE which is comparable to allpurinol and effective xathine oxidase inhibitory activity with ($P<0.05$) produced by both groups of OBLE treated rats compared to hyperuricemic control. Potassium oxonate and yeast extract caused a significant increase in uric acid ($P < 0.01$,) indicating the induction of hyperuricemia. Hyperuricemic control rats also showed a highly significant increase in serum globulins ($P < 0.001$) indicating the occurrence of inflammation. While serum globulin significantly reduced in OBLE treated rats. Serum parameters for both liver and kidney functions were measured. Results were compared with normal rats, hyperuricemic control and standard drug allpurinol.

Keywords: Basil, Hyperuricemia, Anti-inflammatory, Antioxidant, Lamiaceae

Introduction

Xanthine oxidase (XO) catalyses the metabolism of hypoxanthine to xanthine, and xanthine into uric acid, which is responsible for the medical condition leading to painful inflammation called gout (Nile and Khobragade, 2011). Furthermore the enzyme also generates superoxide radical during oxidation of substrates, subsequently plays an important role in various forms of inflammatory diseases (Rohman *et al*, 2010), several types of tissue and vascular injuries (Berry and Hare, 2004), and chronic heart failure (Pacher, 2006). Realizing the importance of plants in the discovery of new and safer therapeutic agents, screening of herbs for pharmacological activities and phytochemical constituents is one of the active fields of research round the world today. The genus Ocimum comes under Lamiaceae family and is found in many part of the world like tropical and sub-tropical regions of Asia, Africa and Central and South America(Prakash and Gupta, 2005). *Ocimum basilicum* is called basil, common or sweet basil (English) and rihan (Arabic) (Bilal *et al*, 2012). Basil leaves are used in folk medicine as a remedy for a large number of diseases, including cancer, convulsion, diarrhea, epilepsy, gout, nausea, sore throat, toothaches, and bronchitis(Khalid *et al*, 2006). Total phenolic compounds in basil accessions were higher than the other Lamiaceae plants (Zheng and Wang, 2001). The main phenolic compounds are rosmarinic acid, lithospermic acid, vanillic acid, coumarinic acid, hydroksibenzoacid, syringic acid, ferulic acid, protocatheuic acid, caffeic acid, and 5-Caffeoylquinic acid (Nguyen *et al*, 2008). Phenolic compounds are currently receiving much attention due to their beneficial health effects related to their antioxidant (Xiao *et al*, 2013). Polyphenols exhibit dual effects which are inhibiting xanthine oxidase and scavenging free radicals. This proposes new discovery in therapeutic approach to diseases like hyperuricemia, oxidative stress triggered from uric acid, inflammation and tissue damage (Gliozzi *et al*, 2014).

Ocimum basilium leaves

Materials and Methods

Plant material

Ocimum basilium leaves were obtained from horticulture section, ministry of agriculture, Khartoum state and were identified in the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. O basilium leaves were washed thoroughly in running tap water, separately shade dried at room temperature. Dried plant materials were milled to make fine powder in a grinder.

Preparation of extract

The powdered plant materials (1000 g) were extracted by the cold maceration method with sufficient quantity of 80% (v/v) methanol at room temperature for 48h. The process of extraction was repeated twice to complete extraction. The extracts were filtered through Whatman No1 and combined, then concentrated using a rotary evaporator under reduced pressure at 40^{e}C. The dry extracts obtained were weighed (Yanti *et al*, 2015).

Phytochemical analysis

Preliminary phytochemical screening was carried out for the identification of secondary metabolities using standard phytochemical methods according to (Harborne, 1998).

Evaluation of free radical scavenging activity

1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity was determined according to the modified method of Shimada *et al*, (1992). It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm. In 96-wells plate, the test samples were allowed to react with DPPH for half an hour at 37θ C. The test samples were dissolved in dimethyl sulfoxide (DMSO) while DPPH was prepared in methanol. After incubation, decrease in absorbance was measured at 517 nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated pyrogallate as control.

Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to Prieto *et al*, (1999). The method is based on the reduction of Mo (VI) to Mo (V) by the action of antioxidant compounds and the formation of a green phosphate - Mo(V) complex with a maximal absorption at 695 nm. Different plant extracts at concentration of 100µg/mlm, 200µg/ml and 300µg/ml were added to each test tube individually containing 3 ml of distilled water and 1 ml of

Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30min and the absorbance of the reaction mixture was measured at 695nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

***In vitro* anti-inflammatory activity**

The anti-inflammatory activity of extracts studied by using inhibition of albumin denaturation technique which was studied according to Sakat *et al*, (2010). The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660nm.(UV-Visible Spectrophotometer). The experiment was performed in triplicate. The percentage inhibition of denaturation was calculated by using following formula (Chandra *et al*, 2012):

$$\text{Inhibition } \% = 100*(1-Vc/Vt)$$

Experimental animals:

Animals

25 Wister albino rats (160±40g) were obtained from the animal house, Biochemistry department, Veterinary medicine collage, Khartoum University. They were kept in the departmental animal house where the experiment was carried out. Animals were provided with standard rodent pellet diet and the food was withdrawn 18 h, before the experiment though water was allowed ad libitum.

Experimental design

The antihyperuricemic activity of the plant extract was investigated using the yeast extract/ potassium oxonate-induced hyperuricemia in the rat's model according to**(**Xilifu *et al*, 2014**)**. Experimental animals (rats) were divided randomly into five groups (n= 5). Animals of control group (GI) each animal received only water as vehicle, hyperuricemic control group (GII) and rats of groups (III–V) were received 20g/kg of yeast extract powder 12- h prior to extracts and standard drug administration and uricase inhibitor (potassium oxonate) dissolved in 0.9% saline at a dose of 250mg/kg was given orally to each animal 1 h before the last oral administration of test compounds, which raised the serum uric acid level by inhibiting the decomposition of uric acid. Standard and expiremental rats were treated for 40 days as follows:

GIII: Allpurinol (5mg/kg) as standard; GIV: PO and YE plus TTFME (200mg/kg) methanol extract, GV: PO and YE plus TTFME (400mg/kg) methanol extract.

Biochemical examination

Uric acid, urea, creatinine levels, total protein albumin, ALP, AST, ALT activities in serum were measured 10 days intervals by the spectrophotometric methods using commercial diagnostic kits (Biosystem Chemicals, Barcelona, Spain). Serum xanthine oxidase was measured by chemical according to the method of Prajda and Weber,(1975).

Statistical analysis

Data obtained were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 22 at a statistical significance level of P <0.05 and 95% confidence interval. All results were expressed as mean ± standard deviation (SD).

Results and Discussions

Phytochemical screening of methanolic leaf extract of *O. basilium* showed the presence of tannins, flavonoids, alkaloids, steroids and carbohydrates (Table1). *O. basilicum* methanolic extract showed strong DPPH scavenging activity(80%), however its lower than the standard pyrogallate(PG) which exhibit (92%) inhibition [Fig 1]. The present result in accordance with(Khalifa *et al,* 2013) who showed that methanolic extract of *O basillicum* plant is a potent antioxidant with 91% activity at 100 ppm. The high antioxidant activity of basil, and the majority of its medicinal properties, have been attributed primarily to rosmarinic acid (Chen and Ho,1997), but some other like caffeic acid derivates, such as cichoric acid (Lee and Scagel, 2009), are also found in substantial concentrations. In the present study, *O. basilicum* leaves inhibit protein denaturation activity in a concentration dependent manner with higher percentage(80.2%) produced at 300µg/ml, however with lower value compared to diclofenac((97.7%) as standard drug[Fig(2)] . Involvement of flavonoids in the reduction of inflammation has been reported(Okokon *et al*, 2012)

Treatment of hyperuricemic rats with 200 and 400 mg/kg OBL methanolic extract at two dose levels caused significant decrease in serum uric acid levels in a non-dose-dependent manner, producing 74% and 73.4% decline in UA levels, respectively, by the end of the study period [P < 0.01 and P < 0.05; Table 2] compared to hyperuricemic control. The hypouricemic effect of 200mg/kg OB extract-treated rats showed the same level of significant (P<0.01), however lower percent of reduction than that produced by

allopurinol-treated rats(84%), whereas 400mg /kg OB extract- treated rats showed lower level of significant produced by allopurinol-treated rats. OBLME treated- rats at 200 and 400 mg/kg doses produced the same level significant ($P<0.05$) in XO inhibitory activity by the end of study period, however lower level than that($P<0.01$) produced by allopurinol [Table 2]. Phenolics compounds seem to have the most prominent role in reducing uric acid production and inhibiting the activity of XO enzymes(Nguyen *et al*, 2017). Altered values of urea, measured together with the creatinine, may indicate altered renal function(Haase-Fielitz *et al*, 2009). Long-term hyperuricemia can lead to deterioration of renal function (Komendarek-Kowalska , 2017). Hyperuricemic group showed significant elevation on both urea and creatinine($P<0.05$) compared to control group[Tables 3], while combination treatment with OBLE at two dose level caused highly significant reduction($P<0.01$) on serum creatinine and urea at the first 30days and without significant difference from the control group at the end experiment, while allopurinol showed significant elevation($P<0.05$) compared to control group. This revealed nephroprotective activity of the plant extract. Also serum globulins showed sharp significant elevation ($P<0.001$) in hyperuricemic rats [Table 4]. Whereas combination treatment with *O basilium* leaves extract and allopurinol caused significant reduction in serum globulins with higher level($P<0.01$) produced by 400mg/kg *O basilium* compared to hyperuricemic rats confirmed anti-inflammatory activity of the extract, while serum albumin significantly decreased($P<0.05$) in allopurinol group compared to control group. In agreement with Chen *et al*, (2016) a positive association between hyperuricemia and elevated ALT($r= 0.852$) and AST($r=0.661$) were reported with significant level of elevation ($P<0.05$) compared to normal group [table 5]. Several studies have demonstrated that hyperuricemia is associated with elevated inflammatory biomarkers levels and development of nonalcoholic fatty liver disease (Ayman *et al*, 2019). Treatment with OBLE at two doses showed no significant change on serum ALT and AST, while allopurinol produced significant increase($P<0.05$) compared to GI. Chinnasamy *et al*, (2007) reported that the protective action of basil was attributed to its antioxidant action. They added that this protection may be also due to anti-inflammatory property of ocimum which reduces formation, release, and activity of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes. *Invivo* studies found that basil extract possesses anti-inflammatory properties, and the mechanism involved is a composed interaction between the inhibition of pro-inflammatory mediator and the stimulation of anti-inflammatory cytokines (Guez *et al*, 2017). Several studies report many properties of rosmarinic acid including cyclooxygenase inhibition (Petersen and Simmonds, 2003).

Conclusions

The outcome of the present study revealed that OBLE possessed significant hypouricemic and xanthine inhibitory activities and had a preventive effect on the biochemical alterations on YE/PO induced hyperuricemic rats. The anti-hyperuricemic properties of 200mg/kg OBLE statistically equals the standard drug, allopurinol($P<0.01$). These actions are attributed to its composition, which is rich in polyphenols and flavonoids such as rosmarinic and caffeic acids which are strong antioxidant constituents of basil. This protection may be also due to its anti-inflammatory property which reduces formation, release, and activity of inflammatory mediators.

Table(1): Preliminary phytochemical screening of ocimum basilim leaves extracts

Extract	Alkaloid	Flavinoids	Phenols	Tanins	Glycosides	Steroids	Triterpense	Saponins	Carbohydrates	Amino-acids
OBM	+	++	++	+++	-	+	-	-	+	-

BM O basilium methanol extract −: Not detected; +: Weak; ++: Moderate; +++: Strong

Table(2): Effects of *Ocimum basilium* leaves extract on serum uric acid and xanthine oxidase on hyperuricemic rats

Time / Group	**Uric acid mg/dl (Mean±SD)**			**Xanthine Oxidase activity U/L (Mean±SD)**		
	Day20	**Day 30**	**Day 40**	**Day20**	**Day 30**	**Day 40**
Control	**1.5120±.29**	**1.225± .69**	**1.12± .14**	**20.20±.2.57**	**18.25± .96**	**17.25± 1.50**
HU	**4.120±1.39***	**4.49± 1.07****	**6.84± 2.14****	**31.58±7.27***	**32.8± 4.99****	**36.67± 5.84****
STD	**3.28±1.160***	**2.74± .6753•**	**1.09 ± .46765••**	**19.78±5.60**	**13.28± 5.12••**	**10.46± 6.12••***
OB200	**3.96±1.40***	**2.25±.75•**	**1.77±1.26••**	**18.78±4.95•**	**13.30±2.19••**	**15.52±2.83•**
OB400	**3.98±1.36***	**3.08±.24•**	**1.82±1.04•**	**15.80±4.95•***	**11.00±3.25••**	**12.6±6.82•**

HU: hyperuricemic control; OB200 : *Ocimum basilium* (200mg/kg); ; OB400 : *Ocimum basilium* (400mg/kg);

* $P<0.05$ **$P<0.01$***$P<0.001$ compared to control •$P<0.05$ ••$P<0.01$ compared to HU group.

Table(3): Effects of *O basilium* on serum urea and creatinine on hyperuricemic rats

Time / Group	Urea mg/dl (Mean±SD)			Creatinine mg/dl (Mean±SD)		
	Day20	Day 30	Day 40	Day20	Day 30	Day 40
Control	38.400±1.52	36.33±1.53	32.8±9.16	.50±.10	.56±.06	.53±.06
HU	47.32±12.267*	45.00±9.38***	50.7±6.604*	.57±.15	.83±.11*	.77±.06*
STD	22.2±5.03••***	18.2±4.92••••	48.3±14.89*	.57±.15	.63±.15	.53±.15
OB200	18.52±6.66••••	25 ±4.89••**	40.4 ±2.08	.53±.06	.53±.15•	.60±.10•
OB400	12.148±2.2••••	25.8±3.96••••	40.75±10.99	.63±.12	.53±.15•	.53±.15•

HU: hyperuricemic control; OB200 : *Ocimum basilium* (200mg/kg); OB400 : *Ocimum basilium* (400mg/kg);

* P<0.05 **P<0.01***P<0.001 compared to control •P<0.05 ••P<0.01 compared to HU group.

Table(4): Effects of *O basilium* on serum abumin and globulins on hyperuricemic rats

Time / Group	Globulins g/l (Mean±SD)			Albumin g/l (Mean±SD)		
	Day20	Day 30	Day 40	Day20	Day 30	Day 40
Control	2.9±.590	3.22±.08	2.76±.24	3.03±.153	3.20±.53	3.47±.55
HU	4.65±.32*	10.40±.04***	14.36±.13****	3.23±.21	3.27±.74*	3.03±.89
STD	3.65±.09	10.08±1.12**	8.89±.1.18•**	3.3±.12*	2.40±.26•	2.23±.32•
OB200	2.83±.29	3.85±.01	8.81±.27•**	3.93±.65	3.47±.29*	2.77±.47
OB400	3.23±.06	5.22±.194••*	5.53±.44•••*	3.40±.46	2.60±.72	2.87±.32

HU: hyperuricemic control; OB200 : *Ocimum basilium* (200mg/kg); ; OB400 : *Ocimum basilium* (400mg/kg);

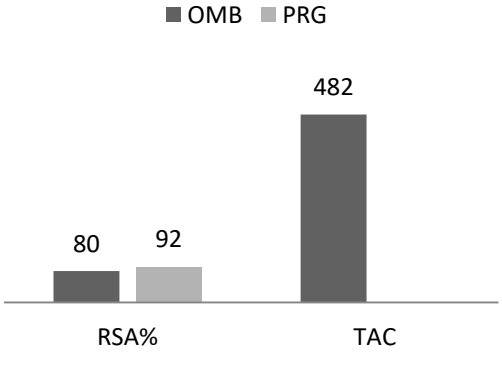
* P<0.05 **P<0.01***P<0.001 compared to cotrol •P<0.05 ••P<0.01 •••P<0.001 compared to HU group.

Table(5): Effect of O basilium on liver enzymes on YE/PO-induced hyperuricemic rats

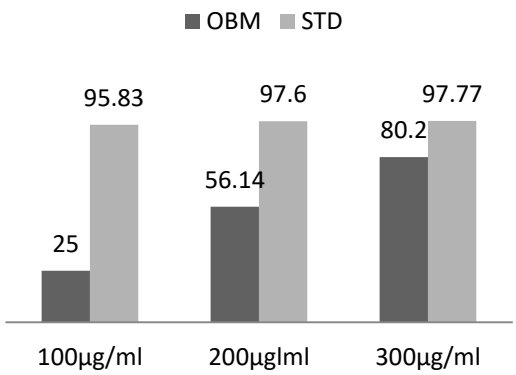
Parameter / Group	ALT U/L(mean±STD)		AST U/L(mean±STD)		ALP U/L(mean±STD)	
	Day 30	Day 40	Day 30	Day 40	Day30	Day40
Control	12.61±.577	12.3±3.84	40.2±7. 68	40.0±4.0	39. ±8.89	43.0±7.87
HU	18.67±2.5*	18.0±6.59	39.67±2.08	53.±15.87*	54.67±6.66	48.4±15.88
STD	18.23±2.08	23.2±8.56*	50.5±1.732•*	53.5±36.65*	50.00±10.0	51.8±38.06
OB200	9.80±2.08	13.3±5.09	47.667±6.43	46.0±23.07	62.0±27.22	50±21.66
OB400	11.87±2.80	15.00±3.00	60.33±12.70	68.3±21.03	78.0±18.0•*	96.3±35.92*

HU: hyperuricemic control; OB200 : *Ocimum basilium* (200mg/kg); ; OB400 : *Ocimum basilium* (400mg/kg);

* P<0.05 **P<0.01***P<0.001 compared to control •P<0.05 ••P<0.01 compared to HU group.



Fig(1): Antioxidant activity of *O basilium* methanol



Fig(2): Anti-inflammatory activity of *O basilium* extract ; OBM: *O basilium* extract; PRG: pyrogallate STD: diclofenac sodium

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