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المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد العاشر– العدد الأول– مسلسل العدد (٢٣) – يناير ٢٠٢٤م

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2974-4423

موقع المجلة عبر بنك المعرفة المصري https://jsezu.journals.ekb.eg

البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

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تاريخ رفع البحث: ٢٦-١١-٢٠٢٣م تاريخ تحكيم البحث: ١٥-١٢-٢٠٢٣م

تاريخ مراجعة البحث: ٥-١-٢٠٢٤م تاريخ نشر البحث: ٧-١-٢٠٢٤م

Abstract:

Zucchini (*Cucurbita pepo* L.) flowers considered as agriculture wastes. Even though its contain high different quantities of nutrients and bioactive components. There isn't much information on the possible nutritional and healthy benefits of zucchini flowers. So, the aim of the current investigation study was to determine zucchini flower extract (ZFE) might had the curative effect of treating renal injured hyperuricemic rats. As well as studying the impact of ZFE on liver and kidney as anticancer. 24 male albino rats were divided into four groups (n = 6) for this study. The first group functioned as the negative control and just received a basic diet. The remaining rats (n = 18) were given a basic diet and injected with gentamicin (100 mg/kg BW/day) for 7 days to cause hyperuricemia, which results in kidney injury. The hyperuricemic rats were subsequently split into three groups; the first group acted as the positive control and did not get any therapy, while the second and third groups each received an oral dose of the solution contained 250 and 500 mg/kg ZFE for 30 days. Interestingly, the findings revealed that the hyperuricemic group receiving a meal supplemented with 500 mg/kg ZFE did not differ substantially from the negative control in the biological parameters assessed and saw a considerable improvement in renal function compared to the hyperuricemic rats (positive control). When compared to rats with hyperuricemia (positive control), the group of hyperuricemic rats given 500 mg/kg ZFE had significantly lower levels of cholesterol, low-density lipoprotein, triglycerides, malondialdehyde, urea, creatinine, AST and ALT, with higher levels of total protein ($p \leq 0.05$). The biologically favorable action of ZFE may be ascribed to its possible increase of antioxidant status through enhancement of glutathione peroxidase and catalase activities. In HepG-2 cells (which represent human hepatocellular carcinoma) and Vero cells (which represent kidney epithelium), ZFE also shown a considerable cytotoxicity. These findings might serve as an experimental foundation for further study into the possible anti-hyperuricemic and anticancer effects of ZFE.

Key words: *Cucurbita pepo*, Hyperuricemia, Anticancer, Gentamicin, Kidney toxicity

1. Introduction

Kidney diseases are defined as a heterogeneous of disorders which affecting on the structure and function of kidney, these can be increase with time and led to kidney failure then mortality **(Levey *et al*., 2013)**. Cause of oxidative stress for polyunsaturated fatty acids in renal lipid composition, the kidney is extremely susceptible to failure and damage by reactive oxygen species, **(Ozbek, 2012)**. Large volumes of blood passing through it and filtering a lot of toxins, which can accumulate in kidney lobules, might also cause big harm **(Begum *et al*., 2011)**. According to **Kadir *et al.* (2013)**, the kidney's influence with toxicants can take many different morphological forms, ranging from tubular or interstitial alterations to nephropathy.

Kidney diseases are divided based on disease duration to two main types. The first, acute kidney injury (AKI) that happens within duration of 3 months or fewer. the other, chronic kidney disease (CKD) that happens within duration of greater than 3 months **(Chawla *et al*., 2017)**. The cumulating of toxic compounds as nitrogen metabolism or creatinine with high levels in serum of the patient leads to loss of kidney function which called AKI or acute renal failure. So, the kidney cannot be able keeping the normal levels of fluid in the body **(Gyurászová *et al*., 2020)**. There are about 13.3 million cases by AKI yearly. AKI causes can be categorizing to three types which start within the glomeruli and tubules (acute tubular necrosis) then disruption of drainage of urine as a result of decreasing blood perfusion to the kidneys **(Dennis and Witting, 2017)**.

Many organs as heart, lungs and brain are affected by AKI. CKD is features with reduction in kidney function gradually, which was occurred during long of time. CKD ranged 7 :12% of patients all over the world **(Gyurászová *et al*., 2020)**. There is correlation between CKD and AKI; AKI can cause CKD, and CKD increases the harm of AKI. The global ratio of prevalence AKI and CKD are highly increasing because of the population aging, the increase of injury with hypertension and diabetes **(Levey *et al*., 2013)**. AKI disease can be prevented and treated at early discovered, but untreated lead to progression hence, occurring with the kidney failure. Until our date, there is no effective medication or supplement for therapeutic AKI. Almost all the treatment strategies involve preventative actions to minimize the AKI occurrence or to opposite the cause of AKI **(Lim *et al*., 2021)**.

Edible plant flowers are used in many food meals as ingredient to promote the nutritional value and the taste or as garnishes to more attractive food, as well as health-promoting **(Pieterse *et al*., 2023)**. The

flowers of some vegetables, ornamental plants, herbs and trees are used by many methods, fresh or processed, in prepare jellies, sorbets, jams, cocktails, tea, ice cream, salads and honey. As well as, they can be boiled, grilled, fried and candied **(Jadhav *et al.*, 2023)**. In traditional medicine, the flowers are widely used as an antitussive and antispasmodic drug, as well as it is used in the treatment of kidney disease, influenza and lung infection. Also, it has a significant protective and inhibitory properties against tissue damage caused by ROS **(Biezanowska-Kope´ *et al.*, 2022)**. The consumption of edible flowers which rich in its content of important nutrients as vitamins especially folic acid, essential amino acids, minerals, phenolic acids, flavonoids, anthocyanins, carotenoids, polyphenols and ascorbic acid (bioactive compounds) which have a significant role for human health protection **(Matyjaszczyk & Śmiechowska, 2019; Dujmovic *et al.*, 2022 and Jadhav *et al.*, 2023)**.

The zucchini (*Cucurbita pepo* L.) is a summer squash which own to the Cucurbitaceae family, it is eldest vegetables common in the world. Flowers, fruits and seeds of zucchini are eatable and involve nutrients which give it a significant health stimulating features **(Seleim *et al.*, 2015)**. It characterized by a delicate flavor and a bright yellow color **(Prabawati *et al.*, 2021)**, Zucchini flowers are consumed as fresh vegetable or cooked (pizza, fried foods and sauces) **(Massantini *et al.*, 2022)**. Hence, this paper was intended to study the nutraceutical and bioactive compounds from zucchini flowers, examine the impact of ethanolic zucchini flowers extract on hyperuricemia and renal injury in rats and use it as anticancer.

2. Materials and methods

2.1. Ethical statement

The current study was performed following the approval from the institutional animal care and research Unit of Zagazig University (Institutional Review Board Number ZU-IACUC/2/F/292/2023).

2.2. Materials and reagents

Fresh zucchini (*Cucurbita peoo* L.) flowers were gathered from regional farmstead in Sharkia Governorate, Egypt, at spring in 2021. 24 adult male Swiss albinos weighing 120±10g each were purchased from the National Research Center's breeding facility in Dokki, Cairo, Egypt. The casein, starch, choline chloride, vitamins, minerals, cellulose, DL-methionine, and a few kits for biochemical analysis were gotten from El-Gomhoria Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. From Sigma (St. Louis, Missouri, USA), dimethyl sulfoxide (DMSO) and gentamycin were brought.

2.3. Preparations of zucchini flowers extract (ZFE)

Zucchini flowers were washed by tap water then desiccated at room temperature for one day and completed drying at oven air (45 ±5°C) for 24hr. then the flowers samples were powdered by an electric blender (Braun MultiQuick, MQ 5245 WH, German) and were cased in polyethylene containers. The dried flower powder was first defatted by n-hexan, and then extracted with aqueous ethanol (70%) at a ratio of 1:10 w/v,left overnight at room temperature then filtered with filter paper. The filtrate was evaporated at 50°C by rotary evaporator (BÜCHI-water bath-B-480, German). Freeze Dryer (France type) was used to freeze-dry the extracts at -58.2°C, then kept at freezer at -20 °C until use.

2.4. Chemical analysis

Moisture, protein, fibre, ash, and oil were measured using the procedures outlined by **AOAC (2005)**. Flavonoid content was evaluated by **Ordon *et al*. (2006)**, Polyphenol content (TPC) was calculated according to **Ragaee *et al*. (2006)** and **Dvorakova *et al*. (2008)** while antioxidant activity was assessed by the method of **Tepe *et al*. (2005).** Some of polyphenolic compounds were separated and identified by HPLC **(Goupy *et al*., 1999).**

2.5. Evaluation the anti-tumor activity (Cell viability evaluation)

HepG-2 cells (human Hepatocellular carcinoma) and the Vero cell line (Vero cells are segregate from kidney epithelial cells of the African green monkey) were commercially gotten from VACSERA Tissue Culture Unit. The cells were promulgated in Dulbecco's modified Eagle's medium (DMEM) provided with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were kept at 37ºC in a humidified atmosphere with 5% CO_2 and were sub cultured two times a week. The cytotoxicity assay was decided with method of **Mosmann (1983) and Gomha *et al*., (2015)**.

2.6. Biological study

Twenty -four albino male adult rats of weighting 120±10 g were used in the current experimental. Rats were retained in normal and idealized condition of cages, feeding, temperature and humidity degree, cleaning and daily checking. Rats were distributed to two main groups. Group 1 contained six rats as a nagative control group (G1). 18 rats were injected with 100 mg/kg BW/day of gentamicin (intraperitoneal) for 7 days to induce hyperuricemia according to **Ismaiel *et al*. (2019)**. The hyperuricemic rats were distributed to three groups. One of them used as positive group (G2) did not receive any treatment only saline. The treated groups (G3) and (G4) were received oral solution contained 250 and 500 mg/kg of zucchini flowers extract for 30 days, respectively. All hyperuricemic rats received saline orally for 30 days.

2.7. Biological analysis

After 30 days of treatment, fasted rats were immolated under ether numbness, then the blood samples were collected and centrifuged. Then, serum samples were kept in dry clean ependorf tubes at freezer -20°C till analysis. Also, the kidney and liver tissues of all rats were excised, weighted and then classified into two parts; the first part was homogenized with saline and used for biochemical assay (determination of antioxidants biomarkers). The second part of kidney was washed in saline and immediately fixed into 10% formalin solution for histological examination.

In kidney tissues for all rat groups, the Malondialdehyde (MDA), Catalase (CAT) and Glutathion peroxidase (GPX) were determined by **Sun *et al*. (1988), Aebi (1984) and Satoh (1978),** respectively. While in blood serum, Aspartate aminotransferase (AST), Alanine Amino transferase (ALT) and total protein were assayed according to **Chawla (2003)**, **Srivastava *et al*. (2002) and Henry (1974),** respectively. Urea, nitrogen, uric acid and creatinine were assayed by methods of **Patton and Crouch (1977)** and **Henry (1974)**. Triglycerides **(Stein, 1987)**, Total cholesterol **(Young, 2001)**, HDL-c (**Lopes *et al*., 1977)**, LDL-c and VLDL-c were estimated according to **Friedewald *et al*. (1972)**.

2.8. Histopathological examination

The histopathological examination of kidney and liver tissues were prepared and carried out according to **Bancroft and Stevens (2013)**.

2.9. Statistical analysis

The results and data were recorded as mean ± SD. Then, statistical analysis system **SAS (2000)** was used at the level of 95% of differences.

3. Results and discussion

3.1. Gross chemical composition and antioxidant properties of zucchini flowers powder

Data in Table (1) presented the chemical composition of the zucchini flowers powder. Which it had 8.50, 23.20, 2.26, 15.85, 35.34 and 14.85 g/100 g dry weight basis of moisture, protein, fat, ash, carbs, and fiber, respectively. These results were fairly consistent with those of **Ghosh and Rana (2021),** who discovered that the pumpkin flower contained 14.86, 1.00, 20.00, 35.20 and 29.00 g/100 g dry weight basis of protein, fat, ash, carbs and fiber, respectively. The ZFP's TPC, TFC, and % DPPH inhibition were, respectively, 280.62 g GAE g-1, 96.56 g QE g-1, and 94.58%. The TPC, TFC and the percentage radical scavengers by DPPH were 250.0 mg GAE/100g, 2-180 mg QE/100g and 96.00%, respectively, according to **López-Agama *et al*. (2021)**

Table (1): Chemical composition and antioxidant properties of zucchini flowers powder

Chemical composition (g/ 100g dry weight basis)	
Moisture	8.50±0.15
Crude protein	23.20±0.89
Crude fat	2.26±0.06
Ash	15.85±0.16
Carbohydrates	35.34±3.74
Crude fiber	14.85±1.11
Phytochemical properties	
TPC (µg GAE g-1)	280.62±0.41
TFC(µg QE g-1)	96.56±0.59
DPPH %	94.58±0.38

3.2. HPLC Identification of phenolic and flavonoids compounds for ethanolic zucchini flowers extract

The typical chromatographic profile of phenolic and flavonoids chemicals that were extracted from ZFE using the HPLC method are shown in Table (2). Sixteen phenolic and flavonoid compounds as Chlorogenic acid, Catechin, Gallic acid, Syringic acid, Methyl gallate , Coffeic acid, Coumaric acid, Rutin , Vanillin, Ferulic acid, Naringenin, Daidzein, Querectin , Cinnamic acid , Kaempferol and Hesperetin were identified by HPLC, the phenolic and flavonoids compounds in ZFE range from 15.68 to 1535.03 µg/g. Gallic acid (1335.03 µg/g) was found in a high amount of ZFE, the result was matched with **Mohamed *et al.* (2009)** who found that, the ZFE had high content of Gallic acid and other bioactives compounds.

Table (2): Polyphenol compounds of ethanolic zucchini flowers extract

Item	**Conc. (µg/g)**
Gallic acid	1335.03
Chlorogenic acid	170.27
Catechin	279.83
Methyl gallate	1114.53
Caffeic acid	280.33
Syringic acid	223.08
Rutin	883.26
Coumaric acid	286.78
Vanillin	328.83
Ferulic acid	467.54
Naringenin	194.70
Daidzein	22.49
Querectin	720.04
Cinnamic acid	36.03
Kaempferol	15.68
Hesperetin	47.26

3.3. Effect of ZFE on lipid profile for hyperuricemic rats

Data illustrated in Table (3), showed a substantial decline in levels of total triacylglyceride, cholesterol and LDL-c to be 80.20, 80.08 and 23.77 mg/dL, respectively, for G4 (which received 500 mg/kg ZFE) compared to the group which didn't receive any treatment G2 (hyperuricemic rats) to be 93.63 mg/dL, 93.07 and 45.35 mg/dL, respectively.

As opposed to all groups, the hyperuricemic rat group's (G2) HDL concentrations were the lowest (29.67 mg/dL). Receiving 250 or 500 mg/kg of ZFE with oral javaging significantly raised HDL-c levels to 37.24 and 40.27 mg/dL. Additionally, therapy with ZFE at both dosages significantly reduced LDL-c, triglycerides, and total cholesterol as compared to G2. By lowering total cholesterol and LDL-c while significantly raising HDL-c, ZFE have a remarkable hypolipidemic impact **(Badr, 2018 and El-Sahar *et al*., 2020).**

An aminoglycoside antibiotic called gentamicin (GM) is used to treat some infections as gram-negative bacterial infections **(Balakumar *et al*., 2010)**. GM is the most popular aminoglycoside due to its high activity, quick effect on microorganism and affordable price, but the drug's clinical use has been constrained by major side effects like nephrotoxicity **(Edson and Terrell, 1999 and Lopez-Novoa *et al*., 2011)**. Moreover, GM can increase of producing reactive oxygen radicals, which can support to occur the oxidation processes in vital cellular composition (such as DNA, lipids, and proteins) and causes cell damage **(Said, 2011 and Tavafi & Ahmadvand, 2011)**.

Aquino-Bolanos *et al*. (2013) and Morittu *et al*. (2021) confirmed that the natural phenolic compounds in ZFE is the responsible for the beneficial hypolipidemic impact. The modulation of lipid metabolism by phenolics and flavonoids has also been linked to this hypolipidemic action. This modulation leads to an increase in HDL levels but not in total cholesterol, triglycerides, or LDL because it upregulates the hepatic peroxisome proliferator-activated receptor α (PPAR-α) **(Zeni *et al*., 2017)**. Moreover, these results are matched with **Badr (2018)** who found that zucchini flowers powder had a hypocholesterolemic effect.

Table (3): Impact of zucchini flowers extract on lipid profile of male rats

Groups	Triglyceride (mg /dl)	Total cholesterol (mg /dl)	HDL-c (mg /dl)	LDL-c (mg /dl)	VLDL-c (mg /dl)
G1	73.89±9.21^{b}	71.67±2.33^{d}	36.74±4.40^{a}	20.14±8.34^{c}	14.78±1.85^{b}
G2	93.07±4.70^{a}	93.63±1.85^{a}	29.67±3.98^{b}	45.35±2.96^{a}	18.61±0.94^{a}
G3	81.38±1.75^{ab}	85.67±2.96^{b}	37.24±2.12^{a}	32.16±2.01^{b}	16.27±0.35^{ab}
G4	80.20±6.84^{b}	80.08±3.39c	40.27±3.65^{a}	23.77±6.83^{bc}	16.04±1.37^{b}
LSD	11.79	5.07	6.86	10.96	2.36

Small variable letters on mean values in each column are significant. Group (1) negative control. Group (2) Hyperuricemic rats (positive control). Group (3) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. Group (4) Hyperuricemic rats received 500 mg/kg zucchini flowers extract.

3.4. Effect of ZFE on liver function in rats with hyperuricemia

Few investigations indicated gentamicin's hepatotoxicity, while many articles described the drug's nephrotoxicity and ototoxicity **(Lee, 2003)**. According to **Al-Kenanny *et al*. (2012)**, the intraperitoneal dose of gentamicin for 8 days resulted in a significant increase in the levels of AST and ALT activity. The effects of ZFE on liver function are illustrated in Table (4). In comparison to the hyperuricemic rat groups, the negative control group displayed considerably lower serum AST and ALT levels and higher serum TP levels. The negative control group's lowest mean AST and ALT readings were 71.77 and 24.76 U/L, respectively. Comparatively to the positive control group (G 2), the two rat groups that received 250 and 500 mg/kg ZFE (Groups 3 and 4) exhibited a significant decline in AST and ALT levels. Additionally, the AST and ALT levels in the hyperuricemic rats were noticeably greater than those in the negative control group. In contrast to the negative control rats, the hyperuricemic rats that received 500 mg/kg ZFE showed non-significant variations in AST and TP levels. The highest improvement was observed in the group which received 500 mg/kg ZFE, which may be due to the general health effects of ZFE, antihepatotoxic effects **(Badr, 2018)** and antioxidant activity **(Morittu *et al*., 2021)**. The hepatoprotective effect may be reffer to the bioactive compounds, such as gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid and coumaric acid, as well as flavonoids, such as kaempferol, catechin, epicatechin, rutin, and quercetin **(Aquino-Bolanos *et al*., 2013 and Morittu *et al*., 2021)**, which have a substantial impact in hepatoprotective **(Coballase-Urrutia *et al*., 2011 and López-Agama *et al*., 2021)**. Protocatechuic acid, rutin, quercetin, sterol, and methyl ester, are among the phytochemicals found in edible flowers that are good for preventing liver damage **(Pinakin *et al*., 2020)**.

Table (4): Impact of zucchini flowers extract on liver function in hyperuricemic rats

Group	Liver function		
	ALT (U/l)	AST (U/l)	Total protein (mg /dl)
G1	24.67±1.64^{c}	71.77±3.19^{d}	6.41±0.26^{a}
G2	51.03±2.53^{a}	121.17±2.07^{a}	5.27±0.10^{d}
G3	35.06±1.72^{b}	90.38±1.02^{b}	5.64±0.03^{c}
G4	27.61±1.44^{c}	80.92±0.37^{c}	5.94±0.05^{b}
LSD	3.54	3.73	0.26

Small variable letters on mean values in each column are significant. Group (1) negative control. Group (2) Hyperuricemic rats (positive control). Group (3) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. Group (4) Hyperuricemic rats received 500 mg/kg zucchini flowers extract.

3.5. Effects of ZFE on renal function in hyperuricemic rats

The effects of treatment hyperuricemic rats by ZFE on urea, creatinine, uric acid, sodium, and potassium levels are shown in Table (5). In comparison to the hyperuricemic rats (G2) and other treated hyperuricemic groups, the positive control had considerably ($P \leq 0.05$) higher levels of urea, creatinine, uric acid, sodium, and potassium. While the negative control group had the lowest levels (21.51 mg/dL, 0.57 mg/dL, 1.59 mg/dL, 132.80 mmol/L, and 3.81 mmol/L, respectively). According to our current research, giving 250 and 500 mg/kg ZFE to hyperuricemic rats significantly decreased the indicators of renal function when compared to hyperuricemic rats. So this striking change might be partially attributed to ZFE's abundance in bioactive substances **(Morittu *et al.*, 2021)**, which may indirectly lower the levels of uric acid and protect the kidney from potential oxidative stress damage. Reactive oxygen species (ROS) and uric acid production are suppressed as a result of these natural antioxidants' ability to act as scavengers for superoxide species **(Lin *et al.*, 2015)**.

According to **Soliman *et al.* (2007)** and **Abdel-Raheem *et al.* (2009)**, morethan 30% of patients whom were treated with GM for longer than one week exhibited nephrotoxicity, which is described with severe renal tubular necrosis, increases in blood urea nitrogen (BUN) and creatinine levels, a decline in renal clearance, variations in body weight gain and urine volume, this lead to renal dysfunction and failure **(Pedraza-Chaverri *et al.*, 2000 and Cuzzocrea *et al.*, 2002)**, despite the fact that the precise mechanism underlying GM-induced nephrotoxicity is still poorly understood. According to investigations by **Tavafi & Ahmadvand (2011) and Said (2011)**, who noticed that gentamicin increases the formation of superoxide anions, hydroxyl radicals, and hydrogen peroxide by renal cortical mitochondria. According to **Nitha & Janardhanan (2008) and Stojiljkovic *et al.* (2012)**, the reactive oxygen species (ROS) cause cell injury and death, which is indicative of both the cell's poor structure and function. Recent research has also suggested that

the endoplasmic reticulum (ER) stress caused by GM is correlated to a rise in ROS generation and, consequently, oxidative stress **(Crow *et al.*, 2004).** Residual nephrons can enhance the excretion of potassium (K^+) and sodium (Na^+) in chronic renal failure (CRF) (**Kim *et al.*, 2010)**.

Table (5): Impact of zucchini flowers extract on kidney function of hyperuricemic rats

Group	Kidney function				
	Urea (mg /dl)	**Creatinine (mg /dl)**	**Uric acid (mg /dl)**	**Sodium (mmol/L)**	**Potassium (mmol/L)**
G1	21.51 ± 0.64^{d}	0.57 ± 0.03^{d}	1.59 ± 0.04^{c}	132.80 ± 2.14^{d}	3.81 ± 0.07^{d}
G2	58.34 ± 1.25^{a}	1.46 ± 0.06^{a}	2.61 ± 0.12^{a}	155.83 ± 1.54^{a}	5.60 ± 0.15^{a}
G3	34.61 ± 2.28^{b}	0.82 ± 0.03^{b}	1.83 ± 0.01^{b}	147.04 ± 0.19^{b}	4.95 ± 0.06^{b}
G4	28.09 ± 1.65^{c}	0.65 ± 0.03^{c}	1.72 ± 0.01^{b}	139.54 ± 1.17^{c}	4.13 ± 0.09^{c}
LSD	2.96	0.07	0.12	2.72	0.18

Small variable letters on mean values in each column are significant. Group (1) negative control. Group (2) Hyperuricemic rats (positive control). Group (3) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. Group (4) Hyperuricemic rats received 500 mg/kg zucchini flowers extract.

3.6. Effect of ZFE on antioxidant enzymatic activities in hyperuricemia rats

The data in Table (6), cleared that hyperuricemic rats (G2) had significantly lower values of both CAT and GPXs enzymes to be 27.42 and 45.63 u/ml, respectively and higher values of MDA (74.48 nmol/ L) than the normal rats, which showed higher values for CAT and GPXs enzymes (86.37 and 107.55 u/ml), respectively and less values for MDA (23.35 nmol/ L). However, hyperuricemic rat groups treated with ZFE exhibited a significant rise in CAT and GPXs enzymes levels, and a significant reduction in MDA activities compared with G2. ZFE have natural and bioactive components such as phenolic compounds, flavonoids, minerals, and vitamins so it has a biological function **(López-Agama *et al.*, 2021)**. Therefore, by achieving a balance between antioxidants and free radicals, oxidative stress in bodily tissues can be eliminated. It should be noted that gallic, ferulic, and caffeic acids are among the principal antioxidant components of ZFE **(Mohamed *et al.*, 2009)**. The rats treated with ZFE showed a rise in the activity of these antioxidants enzymes, confirming their practical potential to prevent the damaging effects of ROS. These results are in harmony with **Badr (2018)** who cleared that alloxan-induced diabetic rats which received cake fortified zucchini flowers powder (ZFP) exhibited increased GST and CAT.

Table (6): Impact of zucchini flowers extract on the levels of MDA, CAT and GPx enzymes of rats

Group	MAD (nmol/ L)	CAT (u/ml)	GPx (u/ml)
G1	23.35±2.64^{c}	86.37±3.29^{a}	107.55±11.27^{a}
G2	74.48±3.17^{a}	27.42±2.35^{d}	45.63±2.93^{d}
G3	42.60±2.11^{b}	45.57±3.25^{c}	68.21±3.00^{c}
G4	26.89±1.24^{c}	79.61±3.95^{b}	89.15±1.59^{b}
LSD	4.52	6.14	11.42

Small variable letters on mean values in each column are significant. Group (1) negative control. Group (2) Hyperuricemic rats (positive control). Group (3) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. Group (4) Hyperuricemic rats received 500 mg/kg zucchini flowers extract.

3.7. Effect of ZFE on liver and kidney weights in hyperuricemic rats

The data showed in Table (7) clarified that the weight of kidney and liver for all groups. G2 showed significant decrease in liver weight (7.56 g) compared with G1 (8.89 g) and all hyperuricemic treated group. Whereas the liver weight of treated groups received 250 and 500 mg/kg ZFE showed increase in liver weight (7.91 and 8.35 g) in comparison to positive control group. No significant differences were observed in the kidney weights of all tested groups, the G2 gave the highest value for kidney weight, while kidney weights decreased slightly in the G1 and hyperuricemic groups treated with ZFE. These effects may be due to a higher phenolic content of ZFE **(López-Agama *et al.*, 2021)**. The results in agreement with **Abdel-Hady *et al.* (2018)** who found that there were significant differences in the liver weight in different experimental rat groups fed on *Lantana camara* and squash (*Cucurbita pepo*) extracts compared with positive control rats.

Table (7): Impact of zucchini flowers extract on whole kidney and liver weights of rats

Group	Whole kidney weight	Liver weight
G1	2.19±0.23^{a}	8.89±1.03^{a}
G2	2.39±0.22^{a}	7.56±0.79^{b}
G3	2.21±0.24^{a}	7.91±0.41^{ab}
G4	2.30±0.12^{a}	8.35±0.92^{ab}
LSD	0.28	1.10

Small variable letters on mean values in each column are significant. Group (1) negative control. Group (2) Hyperuricemic rats (positive control). Group (3) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. Group (4) Hyperuricemic rats received 500 mg/kg zucchini flowers extract.

3.8. Cytotoxic effect of ZFE on against HepG-2 and VERO cell line (MTT Assay).

HepG-2 and VERO cells were used at range of concentrations (from 0.0 to 500 g/mL) for 24 hours for assessing the cytotoxic effects of ZFE. The viability of cells percentage was then assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The HepG-2 cell viability percentages started to decline at a concentration of 7.8 g/mL (99.43%), and the largest decline was estimated at 500 g/mL (3.14%; Figure 1) as compared to the control (100%). While, the VERO cell viability percentages started to decline at a concentration of 15.6 µg/mL (98.72%), and the largest decline was estimated at 500 g/mL (9.87 %; Figure 2) as compared to the control (100%).These finding indicate that a high / mild cytotoxicity induced by ZEF in HepG-2 and VERO cancer cells.

The second biggest cause of mortality and a significant public health issue is still cancer. Medicinal medicines, ionising radiation, environmental toxins, and other factors caused a significant DNA damage. Lung, breast, colorectal and stomach cancer (12.7%, 10.9%, 9.7% and 7.81%, respectivelly) are the cancers that are diagnosed the most commonly overall **(Hazafa *et al.*, 2020)**. Numerous articles published in recent years have discussed its benefits for cancer prognosis **(Di Maso *et al.*, 2021)** and impact on the prevention of colorectal cancer **(Yammine *et al.*, 2021)**, breast cancer **(Laudisio *et al.*, 2020)** and prostate cancer **(Urquiza-Salvat *et al.*, 2019)**. According to epidemiological research, having rich diets of its content of bioactive compounds, especially natural antioxidants, can lower the risk of developing cancer **(Loizzo *et al.*, 2016; Pinakin *et al.*, 2020; Zheng *et al.*, 2021)**. Natural substances have the best chances of preventing cancer because they are effective, accessible, and anti-cancer. According to **Tungmunnithum *et al.* (2018)**, which polyphenols substances are employed a vital role for prevention and treatment different types of cancer. Both flavonoids and many phenolic compounds have been shown to be potent antioxidants, anticancer, cardioprotective, antibacterial, anti-inflammatory, immune system-promoting, skin protection from UV radiation, and intriguing candidates for pharmaceutical and medical use **(Kumar & Pandey, 2013, Chen *et al.*, 2015, Dziao *et al.*, 2016, Andreu *et al.*, 2018 and Meng *et al.*, 2018).** So, the biological activity of a ZFE may be refer to its chemical composition of these compounds as shown in (Table 2).

For the prevention of cancer, edible flowers are considered one of the rich source of phytochemicals **(Pinakin *et al.*, 2020)**. The complementary pathways of oxidative stress, inflammation, interleukins (ILs), tumour

necrosis factor (TNF)-a, nuclear factor kappa-light-chain-enhancer of activated B cells (NFjB), and apoptosis, including Bcl-2-associated Xprotein (Bax), Bcl2, caspase, and cytochrome C, may be targeted by edible flowers for prevent and battle cancer progression **(Fakhri *et al.*, 2021)**.

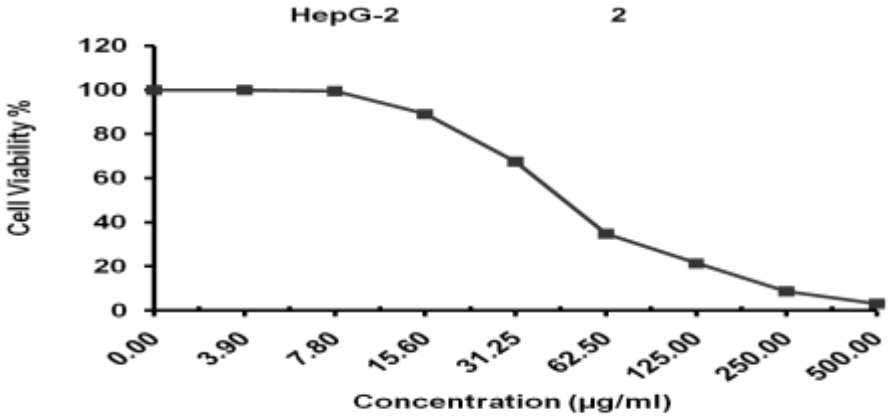

Fig. (1): Viability assessment of ZFE (from 0.0 to 500 µg/mL) in HepG-2 cells at 24 h post-stimulation by MTT assay. The results are presented as cell viability percentage (%) normalized to control (non-stimulated) when compared with control. Data are expressed as the mean values obtained from three experiments in duplicate.

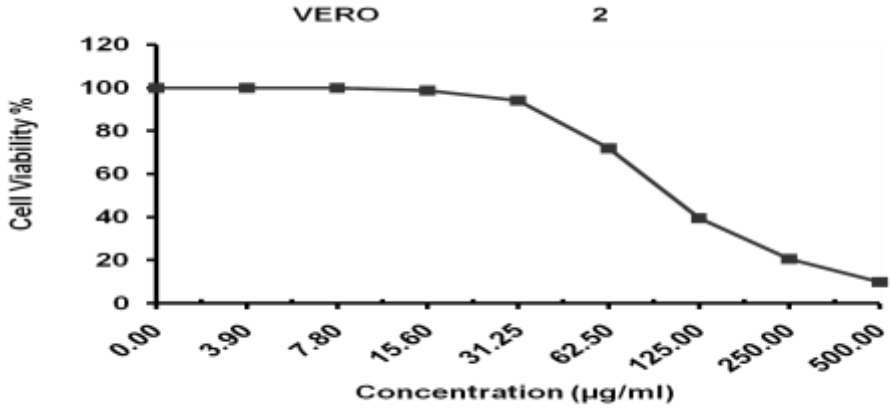

Fig. (2): Viability assessment of ZFE (from 0.0 to 500 µg/mL) in VERO cells at 24 h post-stimulation by MTT assay. The results are presented as cell viability percentage (%) normalized to control (non-stimulated) when compared with control. Data are expressed as the mean values obtained from three experiments in duplicate.

3.9. Kidney histopathological examination

Photo legend No. (1): Photomicrographs presented the histopathological variations for sections of kidney tissue between examined groups as follows: (A) Kidney section of negative control group signified the standard histological structure of renal cortex with normal assembly of proximal convoluted tubule (thick arrow), distal

convoluted tubule (wave arrow), and renal corpuscle (circle) composing of its bordering bowman's capsule (thin arrow), glomerulus (arrowhead), as well as glomerular space in between them. (B) Kidney sections of positive control group highlighted severe degenerative changes along renal tubules losing its normal organization in cortex area (cube). Tubules detected with epithelial desquamation (thick arrow), lining apoptotic cells (arrowhead), and hyaline cast inside its lumen (thin arrow). Renal Corpuscle marked deteriorated bowman's capsule and RBC'S between glomerulus (thin arrow). Inside blood vessels, serious congestion (wave arrow) was noticed. (C) Kidney section of treated group (3) revealed evident regress in renal cortex structure. Renal corpuscle marked with thinning and deterioration (circle), apoptotic cells lining glomerulus (arrowhead), along with significant expanding in glomerular space (wave arrow). Renal tubules posed degeneration (cube), desquamated epithelium (thick arrow) besides dispersed hyaline cast (thin arrow). (D) Kidney section of treated group (4) marked slight improvement along renal cortex area. Bowman's capsule revealed serious thinning and sloughing in other areas (circle). Some distal convoluted tubule existed with obvious degeneration (cube), apoptotic lining cells (arrowhead), epithelial desquamation (thick arrow), as well as hyaline cast inside lumen (thin arrow).

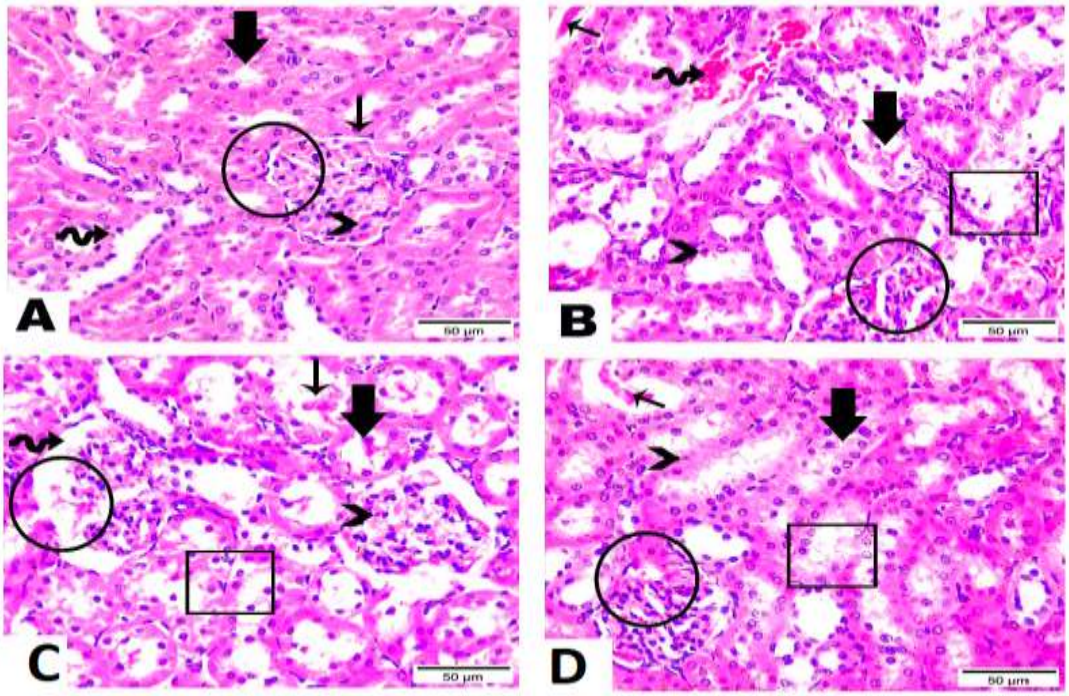

photo 1. Photomicrograph of different H&E-stained kidney section of different groups. (A) negative control. (B) Hyperuricemic rats (positive control). (C) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. (D) Hyperuricemic rats received 500 mg/kg zucchini flowers extract. (Hematoxylin & Eosin Stain, Magnification Power= x400 & Scale Ba r= 50µm).

3.10. Liver histopathological examination

Photo legend No. (2): Photomicrographs highlighted the pathological differences in portal area of liver tissues between studied groups as follows: (A) Liver sections of negative control group exhibited the normal portal area structure with portal vein, hepatic artery, and bile duct (circle). Hepatic cords existed in a regular parallel form (thick arrows) encompassing polygonal hepatocytes with large round central light vesicular nucleus (arrowhead). Cords are split by hepatic sinusoids (wave arrow) in conjunction with Von Kupffer lining cells (thin arrows). (B) Liver sections of Positive control group accentuated serious congestion (cube) and inflammatory cells infiltration (circle), and edema (thin arrow) in portal area. Certain hepatic cords demonstrated necrotic changes (thick arrow). Some hepatocytes observed in an apoptotic form (arrowhead). Hepatic sinusoids existed with accumulated blood inside them (wave arrow). (C) Liver sections of treated group (3) showed degenerative changes with losing the arrangement of hepatic cords. Portal Area revealed inflammatory cells infiltration (circle) and congested blood vessels (wave arrow). Hepatic tissue emphasized necrotic cords (thick arrows), apoptotic hepatocytes (arrowhead), as well as narrowing in hepatic sinusoids (thin arrow). (D) Liver sections of treated group (4) highlighted slight improvement in hepatic tissue structure. Portal Area exposed inflammation, congestion, and deteriorated lining epithelium of portal vein (circle). Hepatic tissue restored cords organization, but still certain area appeared necrotic (thick arrows). Hepatocytes existed in normal, apoptotic (arrowhead), and vacuolated forms (thin arrow). Dilatation in hepatic sinusoids was also noticed (wave arrow).

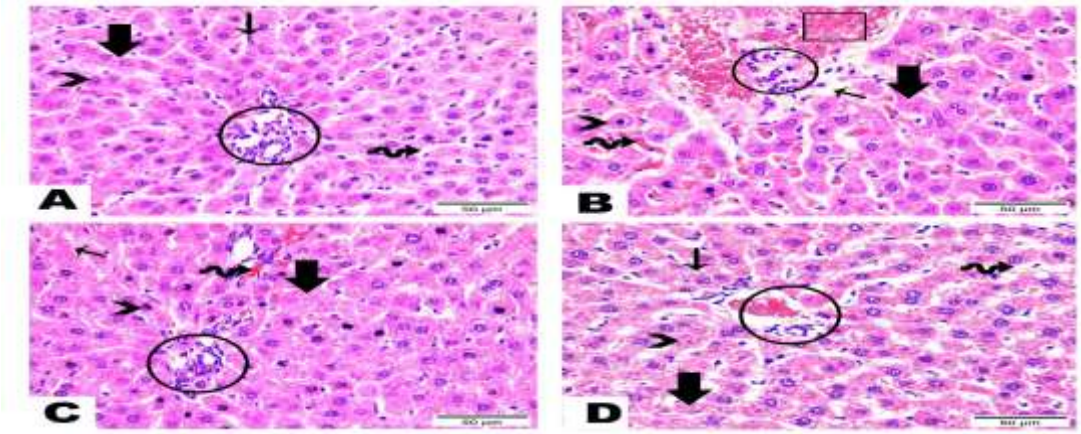

Photo 1. Photomicrograph of different H&E-stained liver section of different groups. (A) negative control. (B) Hyperuricemic rats (positive control). (C) Hyperuricemic rats received 250 mg/kg zucchini flowers extract. (D) Hyperuricemic rats received 500 mg/kg zucchini flowers extract. (Hematoxylin & Eosin Stain, Magnification Power = x400 & Scale Bar = 50µm).

4. Conclusions

Given the information above, ZFE might be regarded as a novel source of nutrition that, recognizes to its high antioxidant content, may have positive health-protective effects. In Albino rats, ZFE showed to provide efficient protection against hyperuricemia. Rat histopathology analysis revealed that the bioactive components of ZFE were effective at preventing hyperuricemia by reducing the concentrations of variables associated with renal dysfunction and severe kidney tissue damage. ZFE significantly damaged HepG-2 cells (which represent human hepatocellular carcinoma) and Vero cells (which represent kidney epithelial cells). ZFE is categorically indicated as an anti-hyperuricemic and anti-cancer drug.

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الملخص:

تعتبر أزهار الكوسة (*Cucurbita pepo L.*) من المخلفات الزراعية الغنية بالعناصر الغذائية والمكونات النشطة بيولوجيا، وبالرغم من ذلك لا توجد معلومات كافية عن فوائدها الغذائية والصحية، لذلك أجريت هذه الدراسة بهدف تقييم التأثير العلاجى المحتمل لمستخلص ازهار الكوسة علي الفئران المصابة بفرط حمض يوريك الدم. وكذلك دراسة تأثيره كمضاد لسرطان الكبد والكلى. واستخدم لذلك ٢٤ من ذكور الفئران الالبينو، تم تقسيمهم إلى أربع مجموعات تحتوى كلا منها على ٦ فئران. كانت المجموعة الأولى بمثابة المجموعة الضابطة السالبة والتى تلقت نظامًا غذائيًا أساسيًا، ثم أعطيت الفئران المتبقية (١٨ فأر) نظام غذائي أساسي وتم حقنها بالجنتاميسين بمعدل ١٠٠ ملجم / كجم من وزن الجسم / يوم لمدة ٧ أيام لاحداث الاصابة بفرط حمض يوريك الدم، ومن ثم إصابة الكلى. وبعد ذلك تم تقسيم الفئران التي تعاني من فرط حمض يوريك الدم إلى ثلاث مجموعات، كانت المجموعة الأولى منهم بمثابة المجموعة الضابطة الموجبة والتى لم تتلق أي علاج، في حين تلقت كلاً من المجموعتين الثانية والثالثة محلول يحتوى على ٢٥٠ و ٥٠٠ ملجم / كجم من مستخلص ازهار الكوسة لمدة ٣٠ يومًا. وأوضحت النتائج أن قيم التحليلات البيولوجية لفئران المجموعة التي تعاني من فرط حمض يوريك الدم والتي أعطيت مستخلص أزهار الكوسة بمعدل ٥٠٠ ملجم/كجم لم تختلف كثيراًعن قيم المجموعة الضابطة السالبة وشهدت أيضاً تحسنًا كبيرًا في وظائف الكلى مقارنةً بالمجموعة الضابطة الموجبة. وكانت مستويات كلا من المالونالدهيد (MDA) والليبوبروتينات منخفضة الكثافة (LDL) والكوليسترول والدهون الثلاثية وAST وALT و الكرياتينين واليوريا أقل معنوياً فى مجموعة الفئران المصابة بفرط حمض يوريك الدم والتي أعطيت مستخلص أزهار الكوسة بمعدل ٥٠٠ ملجم/كجم وكان مستوى البروتين الكلى اعلى معنويا في تلك المجموعة وذلك مقارنةً بالفئران المصابة بفرط حمض يوريك الدم ولم تتلقى اى علاج (المجموعة الضابطة الموجبة)، ويمكن أن يُعزى التأثير البيولوجي لمستخلص أزهار الكوسة إلى زيادة محتواه من مضادات الأكسدة والتى يمكن أن تعزز من نشاط الجلوتاثيون بيروكسيديز والكتاليز. وقد أظهرمستخلص أزهار الكوسة سمية خلوية كبيرة على خلايا HepG-2 (التي تمثل سرطان الخلايا الكبدية البشرية) وخلايا فيرو (التي تمثل الخلايا الظهارية للكلى). ومما سبق يمكن القول بأن هذه النتائج قد تكون بمثابة أساس تجريبي لمزيد من الدراسة عن تأثيرات مستخلص أزهار الكوسة المحتملة المضادة لفرط حمض يوريك الدم والسرطان.

الكلمات المفتاحية: أزهار الكوسة، الجنتاميسين، فرط حمض يوريك الدم، مضادات السرطان، السمية الكلوية.