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Effect of Bisphenol A on Nephrotoxicity in Rats and The protective Role of Giloy and Echinacea Against This Effect

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ABSTRACT

this study aimed to investigate the potential protective effect of Giloy *(Tinospora cordifolia ; T. cordifolia*) (*T. cordifolia*) and Echinacea (*Echinacea purpurea* ;*E. purpurea),* on nephrotoxicity in experimental rats. Thirty six male albino rats (50±10 g) (6 rats each), the first kept as as a negative control group (-v) control received basal diet throughout the experimental period . While second was positive control group (+v) control which treated orally with BPA (25 mg/kg/day) dissolved in olive oil, to induce nephrotoxicity once daily, for 6 weeks while the four others groups fed on basal diet and treated orally with BPA (25 mg/kg/day) and ethanolic extracts of *T. cordifolia* (200, 400 mg/kg/day) and *E. purpurea* (200, 400mg/kg/day) for 6 weeks (as a protective experiment) . At the end of experimental period, biological evaluation, serum antioxidant enzymes, kidney function, Na, K were evaluated and MDA were estimated. Also histological changes of kidney tissues were examined. The obtained results concluded that supplemented diets with plant materials (extract of *T. cordifolia* and *E. purpurea*) improved antioxidant enzymes status, kidney function, Na, K and MDA. The best results found in high doses of *T. cordifolia* and *E. purpurea* followed by low doses of *T. cordifolia* and *E. purpurea*. So, these plants could be used for improves nephrotoxicity and may be other kidney functions.

Keywords: Nephrotoxicity- Bisphenol A - kidney function, antioxidant enzymes- *Tinospora cordifolia* - *Echinacea purpurea*- histological changes

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INTRODUCTION

Nowadays, most of the chemicals that are used in our daily life cause hazardous effects on the environment. Some of these chemicals are severely toxic to humans. Plasticizers are among such chemicals. Plasticizers are substances that are used in disposable dishes, manufacture of polycarbonates, plastic industry, and dental materials to produce or increase plasticity and flexibility as well as to reduce fragility **(Fadda *et al.*, 2019).**

Bisphenol A (BPA) .This contact with food material is due to heating, overuse, contact with an acidic or alkaline substance, and microwave exposure, leading to BPA intake and the food material **(Ahmad and Huei, 2021).**

A variety of chemical constituents such as alkaloids, diterpenoid lactones, steroids, glycosides aliphatic compounds and polysaccharides have been isolated, identified and characterized from different parts of *T. cordifolia*. T. cordifolia has shown antioxidant, anti-hyperglycaemic, anti-

neoplastic, anti-stress, antispasmodic, anti-pyretic, anti-allergic, antileprotic, antiinflammatory and anti hyperlipidaemic activities in experimental animals **(Sharma *et al.*,2019).**

*E. purpurea*is an important medicinal plant with various pharmacological properties. Nevertheless, limited information is available on the potential of *E. purpurea* in combating immunotoxicity of chemotherapeutic drugs, such as cisplatin **(Matthias *et al.*, 2008)**. Its Extracts and dietary supplements exhibited anti-immunosuppressant, Antioxidative, anti-inflammatory, antibacterial, antiviral, and anticancer properties. It has been previously reported that Echinacea compounds improved insulin sensitivity **(Said *et al.*, 2019).**

MATERIALS AND METHODS

Materials:

Plant materials: *(E. purpurea*.) and (*T. Cordifolia*.),were purchased from The Local Company for Herbs and Medicinal Plants, Cairo Governorate, Egypt.

Corn oil and starch were purchased from the local market. Casein, cellulose, vitamins, minerals, dextrin, L-cysteine and choline chloride will be obtained from the Cairo Company for Chemical Trading, Cairo, Egypt.

Experimental animals: A total of 36 adult male albino rats (Sprague_ Dawley strain) were be obtained from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt.

Chemicals: All required chemicals were obtained from El Gomhouria Company for Trading Drugs, Chemicals and Medical Appliances, Cairo, Egypt. BPA (BPA) powder (97%) purity was purchased from Sigma Aldrich Company (USA).

Methods:

Preparation of *T. cordifolia* Ethanolic Extract (TCE)

Dried and powdered stem of *T. Cordifolia* were defatted with petroleum ether to remove lipids and fats, and then the residue was extracted with ethanol using soxhlet apparatus. The ethanolic extract was evaporated under reduced pressure at 40^{oC}, using a rotary vapour evaporator. The extract was kept in 4^{oC} for further analysis and experimental studies according to **Trigunayat and Mishra(2017).**

Preparation of *E. purpurea* Ethanolic Extract (EPE)

The samples were dried and 70% of ethanol was added. The obtained solution was stirred for 24 h at 40 $^{\circ}C$. After, the crude extract was filtered and freeze-dried according to **Ling Kong *et al*., (2021)**

Chemical analysis of plant samples: Phenolic compounds were estimated in the extracts of the plant using a highly efficient HPLC device according to **AOAC, (1995)**.

Experimental design: Animals were kept in clean wire cages under hygienic conditions. Feed was introduced (*ad libitum*) to the rats in special food containers to avoid scattering. Similarly, fresh water was provided *ad-libitum* and checked daily. Adaptation was continued for one week. After that, rats were randomly assigned to 6 equal groups as follows:

The first group: fed on basal diet according to **Reeves *et al* ., (1993)** for 6 weeks as a negative control group.

The second group: treated orally with BPA (25 mg/kg/day) dissolved in olive oil, to induce nephrotoxicity according to **Haroun *et al*.,** (**2016)**.

once daily, for 6 weeks and kept as positive control group.

The third group: treated orally with BPA (25 mg/kg/day) and TCE (200mg/kg/day) for 6 weeks a protective agent .
The forth group: treated orally with BPA (25 mg/kg/day) and TCE (400mg/kg/day) for 6 weeks a protective agent .
The fifth group: treated orally with BPA (25 mg/kg/day) and EPE (200mg/kg/day) for 6 weeks as a protective agent.
The sixth group: treated orally with BPA (25 mg/kg/day) and EPE (400mg/kg/day) for 6 weeks as protective agent .

At the end, animals were weighed, fasted overnight, and then sacrificed under very light ether anaesthesia. Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 rpm (round per minute) for 15 minutes at room temperature, transferred into dry clean ebendorf tubes, then kept frozen at - 20˚C for latter determinations. Livers, kidneys and hearts were removed from rats by careful dissection, washed in saline solution (0.9%), dried using filter paper and independently weighed.

Dietary evaluation:

During the experiment period (6 weeks), the quantities of diet which were consumed (feed intake) and / or wasted were recorded every day. In addition, rats' weight was recorded weekly. At the end of the experiment, feed intake, body weight gain (BWG %), organs weight as a percent of total body weight and feed efficiency ratio (FER) were calculated. According to **(Chapman *et al.*, 1959),** body weight gain and feed efficiency ratio were calculated using the following equations:

Feed intake = Initial Weight of diet (g) - Weight of diet lost (g)

Weight Gain (WG) (g) = Final Weight (g) – Initial Weight (g).

BWG % = Weight Gain (g) / Initial Weight (g) × 100.

Feed efficiency ratio = Gain in body weight (g) / Feed intake (g)

Relative organ weight calculated by the following formula:

Relative organ weight (ROW) % = Organ Weight / Final Body Weight× 100.

Relative weight of internal organs (kidneys):

The organs (kidney) were removed, cleaned by saline solution and dried by filter paper then weighted. Only rats kidney kept in buffered formalin solution (10 % V/V) (For histological examination) according to methods described by **(Drury and Wallington**,**1980)**.

Biochemical analysis:

Serum and tissues were analyzed to determine the following parameters:-

1- Serum levels of uric acid, urea, and creatinine were calculated according to **Barham and Trinder (1972) and Fossati et al., (1980).**

Creatinine clearance was estimated from the levels of both serum and urine creatinine inaddition to the 24 h urinary volumes **(Ali *et al.*, 2013) .**

2- Serum Sodium and Potassium were measured according to the colorimetric method described by **Henry (1974).**

3- Renal levels of antioxidant enzymes (Superoxide Dismutase (SOD), Glutathione Peroxidase (GP_X), catalase (CAT) and level of Malondialdehyde (MDA)by **Okhawa *et al.*, (1979).**

Histopathological examination:

After sacrifice of rats, kidney was removed and immersed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. After that, they were cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with haematoxylin and cosin**(Carleton *et al*., 1980)**.

Statistical analysis:

Statistical analysis were carried out using one way analysis of variance (ANOVA) **(Snedecor and Cochran, 1989**

RESULTS AND DISCUSSION

Materials:

Plant materials: *(E. purpurea*.) and (*T. Cordifolia*.),were purchased from The Local Company for Herbs and Medicinal Plants, Cairo Governorate, Egypt.

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The third group: treated orally with BPA (25 mg/kg/day) and TCE (200mg/kg/day) for 6 weeks a protective agent .
The forth group: treated orally with BPA (25 mg/kg/day) and TCE (400mg/kg/day) for 6 weeks a protective agent .
The fifth group: treated orally with BPA (25 mg/kg/day) and EPE (200mg/kg/day) for 6 weeks as a protective agent.
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At the end, animals were weighed, fasted overnight, and then sacrificed under very light ether anaesthesia. Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 rpm (round per minute) for 15 minutes at room temperature, transferred into dry clean ebendorf tubes, then kept frozen at - 20˚C for latter determinations. Livers, kidneys and hearts were removed from rats by careful dissection, washed in saline solution (0.9%), dried using filter paper and independently weighed.

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Weight Gain (WG) (g) = Final Weight (g) – Initial Weight (g).
BWG % = Weight Gain (g) / Initial Weight (g) × 100.
Feed efficiency ratio = Gain in body weight (g) / Feed intake (g)

Relative organ weight calculated by the following formula:

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The organs (kidney) were removed, cleaned by saline solution and dried by filter paper then weighted. Only rats kidney kept in buffered formalin solution (10 % V/V) (For histological examination) according to methods described by **(Drury and Wallington**,**1980)**.

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2- Creatinine clearance was estimated from the levels of both serum and urine creatinine inaddition to the 24 h urinary volumes **(Ali *et al.*, 2013) .**
3- Serum Sodium and Potassium were measured according to the colorimetric method described by **Henry (1974).**
4- Renal levels of antioxidant enzymes (Superoxide Dismutase (SOD), Glutathione Peroxidase (GP_X), catalase (CAT) and level of Malondialdehyde (MDA)by **Okhawa *et al.*, (1979).**

Histopathological examination:

After sacrifice of rats, kidney was removed and immersed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. After that, they were cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with haematoxylin and cosin**(Carleton *et al*., 1980)**.

Statistical analysis:

Statistical analysis were carried out using one way analysis of variance (ANOVA) **(Snedecor and Cochran, 1989)**.

RESULTS AND DISCUSSION

1. The phenolic compounds profiles in Echinacea and Giloy

Table (1) shows phenolic compounds contents in Giloy and Echinacea. The results show that Giloy is higher than Echinacea in Protocatechuic acid, Chlorogenic acid, Syringic acid, Rutin and Apigenin-7-glucoside.While the Echinacea is higher than Giloy in p-hydroxybenzoic acid, Caffeic acid, Vanillic acid, Ferulic acid, Sinapic acid and Cinnamic acid . It noticed that Giloy doesn't contain Gentisic acid, Catachin, Kaempferol and Chrysin. Also, Echinacea doesn't contain Gallic acid, Catachin, p-coumaric acid, Rosmarinic acid, Qurecetin, Apigenin, Kaempferol and Chrysin

to the presence of different compounds of pharmaceutical importance belonging to various groups as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, and phenolics. These compounds possess pharmacological properties, which make it anti-diabetic, antipyretic, anti-inflammatory, anti-oxidant, hepato-protective, and immuno-modulatory. Also, **Verma *et al.*, (2021)** noticed that the medicinal properties of Giloy extracts were attributed to its phytochemical content including steroids, alkaloids, diterpenoid lactones, and glycosides .

On the other hand **Lin *et al*.,(2023)** found that Echinacea had the highest contents of total polyphenols, 5 caffeic acid derivatives (chicoric acid, caffeic acid, caftaric acid, chlorogenic acid, and 1,5-dicaffeoylquinic acid) in the roots, stems, leaves, and flowers, and the antioxidant activities . Also, **Burlou-Nagy, *et al*.,(2022)** showed that the roots and the aerial part of Echinacea include caffeic acid derivatives alkamides, ketoalkenes, polysaccharides and glycoproteins. Moreover, **Percaccio *et al*.,(2023)** attributed the benefits of Echinacea to the presence of alkamides, glycoproteins, polysaccharides and polyphenols, including caffeic acid derivatives (i.e., chicoric acid, caftaric acid and chlorogenic acid). **Abdel-Wahhab *et al*.,(2024)** revealed that the extract of Echinacea contains significant amount of total phenolic compounds and has a strong antioxidant capacity because of its strong capacity to remove DPPH radicals, which validates the consequent reducing power, which indicated a concentration-dependent relationship. In addition, the phytochemical analysis of the Echinacea by HPLC revealed the identification of 19 phenolic and flavonoid compounds. Among these, chlorogenic acid, naringenin, gallic acid coumaric acid, and caffeic acid were the major phenolic components while querectin, rutin, and apigenin were the major flavonoids compound present in the extract.

Figures and Tables

Table (1):The phenolic compounds profiles in Giloy and Echinacea.

compound	Giloy	Echinacea
Gallic acid	5.64	----------
Protocatechuic acid	26.89	18.66
p-hydroxybenzoic acid	42.88	72.52
Gentisic acid	--------	55.64
Catachin	----------	---------
Chlorogenic acid	5.96	2.46
Caffeic acid	763.52	1224.39
Syringic acid	55.22	5.84
Vanillic acid	7.25	13.93
Ferulic acid	170.66	352.05
Sinapic acid	31.52	56.98
Rutin	74.44	21.91
p-coumaric acid	31.66	--------
Apigenin-7-glucoside	811.08	28.50
Rosmarinic acid	11.24	---------
Cinnamic acid	2.36	23.32
Qurecetin	43.12	----------
Apigenin	88.25	----------
Kaempferol	----------	----------

2. Biological evaluation

Table(2) shows the effect of supplemented diet with TCE (200 and 400 mg/kg) and EPE (200 and 400 mg/kg) on total feed intake, BWG % and feed efficiency ratio against BPA induced Nephrotoxicity in Rats

The results showed that previous mentioned parameters recorded a significant decrease in a positive control group as compared to a negative control group. All

administrated groups with TCE and EPE (200 and 400 mg/kg) showed a significant increase ($p \leq 0.05$) as compared to the positive control group, except administrative group with EPE(200mg/kg) showed non-significant differences compared to positive control group for FI. The best result was found in administrative group With TCE (400 mg /kg) as shown in table (2).

This study agree with **Jiang *et al.*,(2020)** reported that the rats showed less body weight gain after exposure to BPA than in the control group, and this unusual weight gain may be due to increased energy expenditure or decreased feed intake. BPA does not alter the feed intake of rats, suggesting that BPA inhibits rat growth by inducing catabolism, Although BPA-treated rats reduced body weight gain, they developed renal hyperplasia, demonstrating that BPA is nephrotoxic. In these respect **Jiang *et al.*,(2020)** found that the body weight gain of rats was significantly decreased after BPA exposure for five consecutive weeks.

Also **Kobroob et al.,(2021)** showed that all BPA-exposed rats experienced less body weight gain than vehicle-fed rats, although the intake of feed and water was almost the same. In addition to the lower body weight. On the other hand, **Chauhan and Aishwarya(2018)** investigated that the treatment with T. cordifolialeaves powder improved body weight. Also, **Lieshchova and Brygadyrenko(2023)** reported that male rats supplemented with E. purpurea seeds drank 21.4% more water and gained 99.2% more body weight compared to control animals. Moreover **Mohammedsaleh and Aljadani, (2021)**administrated that of E. purpurea, Met, and their mixture to diabetic rats revealed a significant ($p \leq 0.001$) improvement in both FBW and BWG% compared to the diabetic group. These result were in agreement with those reported by **Hmwahba,(2022)** showed that diets fortified with EP, RP and DP and their mixture enhanced body weight improvement and enhanced feed efficiency ratio in diabetic rats.

Table (2): Effect of diets supplemented with Giloy and Echinacea on Total feed intake, BWG % and feed efficiency ratio on Nephrotoxicity in rats (n=6rat).

parameters / Groups	FI (g/day)	BWG %	FER
Control –ve	22.60 ^{a} ± 0.87	46.12 ^{a} ± 8.22	0.079 ^{ab} ± 0.011
Control +ve	19.10 ^{d} ± 0.85	18.91 ^{c} ± 3.47	0.037 ^{d} ± 0.007
BPA+TCE (200mg/kg)	20.70 ^{bc} ± 1.14	32.30 ^{b} ± 6.72	0.059 ^{c} ± 0.011
BPA+TCE (400mg/kg)	21.60 ^{ab} ± 0.78	48.29 ^{a} ± 9.50	0.081 ^{a} ± 0.015
BPA+EPE (200 mg/kg)	20.10 ^{cd} ± 0.83	27.85 ^{b} ± 3.50	0.052 ^{c} ± 0.007
BPA+EPE (400 mg/kg)	21.16 ^{bc} ± 0.83	37.04 ^{b} ± 6.49	0.066 ^{bc} ± 0.010
LSD	1.16	8.75	.014

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

3. Relative Kidney weight

Table (3) shows the effect of supplemented diet with TCE (200 and 400 mg/kg) EPE (200 and 400 mg/kg) on relative Kidney weight against BPA induced Nephrotoxicity in Rats . The mean value of relative kidney weight showed a significant increase in the positive control group as compared to the negative control group. It can be explained by Ehigiator *et al*., (2022) reported that administration of BPA increased kidney weight ($p< 0.01$) when compared with the control group which may be attributed to the accumulation of BPA toxic metabolites and the inability of the kidneys to excrete the metabolites . The accumulated toxic metabolites can alter kidney morphology, thereby impairing kidney function. Also, Jiang *et al*., (2020) found that Kidney/body weight was markedly increased in BPA-exposed rats

All administrative groups with TCE and EPE indicated significant decreases ($p \leq 0.05$) as compared to positive control group. The best result was found in administrative group with TCE (400 mg /kg) followed by EPE (400 mg /kg) which closed to the normal group, as shown in

table (3) . In harmony with these findings, Joladarashi *et al.*, (2012) examined the effect of T. cordifolia(TC) consumption, a potent plant widely used to treat diabetes, on kidney Chondroitin sulphate (CS)/dermatan sulphate (DS). There was a 3-fold increase in kidney index in diabetic rats when compared with non-diabetic control rats, which was significantly decreased at 5 % *T. cordifolia* supplementation. Also, Mohammedsaleh and Aljadani,(2021)found that in diabetic rats, E. purpurea, Met, and their mixture resulted in a substantial reduction in kidney weight and kidney index relative to the diabetic rats($p \leq 0.001$).

Table (3): Effect of diets supplemented with Giloy and Echinacea on relative kidney weight on Nephrotoxicity in rats (n=6rat

parameters Groups	Kidney (g/100g.B.Wt.)
Control –ve	0.50 ^{e} ± 0.03
Control +ve	0.91 ^{a} ± 0.07
BPA+TCE (200mg/kg)	0.65 ^{bc} ± 0.03
BPA+TCE (400mg/kg)	0.54 ^{de} ± 0.06
BPA+EPE (200 mg/kg)	0.71 ^{b} ± 0.06
BPA+EPE (400 mg/kg)	0.59 ^{cd} ± 0.06
LSD	0.07

Means in the same column with different superscript letters are significantly different at $p \leq 0$.

4. Biochemical analysis

4.1. Kidney functions

Table (4) shows the effect of supplemented diet with TCE (200 and 400 mg/kg) and EPE (200 and 400 mg/kg) on kidney functions against BPA induced Nephrotoxicity in Rats. The kidney function was affected as indicated by the increase of serum uric acid, urea and creatinine which the mean values of previous mentioned parameters showed significant increases in positive control group compared to negative control group. All administrative groups recorded a significant decrease ($p \leq 0.05$) compared to positive control group . For Creatinine, the best result was found in administrative group with TCE (400 mg /kg). For urea and uric acid, the best result was found in administrative group with the high dose of TCE and EPE which closed to normal group.

These results reflected that BPA has a negative impact on the glomerular and/or tubular functions resulted in impairment in their ability to excrete waste products as reported by **Jiang *et al*.,(2020)** who hypothesized that BPA causes functional damage to the kidney by inducing oxidative stress, inflammatory response, and mitochondrial dysfunction and found that BPA exposure caused an increase in blood urine nitrogen, serum creatinine, urine protein excretion. Also, **Abdelrazik *et al*.,(2022)** revealed thatThe BPA group showed a significant increase in serum creatinine, urea and BUN levels compared to the control group. Moreover, **Ehigiator *et al*.,(2022)** observed that the BPA-induced alterations in serum renal biochemical markers were accompanied by elevated urea ($p< 0.001$), creatinine ($p< 0.001$), and uric acid levels ($p< 0.001$).

This study agree with Karhib,*et al*.,(2022) who demonstrated that *E. purpurea* has showed a significant antioxidant properties, the bulk of which are attributed to its high phenolic content, which includes flavonoids and phenolic acids. The *E. purpurea* root extract has been shown to have anephroprotective effect against hexavalent chromium toxicity by lowering changes in serum urea, creatinine, and uric acid, as well as ALP and LDH activity in rat kidney homogenates. This could be attributed to E. purpurea's Caffeic acid derivatives, total phenols, and antioxidant properties. Also, Abdel-Wahhab *et al*.,(2024) reported that administration of animals with EPE one-hour prior to bifenthrin intoxication led to a marked down regulation of ALAT, ASAT, urea, and creatinine values near to the corresponding values of the normal control.

Also, Mohammedsaleh and Aljadani,(2021) observed that administration of E. purpurea, Met, and their mixture to diabetic rats caused a significant decline in serum blood urea nitrogen and creatinine levels relative to the diabetic rats ($p \leq 0.001$). These result were in agreement with those reported by Hmwahba.,(2022) who showed that diet supplemented with Echinacea , Rosemary and Dandelion powder and their combination significantly improved kidney function as elevated blood urea nitrogen uric acid and creatinine

In these respect, **Singh *et al*.,(2020)** reported that Creatinine ($p < 0.001$) and microproteinuria levels ($p < 0.05$) in high fat diet supplemented with T. cordifolia stem powder(TCP) HFDX rats showed significant reduction in comparison to high fat diet HFD rats, Urea and Uric Acid levels were reduced in HFDX rats as compared to HFD rats.

Table (4): Effect of diets supplemented with Giloy and Echinacea on Creatinine, Urea and Uric acid on Nephrotoxicity in rats (n=6rat).

parameters / Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control –ve	0.52 ^{e} ± 0.08	13.15 ^{d} ± 2.75	1.63 ^{d} ± 0.15
Control +ve	1.32 ^{a} ± 0.11	79.20 ^{a} ± 18.40	3.28 ^{a} ± 0.17
BPA+TCE (200mg/kg)	0.86 ^{bc} ± 0.12	45.64 ^{bc} ± 7.90	2.66 ^{b} ± 0. 30
BPA+TCE (400mg/kg)	0.67 ^{de} ± 0.10	32.55 ^{c} ± 6.74	2.03 ^{c} ± 0.32
BPA+EPE (200 mg/kg)	0.95 ^{b} ± 0.16	51.38 ^{b} ± 10.08	2.82 ^{b} ± 0.28
BPA+EPE 400 mg/kg)(	0.71 ^{cd} ± 0.11	36.50 ^{c} ± 5.03	2.20 ^{c} ± 0.42
LSD	0.15	12.84	0.38

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

4.2. Creatinine clearance, Urine volume and Urine Creatinine

Table (5) shows the effect of supplemented diet with TCE (200 & 400 mg/kg) and EPE (200 & 400 mg/kg) on kidney function against BPA induced Nephrotoxicity in Rats. The results showed a significant decrease of creatinine clearance, urine volume and urine creatinine in positive control group compared to negative control group. For creatinine clearance, there are a significant increase in administrated groups with TCE(400 mg /kg) and EPE(400 mg /kg) but non-significant differences in TCE(200 mg /kg) and EPE(200 mg /kg) compared to positive control group. For urine volume and urine creatinine, there are a significant increase in all administrative groups with TCE and EPE except EPE(200 mg /kg) group. The best result indicated in administrative groups with the high doses of TCE and EPE which closed to normal group . These results reflect that BPA has a negative impact on the kidney and leads to a deterioration of renal function. The impaired ability to excrete waste products can be caused by a defect in glomerular filtration and/or tubular function that explained by **Kobroob et al., (2018).** This study agree with **Jiang *et al*.,(2020)** who found that BPA exposure caused an increase urine protein to-creatinine ratio, and a decrease in creatinine clearance. Also **Kobroob *et al*., (2021)** found that BPA exposure caused an obvious reduction in creatinine clearance. In addition, a remarkable increase in urine protein excretion.

Also, Uppuluri *et al.*, (2013) demonstrated that the root extract of *T. cordifolia* could help to prevent nephrotoxicity which cisplatin significantly reduced urine to serum creatinine ratio and creatinine clearance. The results of preliminary phytochemical screening of ethanolic extract of T. cordifolia revealed that presence of alkaloid amino acids, flavanoids, glycosides, saponins, steroids, tan- nins, and triterpinoids. Moreover **FILIPETS *et al.*,(2018)** indicated that preventive administration of *E. purpurea* changes kidney reaction in rats with rapid acetylation type, in response to subacute cadmium-nitrate intoxication. The changes are likely to demonstrate the *E. purpurea* participation in volume regulation and maintenance extra cellular fluid volume of the kidneys damaged by exotoxins.

Table (5): Effect of diets supplemented with Giloy and Echinacea on Creatinine clearance, Urine volume and Urin ceartinine on Nephrotoxicity in rats (n=6rat

parameters / Groups	Creatinine clearance (ml/min)	Urine volume (ml/24hr)	Urine ceartinine (mg/dl)
Control –ve	**1.43 ^{a} ± 0.27**	**38.44 ^{a} ± 2. 24**	**25.02 ^{a} ± 1.87**
Control +ve	**0.05 ^{d} ± 0.01**	**9.14 ^{d} ± 2.02**	**11.40 ^{d} ± 0. 72**
BPA+TCE (200mg/kg)	**0.19 ^{d} ± 0.06**	**21.18 ^{c} ± 5.29**	**15.46 ^{c} ± 2. 07**
BPA+TCE (400mg/kg)	**0.95 ^{b} ± 0.20**	**32.28 ^{b} ± 5.26**	**20.56 ^{b} ± 3.52**
BPA+EPE (200 mg/kg)	**0.15 ^{d} ± 0.02**	**14.38 ^{d} ± 1.95**	**14.62 ^{cd} ± 1.91**
BPA+EPE (400 mg/kg)	**0.61 ^{c} ± 0.11**	**29.52 ^{b} ± 7.18**	**19.18 ^{b} ± 3.57**
LSD	**0.19**	**5.84**	**3.24**

Means in the same column with different superscript letters are significantly different at $p \leq 0.0$

4.3. Sodium(Na+) andPotassium(K+)

Table (6) shows the effect of supplemented diet with TCE (200 & 400 mg/kg) and EPE (200 &400 mg/kg) on Sodium(Na+) and Potassium(K+) against BPA induced Nephrotoxicity in Rats. The Sodium(Na+) was affected as indicated by a significant increases in positive control group compared to negative control group and Potassium(K+)was affected as indicated by a significant decreases in positive control group compared to negative control group .For sodium, all administrative groups with TCE and EPE recorded a significant decrease ($p \leq 0.05$) compared to positive control group . The best result was found in administrative group with TCE (400 mg /kg). For Potassium(K+), all administrative groups with TCE and EPE recorded a significant increase ($p \leq 0.05$) compared to positive control group , the best result indicated in administrative groups with the low dose of TCE and EPE which closed to normal group.

This study agree with **Ehigiator *et al.*,(2022)** reported that BPA decreased electrolytes ($p< 0.01$) when compared with the control group. In these respect, **Singh *et al.*,(2020)** showed that K level was reduced in high fat diet supplemented with T. cordifolia stem powder(TCP) HFDX rats as compared to HFD rats. On the other hand, **Turkistan, (2019)** showed that the group f E.

purpurea root extract EPRE (500mg/kg) with Cisplatin CISP showed a significant ($p<0.001$) increase in ionicNa+ level with significant ($p<0.001$) decreased in ionic K+ level as compared CISP group.

Table (6): Effect of diets supplemented with Giloy and Echinacea on Na+ and K+ on Nephrotoxicity in rats (n=6rat).

parameters / Groups	Na+(mmol/L)	K+(mmol/L)
Control –ve	139.12 ^{f} ± 0. 86	5.40 ^{a} ± 0.14
Control +ve	148.38 ^{a} ± 0.44	3.50 ^{d} ± 0.37
BPA+TCE (200mg/kg)	145.00 ^{c} ± 0.07	4.80 ^{b} ± 0.07
BPA+TCE (400mg/kg)	141.60 ^{e} ± 1.45	4.00 ^{c} ± 0.26
BPA+EPE (200 mg/kg)	146.62 ^{b} ± 0.60	4.90 ^{b} ± 0.14
BPA+EPE (400 mg/kg)	143.07 ^{d} ± 1.13	4.20 ^{c} ± 0.17
LSD	1.15	0.28

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

4.4. Antioxidant enzymes and MDA

Table (7) shows the effect of supplemented diet with TCE (200 and 400 mg/kg) and EPE (200 and 400 mg/kg) on antioxidant enzymes against BPA induced Nephrotoxicity in Rats.
The results showed that there are significant decreases of GPX, CAT and SOD in positive control group compared to negative control group.

All administrative groups with TCE and EPE recorded significant increases in previous mentioned parameters compared to positive control group . The best result indicated in administrated group with TCE (400 mg/kg) followed by EPE (400mg/kg).

In contrast in MDA as lipid peroxidation(LPO) indicator, There are significant increases in positive control group as compared to negative control group . All administrative groups with TCE and EPE recorded significant decreases ($p \leq 0.05$) compared to positive control group. The best result was found in administrative groups with high doses of TCE and EPE.

These results consistent with **Jiang *et al*.,(2020)** showed that exposure to BPA for five weeks resulted in significantly increased MDA and significantly reduced SOD, GPx and CAT levels in the kidney tissues. Also**, Abdelrazik *et al*.,(2022)** found that there was a significant increase in

MDA and NO and a significant decline in GSH levels in the BPA group compared to the control group . **Kobroob *et al*.,(2021)**
reported that exposure to BPA for 16 weeks produced an obvious increase in the kidney tissue

The non-enzymatic antioxidant glutathione as well as enzymatic levels of MDA and antioxidant superoxide dismutase were also reduced significantly following long-term BPA exposure. Also, **Saleh *et al*.,(2023)** found that Kidney LPO as MDA. LPO levels were significantly higher in the kidneys of rats in the BPA group than in the control group. **Olukole *et al* .,(2020)** found that BPA caused significant reductions in the activities of SOD, GPX, compared to the control while it caused significant elevations in the levels of MDA when compared with the control group. **Abbas *et al*.,(2021)** revealed a significant reduction of SOD, GPX, and CAT with oral administration of BPA. Also, **Ehigiator *et al*.,(2022)** observed that alteration kidney oxidative stress markers caused by BPA were marked by a significant decrease in glutathione, catalase, superoxide dismutase and glutathione peroxidase levels ($p< 0.001$) with a significant increase in MDA levels ($p< 0.001$) compared with the control group.

In these respect, **Lohanathan *et al*.,(2022)** Excellent antioxidant properties were reported with the ethanolic and n-butanol fractions of T. cordifolia extracts. They have been shown to stabilize the antioxidant status of heart, liver and kidney and inhibit the superoxide, hydroxyl radicals and lipid peroxidation. Studies showed that the methanolic extracts of T. cordifolia-stems prevented cadmium-induced cardiotoxicity, hepatotoxicity and nephrotoxicity by their antioxidant activity via modulation on the cellular antioxidant status of antioxidant enzymes such as superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and GSH levels. T. cordifolia aqueous and hydroalcoholic extracts attenuated arachidonic acid-mediated ROS generation through enhanced enzymic activity of catalase in human monocytic (THP-1) cells. Oral administration of T. cordifolia extract protected against ochratoxin-induced toxicity through increased expression of SOD activity, decreased Asc• and NO• radicals and ROS productions, and Malondialdehyde (MDA) formation. Also, **Bhatia] and Rani (2021)** proved that in light of the counter oxidant activity of its alkaloid parts, its root separate secures against aflatoxin-prompted nephrotoxicit. Cell reinforcement pointers including GPx, SOD, and GSH can be reestablished by taking *T. cordifolia* root separates orally. *T. cordifolia* separates have been accounted for to lessen MDA and receptive oxygen species (ROS) levels while expanding GSH levels in diabetic rodents in maternal livers. and, **SRIVASTAVA, and SINGH (2021)** reported that *T. cordifolia* shows defensive effect by lowering the concentration of thiobarbituric acid reactive substance (TBARS) and enhancing GSH, ascorbic acid, protein and the activities of antioxidant enzymes viz, SOD, CAT, glutathione peroxidase, glutathione S-transferase(GST) and glutathione reductase (GR) in kidney .

This study agree with **Kong *et al*.,(2021)**showed that The GPx activity was increased in EPE groups, The level of SOD was higher in the EPE and control groups, A dramatic decrease in MDA production was observed from EPE1 to EPE5 groups.and, **EL-Sahra *et al*.,(2022)**reported that treatment of hyperthyroid animals with EPE showed significant restoration of the hepatic antioxidant markers (GSH,SOD and GPX), matched with remarkable down regulation in the oxidative markers (MDA).

Also, **Mahmoud *et al*.,(2022)**showed that E.P demonstrated a notable reduction in MDA level and marked increase in CAT activity and GSH level, E. purpurea treatment to lead-injected rats decreased MDA and increased GSH level ,CAT activity. This may be due to the antioxidant effect *of E purpurea* that including scavenging free radicals and chelating transition metal. Furthermore, E.P contains many active components such as caffeic acid and polyphenohics

including cichoric acid and glycosylated flavonoids and polysaccharides that are responsible for the anti-inflammatory and antioxidant effects of E.P. **Karhib*,et al*.,(2022)** found that Antioxidant enzymes (GST, SOD, CAT, GPx, and GR) activities, as well as non-enzymatic (GSH) content in kidney homogenate, were significantly reduced in Cr(VI)-treated rats. In comparison to the control group, rats supplid with EP alone showed a significant increase in enzymatic and non-enzymatic antioxidants.

These result were in agreement with those reported by **Hmwahba (2022)** they showed that feeding diet supplemented with EP, RP and DP and their combination to diabetic rats significantly increased the activity of renal tissue antioxidant enzymes superoxide dismutase (SOD) glutathione peroxidase (GPx), and catalase (CAT). **Abdel‑Wahhab *et al*.,(2024)** showed that treatment of rats with EPE before bifenthrin ingestion resulted in an obvious upregulation in the antioxidant indicators (GSH, SOD & GPx) coupled with a notable downregulation in MDA and NO. Also **Mohammedsaleh and Aljadani (2021)** found that Consumption of *E. purpurea,* Met, and their mixture to diabetic rats produceda substantial rise in renal SOD activity and a substantial reduction in renal MDA content relative to the diabetic rats ($p \leq 0.001$).

Table (7): Effect of diets supplemented with Giloy and Echinacea on GPX , CAT, SOD and MDA on Nephrotoxicity in rats (n=6rat)

parameters / Groups	GPX (U/g.t.)	CAT (U/g.t.)	SOD (U/g.t.)	MDA (nmol/g.t.)
Control –ve	120.32 ^{a} ± 8. 54	3.85 ^{a} ± 0.29	169.22 ^{a} ± 11.42	12.22 ^{d} ± 2.74
Control +ve	55.22 ^{d} ± 4.63	1.53 ^{e} ± 0. 20	73.12 ^{d} ± 7.44	42.10 ^{a} ± 1.99
BPA+TCE (200mg/kg)	81.82 ^{bc} ± 4.46	2.69 ^{c} ± 0.19	127.92 ^{c} ± 9.01	29.92 ^{b} ± 2.09
BPA+TCE (400mg/kg)	108.76 ^{a} ±12.72	3.61 ^{ab} ± 0.22	178.67 ^{ab} ± 19.92	16.62 ^{c} ± 3.53
BPA+EPE (200 mg/kg)	75.08 ^{c} ±4.08	2.36 ^{d} ± 0.23	112.97 ^{c} ± 14.34	33.00 ^{b} ± 2.69
BPA+EPE (400 mg/kg)	93.68 ^{b} ± 19.29	3.41 ^{b} ± 0.21	169.00 ^{b} ± 17.89	18.08 ^{c} ± 4.14
LSD	13.74	0.29	18.38	3.87

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$ .1

5. Histopathological examination of Kidney

Fig.(1) microscopic pictures of hematoxylin and eosin (H&E) stained renal sections showing normal glomeruli and tubules with minimal interstitial tissue in negative control group, normal group (N).While, renal sections from positive control group (BPA group) showing congestion (red arrow), markedly swollen Bowman's capsule with shrunken glomerular tuft (thin black arrows), diffuse severe tubular hydropic degeneration with many nuclear pyknosis (thick black arrow), tubular cast formation (thin blue arrows), perivascular mononuclear cells infiltration (opened arrowhead).

Also, renal sections from BPA+TCE (200 mg/kg) group showing congestion (red arrow), markedly swollen Bowman's capsule with shrunken degenerated glomerular tuft (thin black arrows), diffuse milder tubular hydropic degeneration with few nuclear pyknosis (thick black arrow), tubular cast formation (thin blue arrows), fewer perivascular mononuclear cells infiltration (thin blue arrows).

However, Renal sections from BPA+TCE(400 mg/kg) group showing congestion (red arrow), multifocal moderate tubular hydropic degeneration (thick black arrow). While, renal sections from BPA+EPE (200 mg/kg) group showing congestion (red arrow), degenerated glomerular

tuft (thin black arrows), diffuse moderate tubular hydropic degeneration (thick black arrow) very few perivascular mononuclear cells infiltration (thin blue arrows).

Moreover, renal sections from BPA+EPE(400 mg/kg) group showing congestion (red arrow), slightly swollen Bowman's capsule (thin black arrows), tubular cast formation (thin blue arrows), diffuse mild tubular hydropic degeneration (thick black arrow).

These histopathological changes were in agreement with **Aslanturk and Uzunhisarcikli (2020)** reported that BPA provoked histopathological alterations which were explained by the free radicals generated by BPA which disrupted the integrity along with the perme ability of membranes of various organelles and cells.

Also **Nuñez *et al*.,(2023)** showed that low "safe" doses of BPA induce signs of renal injury, including renal histological changes, increased cel- lular senescence, and activation of cellular repair systems in cortical PTCs. **Abdelrazik *et al*.,(2022)** found that histopathological examination of H&E and PAS-stained renal sections of the BPA group showed that there were already profound changes in the renal morphology including vacuolation, degeneration of renal tubules, hyaline casts within the tubules , lumen with mononuclear interstitial cellular infiltrate congestion of renal blood vessels and interruption ofthe PAS-stained brush border of most of the PCT.

On the other hand **Ehigiator *et al*.,(2022)** BPA caused kidney tubular necrosis, widened bowman's space, collapsed glomerulus, and lipid accumulation. Also, **Eid *et* al.,(2023**) found that sections from BPA group show a thickening of the glomerular basement membrane, mesangial expansion, cellular proliferation, and a discontinuous brush border of the renal tubules. **Kobroob *et al*.,(2021)** found that

H&E-stained kidney section from the long-term BPA-exposed rat demonstrated glomerular structural changes with some of them becoming atrophied a large number of and distributed throughout the kidneys. apoptotic cells were found in the proximal tubules Consistent with light microscopy, podocyte effacement characterized by flattening, widening, reduction in the frequency of filtration slits were and shortening of the foot processes, and detected from electron photomicrographs of the long-term BPA-exposed group. Besides, mitochondria within the proximal tubule appeared swollen, fragmented, with disrupted cristae, and decreased in the number .

Also, **Saleh *et al*., (2023)** found that The kidney tissue lost its architecture, and the lumen of the alterations were seen in the Malpighian ,some renal tubules seemed to be obliterated. Also corpuscles, indicating damaged glomerular capillaries that resulted in a broad capsular gap. Damage to the epithelial lining and its brush borders was observed in several proximal and distal convoluted tubules. Also, necrotic cells and large lumens were observed in these tubules owing to flattening of the epithelial lining. Infiltrated leukocytes and congested blood vessels were observed.

This study agree with **Karhib *et al*.,(2022)** Kidney sections of control (G1) and Echinacea purpura (Ep) (G2) groups showed the normal histological structure of the glomeruli and renal tubules in the cortical region. Examination of kidney sections from Cr(VI)-treated rats (G3) revealed significant changes in the renal cortex (mild to moderate cortical tubular and epithelial degeneration as well as the destruction of upper cells forming castes and atrophied glomeruli. However, rats given EP before being injected with Cr(VI) (G4) demonstrated prominent recovery in kidney architecture.

Also, **Abdel‑Wahhab *et al*.,(2024)** who showed that the group

The results were in agreement with those reported by **Hmwahba.,(2022)** showed that kidneys sections from rats in groups 3a, Echinacea (EP) revealed apparent normal renal parenclyme with no histopathological changes. In these respect, **Singh *et al*.,(2020)** showe ***al*.,(2020)** showed that Concurrent treatment with *T. cordifolia* attenuated HFD-induced histological changes in rat kidneys.

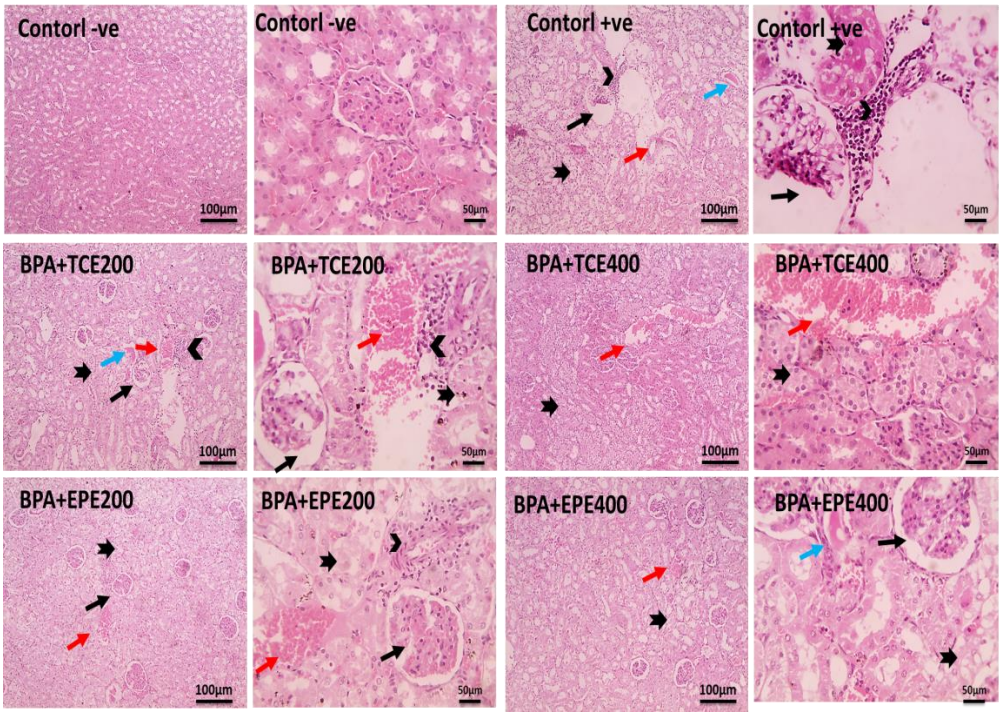

Fig.1: Microscopic pictures of hematoxylin and eosin (H&E) stained renal sections. Low magnification X: 100 bar 100 and high magnification X: 400 bar 50

REFERENCES

Abdelrazik, E., Hassan, H. M., Abdallah, Z., Magdy, A., & Farrag, E. A. (2022). Renoprotective effect of N-acetylcystein and vitamin E in bisphenol A-induced rat nephrotoxicity; Modulators of Nrf2/NF-κB and ROS signaling pathway. *Acta Bio Medica: Atenei Parmensis*, *93*(6).

Abdel-Wahhab, K. G., Elqattan, G. M., El-Sahra, D. G., Hassan, L. K., Sayed, R. S., & Mannaa, F. A. (2024). Immuno-antioxidative reno-modulatory effectiveness of Echinacea purpurea extract against bifenthrin-induced renal poisoning. *Scientific Reports*, *14*(1), 5892.

Abbas, M. A. M., Elmetwally, S. A. F., & Mokhtar Abo-Elfotoh, M. A. (2021). Effect of Oral Exposure to Bisphenol A on the Liver and Kidney of Adult Male Albino Rats. *International Journal of Medical Arts*, *3*(1), 930-937.

Ahmad, A.; Huei, L.C .(2021): Physical and Electrochemical Characterizatioof Modified Graphite NanoparticlesPhosphotungstic Acid-Nafion on Glassy Carbon Electrode for Bisphenol A Determination. *BiointerfaceResearch in Applied Chemistry, 11*, 9266- 92 77.

AOAC (1995): Association of Official Analytical Chemists. Official Methods of Analysis.16th ed. Washington, Dc. USA.

Ali, B. H., Al-Husseni, I., Beegam, S., Al-Shukaili, A., Nemmar, A., Schierling, S., ... & Schupp, N. (2013). Effect of gum Arabic on oxidative stress and inflammation in adenine induced chronic renal failure in rats. *PloS one*, *8*(2), e55242.

Aslanturk, A., & Uzunhisarcikli, M. (2020). Protective potential of curcumin or taurine on nephrotoxicity caused by bisphenol A. *Environmental Science and Pollution Research*, *27*, 23994-24003.

Barham, D. and Trinder, P. (1972) : "Quantitative enzymatic colorimeter determination of uric acid in serum , plasma or urine ". Analyst, 97: 142.

Bhatia, D., & Rani, S. (2021). *Tinospora cordifolia*: a literature review on therapeutic uses and pharmacological actions. *Journal of Pharmaceutical Research International*, *33*(57A), 330 343.

Burlou-Nagy, C., Bănică, F., Jurca, T., Vicaș, L. G., Marian, E., Muresan, M. E., ... & Pallag, A. (2022). *Echinacea purpurea* (L.) Moench: Biological and pharmacological properties. A review. *Plants*, *11*(9), 1244.

Carleton, H. M.; Drury, R. A. B. and Wallington, E. A. (1980): Histological Techniques. 5th edition, Drury, London.

Chapman, D.G.; Castilla , R . And Campbell, J .A. (1959): Evaluation of protein in food .I. A .Method for the determination of protein efficiency ratio can .J. Biochem . plysiol., 37: 679- 68

Chauhan, E. S., & Aishwarya. J. ,(2018). Antidiabetic Lipid Lowering and Oxidative Stress Reducing Potential of "*Tinospora cordifolia*" Leaves Powder in Alloxan Induced Diabetic Albino Rats. International Journal of Creative Research Thoughts (IJCRT) 6: 2320-2882

Drury, R.A. and Wallington, E.A.(1980): Carton is Histological Technique.5 th ed.oxford univ.

Ehigiator, E. B., Adikwu, E., & Chiwendu Ifeduba, A. (2022). Protective activity of glutamine against bisphenol A-induced nephrotoxicity in rats. *Journal of Clinical and Basic Research*, *6*(3), 23-26.

Eid, A.R., Alaa Edeen, M., Soltan, M. A., Al-Shraim, M., Samir A. Zaki, M., M. Al Qahtani, S., ... & M. Hassan, H. (2023). Integration of Ultrastructural and Computational Approaches Reveals the Protective Effect of Astaxanthin against BPA-Induced Nephrotoxicity. *Biomedicines*, *11*(2), 421.

EL-Sahra, D. G., Elqattan, G. M., Hassan, L. K., & Abdel-Wahhab, K. G. (2022). Modulatory efficiency of *Echinacea purpurea* extract on hyperthyroidism modeled rats. *Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry*, *14*(2), 165-179.

Fadda, W. A., Essawy, A. S., & Faried, M. A. (2019). Green tea extract protects the renal cortex against bisphenol A-induced nephrotoxicity in the adult male albino rat: a histological and immunohistochemical study. *Eur J Anat*, *23*(6), 415-424.

FILIPETS, N. D., KMET, T. I., HRACHOVA, T. I., BULYK, T. S., & KRYVCHANSKA, M. I. (2018). Functional kidney state of mature rats with rapid acetylation type under conditions of subacute cadmium-nitrate intoxication and preventive introduction of echinacea purpurea. *Archives of the Balkan Medical Union*, *53*(4), 512-518.

Fossati, P.; Prencipal, L. and Berti, G.(1980): Egyption colorimetric method of determination of uric acid in serum.Clin.Chem.;26:227

Haroun, M.D, **Zamzam, M.D.**, **Metwally, M.D**, **and EL-Shafey, M.S.C.(2016)**: EFFECT OF VITAMIN C ON BISPHENOL A INDUCED HEPATO& NEPHROTOXICITY IN ALBINO RATS Egypt J. Forensic Sci. Appli. Toxicol Vol 16 (2) December suppl 2016

Henry RJ. (1974) Creatinine Measurements with the Colorimetric Method Clinical Chemistry Principles and Techniques. New York, NY

Hmwahba. (2022). Protective effect of Echinacea (*Echinacea Angustifolia*), Rosemary (*Rosmarinus officinalis*, L.) and Dandelion (*Taraxacum Officinal*) powder in Alloxan Diabetic Rats.24-1,(69)2022,

Jiang, W., Zhao, H., Zhang, L., Wu, B., & Zha, Z. (2020). Maintenance of mitochondrial function by astaxanthin protects against bisphenol A-induced kidney toxicity in rats. *Biomedicine & Pharmacotherapy*, *121*, 109629.

Joladarashi, D., Chilkunda, N. D., & Salimath, P. V. (2012). *Tinospora cordifolia* consumption ameliorates changes in kidney chondroitin sulphate/dermatan sulphate in diabetic rats. *Journal of nutritional science*, *1*, e7.

Karhib, M. M., El-Sayed, R. A., Ghanem, N. F., & El-Demerdash, F. M. (2022). Nephroprotective role of *Echinacea purpurea* against potassium dichromate-induced oxidative stress, inflammation, and apoptosis in rats. *Environmental Toxicology*, *37*(9), 2324-2334.

Kong, Z. L., Johnson, A., Ting, T. L., Cheng, P. J., & Mao, C. F. (2021). Protective effects of *Echinacea purpurea* ethanol extract on male reproductive dysfunction in obese rats. *Applied Sciences*, *11*(5), 2392.

Kobroob, A., Peerapanyasut, W., Chattipakorn, N., & Wongmekiat, O. (2018). Damaging effects of bisphenol A on the kidney and the protection by melatonin: emerging evidences from *in vivo* and in vitro studies. *Oxidative medicine and cellular longevity*, *2018*(1), 3082438.

Kobroob, A., Peerapanyasut, W., Kumfu, S., Chattipakorn, N., & Wongmekiat, O. (2021). Effectiveness of N-acetylcysteine in the treatment of renal deterioration caused by long term exposure to bisphenol A. *Biomolecules*, *11*(5), 655.

Kumar, P., Kamle, M., Mahato, D. K., Bora, H., Sharma, B., Rasane, P., & Bajpai, V. K. (2020). *Tinospora cordifolia* (Giloy): phytochemistry, ethnopharmacology, clinical application and conservation strategies. *Current pharmaceutical biotechnology*, *21*(12), 1165-1175.

Ling Kong,Athira Johnson, Tzu-Ling Ting, Po-Jen Cheng and Chien-Feng Mao.,(2021): Protective Effects of *Echinacea purpurea* Ethanol Extract on Male Reproductive Dysfunction in Obese Rats*Appl. Sci.* 2021, *11*, 2392 2 of 20

Lin, X. J., Lai, Z. S. Y., Luo, Q., Kong, M., Liang, M. J., Wu, H., & Bai, M. (2023). Correlation between Polyphenol Contents and Antioxidant Activities in Different *Echinacea Purpurea* Varieties. *Current Medical Science*, *43*(4), 831-837.

Lohanathan, B. P., Balasubramanian, B., Shanmugaraj, B., Subbiah, S., Hu, R. M., Chih Yang, H., & Baskaran, R. (2022). Therapeutic potential of the medicinal plant *Tinospora cordifolia*–minireview. *Phyton*, *91*(6), 1129.

Lieshchova, M. A., & Brygadyrenko, V. V. (2023). Effect of *Echinacea purpurea* and Silybum marianum seeds on the body of rats with an excessive fat diet. *Biosystems Diversity*, *31*(1), 90-99.

Mahmoud, A. H., Abbas, M. M., & AbdElmonem, H. A. (2022). The Antioxidant effects of cerium oxide nanoparticles and *Echinacea purpurea* against lead-induced immunosuppression in male albino rats. *The Egyptian Journal of Hospital Medicine*, *89*(2), 6106-6114.

Matthias A, Banbury L, Bone KM, Leach DN, Lehmann RP. (**2008)** Echinacea alkylamides modulate induced immune responses in Tcells. Fitoterapia.;79:53–8**.**

Mohammedsaleh, Z. M., & Aljadani, H. M. (2021). Echinacea purpurea root extract modulates diabetes-induced renal dysfunction in rats through hypoglycemic, antioxidants, and anti inflammatory activities. *Medical Science*, *25*, 1033-43.

Nuñez, P., Arguelles, J., & Perillan, C. (2023). Effects of short-term exposure to low doses of bisphenol A on cellular senescence in the adult rat kidney. *Histochemistry and Cell Biology*, *159*(5), 453-460.

Okhawa, H.; Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry. 95:351–358.

Olukole, S. G., Ola-Davies, E. O., Lanipekun, D. O., & Oke, B. O. (2020). Chronic exposure of adult male Wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid. *Endocrine regulations*, *54*(1), 14-21.

Percaccio, E., De Angelis, M., Acquaviva, A., Nicotra, G., Ferrante, C., Mazzanti, G., ... & Di Sotto, A. (2023). ECHOPvir: A Mixture of Echinacea and Hop Extracts Endowed with Cytoprotective, Immunomodulatory and Antiviral Properties. *Nutrients*, *15*(20), 4380.

Reeves, P. G., Nielsen, F. H., & Fahey Jr, G. C. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on thereformulation of the AIN-76A rodent diet. *The Journal of nutrition*, *123*(11), 1939-1951.

Saleh, S. M., Mahmoud, A. B., Al-Salahy, M. B., & Mohamed Moustafa, F. A. (2023). Morphological, immunohistochemical, and biochemical study on the ameliorative effect of gallic acid against bisphenol A-induced nephrotoxicity in male albino rats. *Scientific Reports*, *13*(1), 1732.

Said, H., Abdelaziz, H., Abd Elhaliem, N., Elsherif, S. (2019): A Comparative Study between Ginger and Echinacea Possible Effect on the Albino Rat Spleen of Experimentally Induced Diabetes. *Egyptian Journal of Histology*, 43(3), 763-776. doi: 10.21608/ejh.2019.16107.1156

Sharma , M.; Pundir , J.; Vishwakarma, P.; Goel ,R., Saini ,M. and Saxena K. K.(2019): Evaluation of nephroprotective activity of *Tinospora cordifolia* against gentamicin inducednephrotoxicity in albino rats: an experimental study. International Journal of Basic & Clinical Pharmacology. ; 8(6): 1179-1184

Singh, H., Sharma, A. K., Gupta, M., Singh, A. P., & Kaur, G. (2020). *Tinospora cordifolia* attenuates high fat diet-induced obesity and associated hepatic and renal dysfunctions in rats. *PharmaNutrition*, *13*, 100189.

Snedecor, G. W. and Cochran, W. G. (1989): Statistical Methods. 8th ed., Iowa State University Press, Ames, Iowa 50014, USA.

SRIVASTAVA, A. K., & SINGH, V. K. (2021). *Tinospora cordifolia* (Giloy): a magical shrub. *Asian Journal of Advances in Medical Science*, 93-101.

Trigunayat, A., & Mishra, S.K. (2017). EFFECT OF ETHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA ON OXIDATIVE STRESS INDUCED BY CEREBRAL ISCHEMIA-REPERFUSION IN RATS.

Turkistani. A. M.(2019). Modulatory Effect of Echinacea PurpureaRoot Extract on Cisplatin Induced Renal Toxicity in Rats: Antioxidant and Anti inflammatory Pathways International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR) | October 2019| Volume 9| Issue 5| Page 88-96.

Uppuluri, S., Ali, S. L., Nirmala, T., Shanthi, M., Sipay, B., & Uppuluri, K. B. (2013). Nephroprotector activity of hydro alcoholic extract of *Tinospora cordifolia* roots on cisplatin induced nephrotoxicity in rats. *Drug invention today*, *5*(4), 281-287

Verma, D. K., Kumar, P., & El-Shazly, M. (2021). Unmasking the many faces of Giloy (*Tinospora cordifolia L.*): a fresh look on its phytochemical and medicinal properties. *Current Pharmaceutical Design*, *27*(22), 2571-2581.