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FULL ARTICLE

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Assessment of antioxidant traits and protective action of Egyptian acacia pods extracts against paracetamol-induced liver toxicity in rats

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Abstract

This study investigates the protective effect of Egyptian acacia pod extracts against overdose of paracetamol-induced liver damage. Egyptian acacia green and brown pods were extracted by mixture of ethanol 80%: HCl (6 M) (99:1 v/v). In extracts of green and brown pods, total phenolic content in hydrolyzed ethyl acetate fraction (HEF) at pH 4, was 649.89 and 712.14 mg GAE/g while antioxidant activity was 95.55% and 97.35%, both being the highest than any fraction. HEF (pH 4) in brown pods was analyzed by HPLC, there were 22 phenolic compounds rich in ethyl vanillin about 227 mg/g and 11 flavonoids rich in catechin 48.70 mg/g. A biological experiment was conducted using HEF (pH4) in brown pods against overdose of paracetamol in albino rats induced to hepatotoxicity. Thirty rats were divided into five groups; a control group, a paracetamol group, and the other three received paracetamol plus silymarin or two doses of HEF. Animals were received paracetamol and treated with either silymarin or HEF showed reduced levels of liver (ALT, AST, and ALP) and kidney (urea, creatinine, and uric acid) markers compared with the control group as well as reduction of oxidative stress and increment antioxidant enzyme activity in liver tissue when compared with the paracetamol group. It could be concluded that both HEF and silymarin are considerably high hepatoprotector against paracetamol-induced hepatotoxicity in rats due to their strong antioxidant activity.

Practical applications

Both HEF and silymarin improved liver functions and exerted strong antioxidant activities. This antioxidant activity would have a positive effect against oxidative liver damage caused by parcetamol. Thus, it may be concluded that the liver plasma membranes were protected and the regenerative and reparative capacity of liver by phenolic compound in HEF treatment. The study demonstrated the HEF hepatoprotective activity and recommends using Egyptian acacia pods for treatment of liver disorders.

KEYWORDS

antioxidant, Egyptian acacia pods, hepatotoxicity, liver, paracetamol, silymarin

1 | INTRODUCTION

Acacia (*Vachellia nilotica* L.) is an herbal medicinal plant which belongs to the family *Fabaceae;* it grows in tropical and subtropical regions and is known as Arabic gum, Egyptian acacia, or scented thorn in South Africa. It is also used as an ornamental tree in Egypt and is a source of several secondary metabolites and possesses many pharmacological properties enabling it to be a good source of several important secondary metabolites. Its pods and seeds are dried and ground into flour eaten cooked or baked and eaten by humans. It has high protein content, tasty, and flexible. Also, the seeds can be roasted and ground to make a coffee substitute. Being a legume plant, its seeds have high protein contents and the young plants can serve both as human food and animal feed (Adiamo, Netzel, Hoffman, & Sultanbawa, 2020). It is used as antioxidant, antimicrobial (Sadiq, Tarning, Cho, & Anal, 2017), anti-inflammatory (Chaubal, Mujumdar, Puranik, Deshpande, & Deshpande, 2003), antidiarrhea, antidiabetic, anticancer antihypertensive (Sakthive, Kannan, Angeline, & Guruvayoorappan, 2012), antispasmodic agent; as diuretic effective in removing catarrhal matter and phlegm from bronchial tubes (Rather, Islam, & Mohammad, 2015). The pods are in different colors (greenish, dark brown, and black). It contains seeds which possess many active components such as phenolic constituents (gallic acid, ellagic acids, 3, 5-dihydroxy-4-methoxy benzoic acid, and protocatechuic), flavonoids (3,4,5,7-tetrahydroxy flavan-3-ol, catechin, epicatechin, kaempferol, 3,4,7- trihydroxy flavan 3,4-diol, rutin, naringenin, 3,4,5,7-tetrahydroxy flavan-3-oland apigenin) as well as, for example, alkaloids, saponins, and terpenoids (Leela, Kokila, Lavanya, Saraswathy, & Brindha, 2010; Salem, Davidorf, & Abdel-Rahman, 2011).

Liver is the major organ responsible for drug detoxification and chemical toxicity; besides it plays important roles in the synthesis of major macro biomolecules; lipids, carbohydrates, and proteins, besides detoxifying blood from endogenous compounds as bilirubin and exogenous ones as drugs and alcohol (Braeuning et al., 2020; El-Hadary and Hassanien, 2016).

Hepatic injury is usually induced by many toxic chemical such as carbon tetrachloride, thioacetamide, galactosamine (El-Hadary, Elsanhoty, & Ramadan, 2019), and drugs, particularly diclofenac sodium and acetaminophen (El-Hadary and Ramadan, 2019) or by infectious liver diseases.

Paracetamol (N-acetyl-para-aminophenol (APAP) or acetaminophen) is normally used to reduce pains and as antipyretic or analgesic after surgery or with fever in human. It may be utilized in combination with other material in cold medicine (Jóźwiak-Bebenista and Nowak, 2014). It has a reliable safety profile when administered in suitable therapeutic doses. However, overdoses, as in suicide attempts or in unintended accidents, can cause hepatotoxicity leading to acute liver failure and renal necrosis (Mahadevan, McKiernan, Davies, & Kelly, 2006). Recommended doses for children do not usually not exceed 150 mg/kg daily; however, greater doses could induce hepatotoxicity and renal tubular necrosis (Mahadevan et al., 2006).

Excessive amounts of paracetamol can induce severe hepatotoxicity in animals and humans after being metabolized in liver microsomal through cytochrome P450 CYP2E1 pathway into a reactive toxic metabolite, N-acetyl-para-benzoquinone imine (NAPQI), caused cellular necrosis (Cover et al., 2006). Rats are the most susceptible species frequently used in clinical studies for acetaminophen toxicity as administered at 500 mg/kg body weight (bw). Monitoring liver damage is evidenced by elevated levels of serum liver function enzymes such as alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), damage is also shown also by decreased serum protein profile and antioxidant enzyme markers (glutathione peroxidase, glutathione–S-transferase, and super oxide dismutase) would be decreased (El-Hadary, 2016**)**.

Silymarin is a natural product extracted from milk thistle *Silybum marianum* and has shown not only pharmacological properties, but also cardioprotective, antioxidant, anticancer, and anti-inflammatory agent. Silymarin is one of the natural medical products. It is an active component of milk thistle seed extracts with potent antioxidant properties by scavenging free radicals that inhibit lipid peroxidation. Besides, it has an anti-inflammatory action as well as hepatoprotective properties through enhancing superoxide dismutase activity and glutathione activity coupled with high antitumor promoting activity (Anthony, Subramanya, Uprichard, Hammouda, & Saleh, 2013).

Silymarin consists of seven flavonolignans compounds with major constituents of Silibinin 50%–60%, Silychristin 20%, Silydianin 10%, Isosilibinin 5%, and other components as 5% of polyphenols and fatty (acids such as palmitic and oleic acids) (Polyak, Ferenci, & Pawlotsky, 2013).

The general goal of the current study was to evaluate the antioxidant activity of *Egyptian acacia* pod extracts in vitro. The study also aimed at comparatively and comprehensively evaluating the hepatoprotective effects of the extracts on over doses of paracetamol which induce hepatic damage in albino rats compared to silymarin as a standard drug.

2 | MATERIALS AND METHODS

2.1 | Material

Egyptian acacia (*Vachellia nilotica* L.) pods were collected in summer season from Zagazig governorate, Egypt. All chemical, solvent, reagents, and standards were analytical grade and purchased from SIGMA-ALDRICH Co. (Louis, Missouri, USA). Silymarin and paracetamol were provided as gifts from Sedico Pharmaceutical Company, Egypt. Diagnostic kits, that is, lipid profile, liver, and kidney function were purchased from Bio Meriêuex Laboratory Reagents and Products, France.

2.2 | Methods

2.2.1 | Preparation of extracts/fractions

Preparation of hydrolyzed and un-hydrolyzed of green and brown Egyptian acacia pods extracts, 0.5 kg pods were done by washing

with distilled water then drying at 45°C for one day and pulverizing. Powdered samples were divided into two part the first part was extracted by a mixture of ethanol (EtOH) 80%: HCl 6 M (99:1 v/v) 1.25 L and heating in water bath under reflux for two hours (hydrolyzed EtOH extract) (HEE). The second part was soaked in EtOH 80% 1.25 L in brown bottle at room temperature (un-hydrolyzed EtOH extract) (UEE). All extracts were filtered using Buchner funnel and evaporated at 45°C using rotary evaporator (IKA-WERKE, Germany). Extracts were partitioned by separating funnel in distilled water (DW) and ethyl acetate (EtOAC) to obtain un-hydrolyzed EtOAC fraction (UEF) and hydrolyzed EtOAC fraction (HEF). The water partitions were treated with sodium bicarbonate (20:80 w/v) adjusted to pH 8 and obtaining UEF (pH 8) and HEF (pH 8) then fractions were extracted with EtOAC. The water partition was adjusted to pH 4 by HCl 6 M, and then, extracted one more time with EtOAC to obtain UEF (pH 4) and HEF (pH 4). All extracts and fractions were concentrated using rotary evaporator (IKA-WERKE, Germany) until obtain semi-dry extracts (Singh et al., 2009).

2.2.2 | Determination of total phenolic contents

Phenolic content was determined according to (Singleton and Rossi, 1965) using Folin–Ciocalteu reagent and gallic acid as standard. The content were expressed as mg gallic acid equivalent.

2.2.3 | Determination of antioxidant activity

The obtained extracts and fractions of Egyptian acacia pods were assayed for antioxidant activity against 2, 2-diphenylpicrylhydrazyl (DPPH) according to (Feresin et al., 2002). The inhibition percent was calculated by the following equation: % inhibition = [Abs blank – Abs test / Abs blank] × 100.

2.2.4 | HPLC analyses of phenolic compounds

Hydrolyzed EtOAC fraction (HEF at pH4) was dissolved in ethanol (1.0 mg/ml) then, filtered in 0.45 μm filter before injection. Identification of the biochemical consistent of the extract was performed using HPLC (Perkin-Elmer, Series 200, USA) having C18 column (250 mm × 4.6 mm, 5 μm), equipped with a Serie 200 diode array detection (DAD). Throughout analysis, mobile phase water/acetonitrile/glacial acetic acid (980/20/5, v/v/v, pH 2.68) was used as mobile phase A; and acetonitrile/glacial acetic acid (1000/5, v/v) for mobile phase B at flow rate at 1.0 ml/min. The gradient profile, started with 4% changing to 33% mobile phase B linearly in 15 min, and finally, to 50% in 60 min. Compound identification was carried out by comparing peak areas (λ max = 325 nm) of the samples (mg/g dry extract) with that of standards (Prakash, Singh, & Upadhyay, 2007).

2.2.5 | Animals

Wister albino rats with about same age, weighing 130 to 150 g, were brought from the Faculty of Veterinary, Zagazig University, Zagazig, Egypt. Care of animals conformed the WHO guidelines, approved by the faculty of agriculture and the Use Committee, Benha University (BDFABU) with an ethical No. BD /18/02/2016. Animals were maintained control (25°C ± 3°C, 40% to 50% relative humidity and 12-hr light/ dark) and were allowed free access to water and fed on standard diet (Reeves, Nielsen, & Fahey, 1993).

2.2.6 | Treatment protocol

Animals were adapted to the laboratory environment for two weeks, 30 rats were used. There were five treatments six rats in each (El-Hadary and Hassanien, 2016). Group I, control group is healthy control which received vehicle (distilled water). Group II, a paracetamol group received paracetamol daily at 750 mg/kg body weight (bw) for a month. Group III received paracetamol daily at 750 mg/ kg bw, followed by 50 mg silymarin mg/kg bw given orally through gastric gavages for a month. Group IV received paracetamol daily at 750 mg/kg bw, followed by 100 mg/kg bw (HEF at pH 4) of Egyptian acacia brown pod extract for a month. Group V received paracetamol daily at 750 mg/kg bw, followed by 200 mg/kg bw (HEF at pH 4) of acacia pod extract for a month given orally through gastric gavages.

2.2.7 | Biochemical blood parameter estimation

At the end of study, blood samples were taken from the retro-orbital plexus veins by fine capillary heparinized tubes, allowed to clot before separating serum by centrifuging at 1,008 ×*g* for 15 min for biochemical blood estimation and sacrificed by cervical decapitation to obtain organ tissue.

1. **Liver enzyme activity:** Alanine amino transaminase (ALT) aspartate amino transaminase (AST), alkaline phosphatase (ALP) and liver total Bilirubin were determined according to (Reitman and Frankel, 1957; Tietz, 1986).
2. **Serum protein profile:** Total protein and serum albumin levels (Doumas, 1975; Doumas, Biggs, Arends, & Pinto, 1971). Globulin was calculated by subtracting the albumin from serum total protein.
3. **Renal function:** Urea and creatinine levels (Tabacco, Meiattini, Moda, & Tarlip, 1979).

2.2.8 | Evaluation of hepatic antioxidant activity and oxidative stress

After animals sacrifice, liver specimens were washed with saline solution to remove blood, dried by blotting with filter paper, and

weighed. Preparation of liver homogenates (10% w/v) by homogenizing tissue in 100×10^{-3} M of potassium phosphate buffer (pH 7.4), then centrifuged at 1,500 ×*g* for 10 min at 4°C (centurion scientific Ltd, K2015R, UK). Oxidative stress was estimated by measuring the concentration of nitric oxide by the method of (Bryan and Grisham, 2007).

Antioxidant enzyme activity such as Glutathione peroxidase (Gpx), was assayed according to (Flohe and Gunzler, 1984), Glutathione-S-transferase was performed according to (Habig, Pabst, & Jakoby, 1974). Glutathione was reduced as described by (Owens and Belcher, 1965). Catalase activity was performed according to (Aebi, 1984).

2.2.9 | Statistical analysis

Data were subjected to ANOVA and one-way model statistical analyses using the SPSS Statistics software (version 18.0) IBM company 2010, (SPSS Ltd., Surrey, UK) (Steel, Torrie, & Dicky, 1997).

3 | RESULTS AND DISCUSSION

3.1 | Biochemical constituents

Data in Table 1 indicate that the hydrolyzed ethanol (EtOH) extract in both green and brown pods were higher than any fractions, higher by 21.42% and 25.56% for each, respectively. Total phenolic content of Egyptian acacia pods extracts was higher in hydrolyzed ethyl acetate (EtOAC) fraction at (pH 4) in both green and brown pods with approximately 649.89 and 712.14 mg GAE/g extract, respectively. Assessment of antioxidant activity of extracts and fractions of Egyptian acacia green and brown pods showed higher activity in both un-hydrolyzed EtOAC fraction and hydrolyzed EtOAC fraction at (pH 4) with 97.23% and 97.35%, respectively. These results are in agreement with those of (Singh et al., 2009) who found that *Acacia nilotica* L. green pods showed high total phenolic compound as well as high antioxidant activity. Also the aforementioned results are similar to findings obtained by (Sultana, Anwar, & Przybylski, 2007), who found that *Acacia nilotica* possess high inhibition of linoleic acid oxidation and DPPH scavenging activity of different extracts.

3.2 | HPLC analysis of hydrolyzed EtOAC fraction at pH4 of Egyptian acacia brown pods

The data preformed in Table 2 show presence of 33 known compounds forming the majority of hydrolyzes EtOAC fraction at pH 4 of Egyptian acacia brown pods.

The predominant compounds in hydrolyzes EtOAC fraction at pH 4 are 22 phenolic acids which represented about 84% of the total extract, and 11 flavonoids which represented about 8% of the total extract in hydrolyzed EtOAC fraction at pH 4 of Egyptian acacia brown pods. Ethyl vanillin (4-(Hydroxymethyl)-2-methoxyphenol) represented more than 227 mg/g of total extract as a major phenolic compound but catechin 48.70 mg/g is the major flavonoid as compared to standard compound. The aforementioned data are similar to findings obtained by (Singh et al., 2009; Salem et al., 2011) reporting the presence of phenolic acids, gallic acid (105 mg/g), caffeic acid (1.3 mg/g), ellagic acid (18.2 mg/g), and ferulic acid (9.3 mg/g) as well as rutin as flavonoids (27.7 mg/g).

TABLE 1 Yields, phenolic contents, and antioxidant activities of Egyptian acacia green and brown pods extracts

	% Total of extracts/ fractions (yields g/100 g material)		Phenolic content (mg GAE/g extract)		Antioxidant activity (% DPPH inhibition)	
Parameter material	Green pods	Brown pods	Green pods	Brown pods	Green pods	Brown pods
Un hydrolyzed EtOH extract	15.76 ± 1.22	19.42 ± 1.22	114.9 ± 2.11	316.94 ± 2.11	75.33 ± 6.44	85.75 ± 6.44
Hydrolyzed EtOH extract	21.42 ± 1.28	25.56 ± 1.28	382.79 ± 29.18	409.43 ± 29.18	93.98 ± 8.55	95.45 ± 8.55
Un hydrolyzed EtOAC fraction	10.58 ± 1.18	17.87 ± 1.18	326.95 ± 24.12	395.55 ± 24.12	92.54 ± 8.56	93.27 ± 8.56
Hydrolyzed EtOAC fraction	18.81 ± 1.09	14.81 ± 1.09	405.56 ± 32.82	512.22 ± 32.82	94.23 ± 8.90	96.97 ± 8.90
Un hydrolyzed EtOAC fraction (pH 8)	1.02 ± 0.88	1.89 ± 0.88	26.24 ± 2.88	32.92 ± 2.88	38.4 ± 2.65	45.7 ± 2.65
Hydrolyzed EtOAC fraction (pH 8)	0.88 ± 0.13	2.05 ± 0.13	44.92 ± 3.45	47.66 ± 3.45	65.67 ± 5.08	70.93 ± 5.08
Un hydrolyzed EtOAC fraction (pH 4)	1.45 ± 0.15	0.87 ± 0.15	587.73 ± 38.96	690.09 ± 38.96	94.34 ± 8.96	97.23 ± 8.96
Hydrolyzed EtOAC fraction(pH 4)	0.76 ± 0.11	2.05 ± 0.11	649.89 ± 40.16	712.14 ± 40.16	95.55 ± 9.72	97.35 ± 9.72

Abbreviation: mg GAE, mg gallic acid equivalent.

TABLE 2 HPLC analysis of hydrolyzed EtOAC fraction at pH4 of Egyptian acacia brown pods

No.	Phenolic compound	mg/g	No.	Flavonoid	mg/g
1	Ethyl vanillin	227.28	1	Catechin	48.70
2	Protocatchuic	141.56	2	Naringin acid	21.88
3	3-OH-Tyrosol	119.97	3	Quercetrin	2.60
4	Catechol	111.02	4	Hesperidin	2.56
5	Pyrogallol	83.24	5	Rutin	1.65
6	Chlorogenic	40.19	6	Hispertin	0.29
7	P-OH-benzoic	38.61	7	Rosmarinic acid	0.27
8	4-Amino-benzoic	12.55	8	Quercetin	0.06
9	Vanillic	10.99	9	Kaempferol	0.06
10	Caffeic	9.74	10	Apigenin	0.01
11	Benzoic	7.84	11	7-OH flavone	0.01
12	Iso-ferulic	6.80	12	Total flavonoids	78.09
13	Salicylic	6.42	13	unknown	85.19
14	Alpha-coumaric	6.36			
15	Ellagic	4.68			
16	Ferulic	3.08			
17	Reversetrol	2.13			
18	Gallic	1.48			
19	3,4,5-methoxy-cinnamic	1.09			
20	Coumarin	0.98			
21	p-coumaric	0.56			
22	Cinnamic	0.15			
23	Total phenolic	836.72			

TABLE 3 Effect of Egyptian acacia pods extracts on liver enzymes markers in rats as affected by hepatic injury induced by paracetamol

					Bilirubin(mg/dL)		
G	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total	Direct	Indirect
I	Control group	98 ± 0.12^{d}	36 ± 0.11^{c}	282.14 ± 5.62^{c}	0.37 ± 0.02^{c}	0.10 ± 0.01^{cd}	0.27 ± 0.02^{c}
II	Paracetamol group (paracetamol 750 mg/kg)	214 0.57 ± 1.04^{a}	61.03 ± 0.5^{a}	505.00 ± 5.35^{a}	0.84 ± 0.02^{a}	0.29 ± 0.01^{a}	0.55 ± 0.02^{a}
III	Silymarin 50 mg/ kg + paracetamol 750 mg/kg	124.91 ± 1.52^{b}	42.59 ± 0.78^{b}	298.43 ± 1.63^{b}	0.42 ± 0.02^{b}	0.12 ± 0.01^{c}	0.30 ± 0.02^{b}
IV	Egyptian acacia 100 mg/ kg + paracetamol 750 mg/kg	102.76 ± 2.72^{C}	$38.43 \pm 0.37c$	275.71 ± 2.96^{c}	0.39 ± 0.02^{b}	0.14 ± 0.01^{b}	0.25 ± 0.01^{c}
V	Egyptian acacia 200 mg/ kg + paracetamol 750 mg/kg	$68.31 \pm 0.65e$	36.96 ± 0.34^{c}	269.86 ± 1.84^{c}	0.37 ± 0.01^{c}	0.12 ± 0.01^{c}	0.25 ± 0.00^{c}

3.3 | Principles and procedures of statistics: A biometrical approachLiver function markers

The study explored protection impact when applying hydrolyzed EtOAC fraction at pH 4 of Egyptian acacia brown pods extract 100 and 200 mg/kg against paracetamol -induced liver toxicity. Data in Table 3 indicate that paracetamol group rats (group II) were associated with significantly ($p > .05$) higher levels of the liver function markers AST, ALT, ALP, total bilirubin, direct bilirubin, and indirect bilirubin as compared with the control group (group I). Oral subjection of hydrolyzed EtOAC fraction at pH 4 of acacia brown pods extract at 100 and 200 mg/kg as well as standard drug silymarin significantly ($p > .05$) decreased AST, ALT, ALP, total bilirubin, direct bilirubin, and indirect bilirubin as compared with the control group (group I).

Overdose of paracetamol or longer than required period of use metabolized in liver microsomal through cytochrome P450 CYP2E1 pathway into a reactive toxic metabolite, N-acetyl-para-benzoquinone imine (NAPQI), caused cellular necrosis, and elevated the permeability of hepatocytes membrane. Thus, there was a leakage of liver enzymes like alanine aminotransferase enzymes (ALT & AST) and a raising of ALP activity and bilirubin levels which could be taken as an index of liver failure (Cover et al., 2006).

Administration of hydrolyzed EtOAC fraction at pH 4 of brown pods extract at 100 and 200 mg/kg showed the capability to reduce liver enzyme levels such as alanine aminotransferase enzymes, ALP and bilirubin to its basal levels. Such effect might be attributed to the antioxidant capacity of these extracts, which would be related to having phenolic and flavonoid compounds (Afsar, Razak, & Almajwal, 2019; Taha, Kamal, & Ibrahim, 2019).

3.4 | Renal marker and protein profile

Table 4 shows that rats treated with paracetamol (750 mg/kg bw) for one month, that is, the paracetamol group (group II) showed higher levels in creatinine and urea than those of the control group (group I). According to (Ogutcu, Suludere, & Kalender, 2008) paracetamol overdose causes renal toxicity revealed by increasing kidney function such as rising serum creatinine and urea due to increased formation of reactive oxygen species (ROS) and decreased urea synthesis by inhibiting urea recycle enzymes.

On the contrary, treatment with hydrolyzed EtOAC (pH 4) using the brown pods extract at 100 and 200 mg/kg or the silymarin drug significantly ($p > .05$) decreased serum creatinine and urea more than paracetamol group (group II) which changed toward the normal values of these parameters. The protective action of hydrolyzed EtOAC using the brown pods extract may have been due to the phenolic and flavonoid compounds which have many biological activities, such as reducing formation of ROS and inhibiting lipid peroxidation (Ahmed and Ali, 2010).

Data in Table 4 show a significant ($p < .05$) decrease in total protein, albumin, and globulin in rats' serum treated with paracetamol (750 mg/kg bw) paracetamol group (group II) for one month compared with control group (group I). Paracetamol overdose metabolites (NAPQI) lead to disturbance in metabolism of proteins in hepatocytes (El-Hadary, 2016). On the contrary, treatment with hydrolyzed EtOAC fraction at pH 4 acacia brown pod extract at 100 and 200 mg/kg or standard drug silymarin enhanced protein profile levels nearly to the normal level. The ameliorative action resulting from the active phytochemical constituents, that is, phenolic and flavonoid compounds would restore the functionality of the hepatocytes (Afsar et al., 2019).

3.5 | Impact of Egyptian acacia pods extracts on oxidative stress and antioxidant marker

Data in Table 5 indicate that oxidative stress marker (nitric oxide) in liver tissue of rats which ingested paracetamol at above the therapeutic dose (group II) to normal rats increased by 198% of the control group while decreases in the antioxidant enzyme marker amounted to 11%, 35%, 6%, and 3% in the activities of glutathione peroxidase, reduced glutathione, glutathione S-transferase, and catalase, respectively.

For rats treated with Silymarin (50 mg/kg), the production of nitric oxide, showed a decrease of 41% relative to paracetamol group and increases of antioxidant enzyme marker of 34%, 128%, 4% and 15% in the activities of glutathione peroxidase, reduced glutathione, glutathione –S-transferase, and catalase, respectively when compared to paracetamol group. However, rats treated with hydrolyzed EtOAC fraction at pH 4 with acacia brown pods extract at 100 and 200 mg/kg ameliorated the activity of antioxidant enzymes by 44–49, 131–146, 7–8, and 28%–33% side by side with 84%–91% reduction in nitric oxide compared to paracetamol group.

Therapeutic with paracetamol over dose metabolized to NAPQI through cytochrome P450 CYP2E1 pathway as well as NAPQI conjugated by hepatic glutathione, through glucuronidation and sulfonation reactions resulted in glutathione depletion as a main factor that allows lipid peroxidation (Durairaj, Vaiyapuri, Kanti, & Malaya, 2008).

The present work revealed that applying hydrolyzed EtOAC fraction at pH 4 of acacia brown pods extract exhibited antioxidant capacity manifested by a decrease in nitric oxide and increase in glutathione and catalase enzyme activities in liver. This may be attributed to presence active phytochemical constituents, that is, phenolic and flavonoid compounds that scavenging free radicals (Singh et al., 2009).

TABLE 4 Effect of Egyptian acacia pods extracts on renal marker and protein profile in the serum of rats as affected by hepatic injury induced by paracetamol

G	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)
I	Control group	6.12 ± 0.04^{a}	3.16 ± 0.01^{a}	2.96 ± 0.04^{a}	0.54 ± 0.01^{b}	26.04 ± 0.68^{g}
II	Paracetamol group (paracetamol 750 mg/kg)	5.10 ± 0.04^{c}	2.75 ± 0.05^{b}	2.35 ± 0.08^{d}	0.96 ± 0.07^{a}	43.83 ± 1.05^{a}
III	Silymarin50 mg/kg + paracetamol 750 mg/kg	5.89 ± 0.04^{b}	3.19 ± 0.04^{a}	2.73 ± 0.08^{c}	0.54 ± 0.02^{b}	26.73 ± 0.75^{f}
IV	Egyptian acacia100 mg/kg + paracetamol 750 mg/kg	5.91 ± 0.04^{b}	3.15 ± 0.03^{a}	2.82 ± 0.04^{b}	0.53 ± 0.01^{b}	28.31 ± 1.26^{b}
V	Egyptian acacia200 mg/kg + paracetamol 750 mg/kg	6.02 ± 0.04^{a}	3.21 ± 0.01^{a}	2.81 ± 0.04^{b}	0.52 ± 0.1^{b}	27.76 ± 0.66^{d}

TABLE 5 Effect of Egyptian acacia pod extracts on oxidative stress and antioxidant marker in liver tissue of rats treated with overdoses of paracetamol

G	Treatment	Nitric oxide (μmol/ L)	Glutathioneperoxidase (U/g tissue)	GlutathioneReduced (μmol/ g tissue)	Glutathione-S-transferase (U/ g tissue)	Catalase (μmH_2O_2/ Sec/g tissue)
I	Control group	16.40 ± 0.06^{e}	193.60 ± 0.06^{d}	83.60 ± 0.06^{f}	4.26 ± 0.00^{a}	582.80 ± 0.06^{d}
II	Paracetamol group (paracetamol 750 mg/kg)	48.90 ± 0.06^{a}	171.40 ± 0.06^{e}	54.10 ± 0.06^{g}	4.0 ± 0.06^{c}	566.03 ± 0.03^{e}
III	Silymarin50 mg/kg + paracetamol 750 mg/kg	28.40 ± 0.06^{b}	230.20 ± 0.06^{c}	123.40 ± 0.06^{e}	4.16 ± 0.00^{b}	651.00 ± 0.58^{c}
IV	Egyptian acacia100 mg/kg + paracetamol 750 mg/kg	25.600 ± 0.06^{d}	246.70 ± 0.06^{b}	125.20 ± 0.06^{d}	4.27 ± 0.00^{a}	723.30 ± 0.00^{b}
V	Egyptian acacia200 mg/kg + paracetamol 750 mg/kg	27.90 ± 0.06^{c}	256.10 ± 0.06^{a}	133.40 ± 0.06^{b}	4.32 ± 0.00^{a}	752.80 ± 0.06^{a}

4 | CONCLUSION

This study demonstrated using natural extracts of Egyptian acacia brown pods played a significant positive effect as an antioxidant agent against DPPH radical scavenger. This investigation indicates a protective effect of Egyptian acacia brown pods extract against paracetamol-induced liver toxicity in experimental albino rats. The results demonstrate that the subjection of hydrolyzed EtOAC fraction at pH 4 Egyptian acacia brown pods extract at 100 and 200 mg/kg can ameliorate liver and kidney function as well as heal oxidative stress and antioxidant enzymes marker in comparison with the standard drug of silymarin. It could be concluded that both HEF and silymarin are good candidates for hepatoprotector against paracetamol-induced hepatotoxicity and probably other toxicity models due to their strong antioxidant activity. Thus, more scientific studies should be conducted to investigate Egyptian acacia pods extract may be useful against other models toxicity.

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CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest.

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