تقييم فعالية مستخلص الجنكو بيلوبا في تحسين الإجهاد التأكسدي والسمية القلبية الدموية الناتجة عن الفينيل هيدرازين لدى ذكور الفئران

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المستخلص

نظراً لما تمتلكه الجنكو بيلوبا من أنشطة كيميائية نباتية وبيولوجية واسعة النطاق؛ تهدف الدراسة الحالية إلى تقييم فعالية مستخلص الجنكو بيلوبا في تحسين السمية القلبية الدموية الناجمة عن فينيل هيدرازين. تم تقسيم ذكور فئران ويستار البيضاء البالغة (١٦٠-١٨٠ جم) إلى أربعة مجموعات عشوائيًا (٨حيوانات في كل مجموعة)، على النحو التالي: المجموعة (١) مجموعة ضابطة سالبة، المجموعة (٢) تتغذى على الوجبة الغذائية الأساسية +١٠٠مجم/كجم وزن الجسم/مذاب في الماء) من الجينكو بيلوبا لمدة أربعة أسابيع لدراسة تأثير الجينكو بيلوبا على الحالة الصحية للفئران السليمة، المجموعة(٣) الفئران المصابه بسمية القلب بفينيل هيدرازين بجرعة٤٠ مجم/كجم يومًا بعد يوم لمدة يومين وتتغذى على الوجبة الغذائية الأساسية كمجموعة ضابطة موجبة, والمجموعة (٤) الفئران المصابه بسمية القلب و تتغذى على الوجبة الغذائية الأساسية +١٠٠مجم/كجم وزن الجسم/مذاب في الماء) من الجينكو بيلوبا لمدة ٤ أسابيع. بعد أربعة أسابيع،أثبتت النتائج التي تم الحصول عليها أن GAE عزز بشكل كبير التدهورات القلبية الوعائية والدموية والمناعية الناجمة عنPHZ؛ وقد تجلى ذلك من خلال الانخفاض الكبير في مصلALT وAST وLDH وCK-MB والكوليسترول الكلي والدهون الثلاثية وTNF-α و IL 1β، بالإضافة إلىMDA إلى جانب تحسن ملحوظ فيGSH وCAT. وأظهرت المؤشرات الدموية ومشتقات الهيموجلوبين تحسنات كبيرة. علاوة على ذلك، خضعت عضلة القلب لتجديد كبير بسببGAE. خلصت الدراسة إلى أنGAE أظهر خصائص مضادة للتسمم القلبي والدموي، والتي قد تُعزى إلى خصائص مضادات الأكسدة وإزالة الجذور الحرة لمكونه النشط عالي الفينول. لذا خلصت النتائج إلى أن GAE لديه إمكانات كمكمل غذائي وقائي للقلب والدم.

الكلمات المفتاحية: سمية القلب، مكونات الدم، فينيل هيدرازين، الجنكو بيلوبا.

Evaluation of *Ginkgo biloba* Extract Efficacy in Ameliorating Oxidative Stress and Cardio-hepatotoxicity Induced by Phenylhydrazine in Male Rats

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Abstract

As *Ginkgo biloba* possesses wide phytochemical and biological activities; therefore, the current study aimed to evaluate the ameliorating effectiveness of *Ginkgo biloba* aqueous extract (GAE) in phenylhydrazine-induced cardio-hematotoxicity. Four groups of adult male Wistar albino rats (160–180 g), 8 animals each, were randomly divided as follows: group (I) negative control group, group (II) fed on basal diet + GAE (100 mg/kg, dissolved in water) four 4 weeks to study the effect of *Ginkgo biloba* on healthy status of rats, group (III) rats cardio-intoxicated with phenylhydrazine (PHZ) at a dose 40 mg/kg on alternate days for 2 days and fed on basal diet as a positive control group, group (IV) cardio-intoxicated rats treated orally fed on basal diet + with GAE (100 mg/kg, dissolved in water) for 4 weeks. After four weeks of treatment, the results acquired proved that GAE substantially enhanced the cardiovascular, hematological, and immunological deteriorations induced by PHZ; this was evidenced by the significant reduction of serum ALT , AST, LDH, CK-MB, total cholesterol, triglycerides, TNF-α, IL 1β, as well as cardiac MDA coupled with marked improvement in cardiac GSH and CAT. Hematological indicators and hemoglobin derivatives were substantial improvements. Furthermore, the cardiac muscle underwent significant regeneration due to the GAE. The study concluded that GAE demonstrated anti-cardio-hematotoxic properties, which might potentially be attributed to the antioxidant and radical scavenging properties of its high phenolic content active ingredient. Thus, the results concluded that GAE has potential as a cardio-hematoprotective supplement.

Keywords: Cardiotoxicity, Hematopoietic, Phenylhydrazine, *Ginkgo biloba*.

Introduction

A common cardiovascular event that puts patients' lives at jeopardy is myocardial infarction (MI). Age has been shown to be a significant risk factor for myocardial infarction, according to the Framingham Heart Study **(Ngwa et al., 2021).** Age is one of the factors that determine the

incidence of myocardial infarction. Elderly males (aged 85–94) had a MI that was more than twice as high, while women (aged 55–64) had a MI that was more than five times higher **(Qipshidze Kelm et al., 2018).** Following MI, the death rate is likewise significantly higher. With a 6% increase in mortality for every year of age above sixty, the death rates of both hospitalized and post-discharge patients rise with decreasing age **(Maggioni et al., 1993; Metra et al., 2023).** Heart arrest following MI is more common in elderly people. It can result in problems such as rupture of the papillary muscle, rupture of the left ventricle, and acquired ventricular septal defect **(Ornato et al., 2001).** From 10.0% in 2000 to 21.8% in 2050 and 32.2% in 2,100, the percentage of the world's population over 60 will rise **(Lutz et al., 2008).** These forecasts have motivated scientists to look for more potent MI therapies.

Phenylhydrazine (PHZ), a potent oxidizing agent, is widely utilized in various industrial applications **(Nicolas et al., 2002; Sheikhsamany & Faghihian 2020).** PHZ is a powerful chemical that induces toxicity in various tissues. Administration of phenylhydrazine mainly causes haematotoxicity **(Pandey et al., 2018).** PHZ induced haemolytic anaemia, exerted potent genotoxic effects and significantly increases iron absorption in the spleen, liver and duodenum and disrupted iron metabolism **(Shwetha et al., 2019),** which subsequently free radical generation that PHZ induces oxidative damage to hemoglobin **(Qin et al., 2022)** and it generates free radicals and reactive oxygen species **(Berger, 2007).** The imbalance between the cellular production of reactive oxygen species and the counteracting antioxidant mechanism leads to Oxidative stress **(Ramani et al., 2021).** Oxidative stress is considered a main factor contributing to numerous health conditions, including cardiovascular diseases **(Castro and Freeman, 2001).** Hematotoxicity is generally encountered in various therapeutic regimens as Adverse Drug Reactions (ADRs) **(Shukla and Singh, 2015).** Studies demonstrated that natural medicinal plants with potent antioxidant activity and potential protective effects can mitigate oxidative stress-related diseases by inhibiting reactive oxygen species (ROS) generation and enhancing antioxidant defense mechanisms **(Forni et al., 2019).**

Ginkgo biloba is among the most widely utilized herbal medicines globally, with a history of use spanning several centuries **(Ude et al., 2013).** *Ginkgo biloba* is rich in bioactive compounds, contributing to make it chemical diversity **(Tabassum et al., 2022).** Sugars, amino acids, organic acids, polysaccharides, sterols, and inositols are among the many constituents in GB extract. Traces of organic acids, terpenoids, and flavonoids are among the active ingredients with a variety of chemical compositions **(Maclennan et al., 2002; Serrano-García et al., 2013).** It

is the most widely used herbal treatment, aiding in the scavenging of free radicals, reducing oxidative stress, and helping in the treatment of several cardiovascular diseases (CVD), and cancer, Additionally, it inhibits the apoptosis of myocardial cells, protects the myocardium, decreases oxidative stress, and suppresses inflammatory responses **(Kuller et al., 2010; Chen et al., 2019; Barbalho et al., 2022).** Most studies have attributed *Ginkgo biloba's* cardioprotective effects to its enhanced antioxidant activity **(Wang et al., 2016).**

Therefore, the current study was carried out to investigate the protective potential of *Ginkgo biloba* aqueous extract (GAE) to ameliorate the pathophysiological induced cardio-hematotoxicity and to assess anti-oxidative damage in rats intoxicated with Phenylhydrazine.

MATERIALS AND METHODS

MATERIALS

Source of *Ginkgo biloba*

The source of *Ginkgo biloba* was from a local supplier, Abd El-Rahman Harraz, located in the Bab El-Khalk area, Cairo, Egypt, in 2024**.**

Chemicals and kits

- The supplier of phenylhydrazine was Sigma in St. Louis, USA.
- Biochemical assays using reagent kits acquired from Human Gesell Schaft für Biochemical und Diagnostica mbH, Germany.
- The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), the lipid profile and iron profile using kits that bought from DiaSys Diagnostic Systems GmbH in Germany. The levels of creatinine and serum urea were measured with kits purchased from Biodiagnostic, Dokki, Giza, Egypt. LDH and CK-MB activity reagent kits were obtained from BioVision, located in South Milpitas, California US.
- Kits from Biodiagnostic, Dokki, Giza, Egypt were used to assess the levels of cardiac malondialdehyde (MDA), catalase (CAT), and glutathione (GSH).
- Tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) measured utilizing reagent kits from SinoGeneClon Biotech Co., Ltd., located at No. 9 BoYuan Road, YuHang District, Hang Zhou, China.

Experimental rats

Thirty-two adult male albino rats, weighing (160-180g), were obtained from the Animal Colony, National Research Centre, Egypt.

Ethical approval

The researchers got the approval of the Ethical Committee, (No.459181224) Faculty of Specific Education, South Valley University, Qena, Egypt, and it complies with the International Guidelines for Research Ethics.

METHODS

Chemical composition of *Ginkgo biloba*

Moisture, protein, ash, fat, crud fiber and minerals were determined according to the methods outlined in the **AOAC (2016)** official methods. Total carbohydrates were calculated by difference as mentioned by **Abd El-Latif, (1990)** according to the following equation: Total carbohydrates =100 – [moisture (%) + crude protein (%) + crude fat (%) + ash (%)]. While total energy was calculated as mentioned by **Merrill & Watt, (1955)** according to the following equation: Energy (kcal) = [4 × (g protein + g carbohydrates)+ 9 × g fat].

Extraction of *Ginkgo biloba*

The following procedure was used to create the aqueous *Ginkgo biloba* extract: For two days, a mixture of 0.5 g bee glue and 10 mL deionized water was stored in a refrigerator at 4 ± 1 °C. After that, the sample was centrifuged for 20 minutes at 10,000 rpm. After filtering the supernatant using a Whatman paper filter No. 41, the water extract was placed in an oven drier set at 50 °C for 24 hours, resulting in a semisolid extract. The water extract was preserved for additional examination in a freezer **(Paviani et al., 2013).**

Determination of total yield

After the mixed extracts were moved to a quick-fit round-bottom flask with a known weight (W1), they were freeze-dried and weighed once again (W2), and the yield was eventually computed using the formula below:

$$\text{Extract yield (g/ g crude herb)} = (W2 - W1)/W3$$

Where, W1 is the weight of a clear and dry quick-fit flask in grams, W2 is the weight of the flask after lyophilization in grams; W3 is the weight of the crude powdered herb in grams **(Muhamman et al., 2013).**

Determination of total phenolic content

Estimation of phenolic compounds was ascertained using the methodology outlined by **Jayaprakasha and Jaganmohan (2000).**

Determination of radical scavenging (RSA) activity by DPPH assay

The tests were made in triplicate and the amount of DPPH that was scavenged was used to calculate the radical scavenging activity **(Nogala-Kalucka et al., 2005)**.

Estimation of reducing power

The extract's reducing power was ascertained using the methodology outlined by **Sethiya et al., (2014).**

Animals and Experimental Design

The animals were kept in suitable plastic cages and given unlimited access to food and water one week before the experiment. They received human care in compliance with the normative guidelines established by the organization for the handling and application of experimental animals. Following the animals' acclimation to the circumstances of the experimental room, they were split into four groups at random, each with eight animals. As a normal control, the first group of healthy animals received conventional food and an intraperitoneal injection of 1 ml of isotonic saline without any therapies, the second group consisted of healthy animals that received four weeks of consecutive oral administration of GAE at a dose of 100 mg/kg/day to study the effect of *Ginkgo biloba* on the healthy rats, The third group of animals consisted of positive control animals that received intraperitoneal injections of phenylhydrazine (PHZ) at a level of 40 mg/kg for four weeks, and the fourth group consists of rats that were given GAE extract orally every day for four weeks after becoming inebriated with phenylhydrazine.

Measurement of body weight

The initial and final body weights of the rats were measured and recorded.

Blood and tissue sampling

Following an overnight fast and weight measurement after the treatment period, blood samples were taken from the retro-orbital plexus using sterile, heparinized glass capillaries. Each blood specimen was split into two parts: the first was collected for hematological measurements in a heparinized tube, and the second part underwent a 10-minute cool centrifugation at 3000 rpm to separate and divide the sera into aliquots, which were then stored at -80°C until biochemical measurements could be performed right away. Following blood collection, the animals were quickly sacrificed by abrupt decapitation, and all of the rats' hearts were removed. The hearts from each group were then dried, rolled in aluminum foil, and stored at -80°C to evaluate oxidative stress markers. The remaining portions of each group's hearts were then submerged in a 10% v/v formaldehyde-saline buffer for histopathological analysis.

Assessment of complete blood count

A full automatic cell blood counter (Model PCE-210 N, Japan) was used to measure the following parameters: red blood corpuscle count (RBC), hemoglobin content, hematocrit (Hct) percentage, mean corpuscular volume (MCV) , mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT)

count , and total leucocytes count (TLC) according to **(Lee and Kang, 2016).**

Biochemical determinations

The Shimadzu spectrophotometer (UV–vis 1201, Japan) was used for all biochemical assays. The activities serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of **Reitman and Frankel, (1957).** Alkaline phosphate (ALP), GGT and lactate dehydrogenase (LDH) were determined according to **Dumas et al., (1971), William (1980) and Szasz et al., (1974).** Total proteins, albumin and bilirubin level were estimated according to **Weichselbaum (1946), Eastham (1976) and Walter and Gerade, (1970),** respectively. Total cholesterol, triglycerides, LDL, HDL and VLDL (very low density lipoprotein) concentrations were measured according to **Richmond (1973), Lopes-Virella et al. (1977), Fossati & Prencipe (1982), Assmann (1979), and Warnick & Albers (1978).** respectively. Hepatic MDA as well as CAT activities were estimated according to **Ohkawa et al. (1979).** Colorimetric assays were used to measure LDH and CK-MB activity.

Oxidative stress markers of heart tissue

The levels of cardiac malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) were measured by the method **Buege and Aust, (1978); Beuter (1982) and Beutler, & Gelbart, (1969).**

Determination of TNF-α and IL 1β

Tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) were measured in serum using ELISA according to **(Ismail et al., 2024).**

Histopathological examination

Every animal heart sample was stored in 10% neutral formalin, and then it was cleaned with xylene, dried with ethanol, and embedded in paraffin. Sections (5 µm thick) were then stained for the histological investigation using hematoxylin and eosin (H&E) **(Ashry et al., 2022).**

Statistical analysis

Using the statistical analysis system's general linear model approach, all data were subjected to a one-way analysis of variance (one way ANOVA) (SAS, 1982). The Waller-Duncan k-ratio was used to assess the importance of the variations between the various treatment groups **(Ashry et al., 2022).** Every significance claim was predicated on the likelihood that $p \leq 0.05$.

Results

1- Chemical composition of *Ginkgo biloba* leaves

Table (1) demonstrates the contents of *Ginkgo biloba* leaves in terms of moisture, ash, crude fat, protein, crude fiber, and total carbohydrates. All results were calculated as (g/100g on dry weight). Carbohydrates, (calculated by difference), were the most abundant macronutrients (58.38 g/100 g dw). Otherwise, protein and fat were the macronutrients present in a smaller quantity, (12 and 15.94g/100g dw) respectively. The levels of ash, crude fiber, and energy were (5.68, 8, 12.15 %, and 424.98 kcal), respectively.

Table (1): Chemical composition of *Ginkgo biloba* leaves on a dry weight basis (mean ± SD) (g/100 g)

Sample	Moisture %	Ash %	Crude fat %	Protein %	Crude fiber %	Carbohydrate %	Energy kcal
Ginko biloba	5.68±0.14	8±0.25	15.94±0.05	12±0.33	12.15±0.23	58.38±0.10	424.98±0.15

- Mean three replicates
- dw: on dry weight
- % Protein = % Nitrogen × 6.25
- Total carbohydrate = 100 – (Moisture +Ash+ Crude fat + Protein)
- Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

2- Yield, TPC, RSA, and reducing power of the GAE

The *in vitro* results showed that GAE has a considerable amount of phenolic compounds, and exhibited higher radical scavenging activity and reducing power **(Figure 1).**

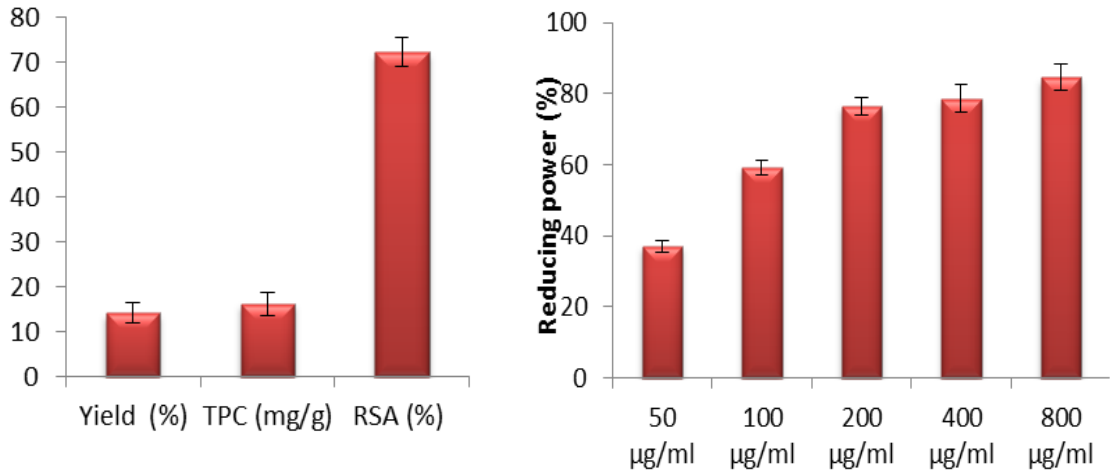

Figure (1): *In vitro* results (Yield, TPC, RSA, and Reducing power) of the GAE

3- Body weight gain of experimental rats

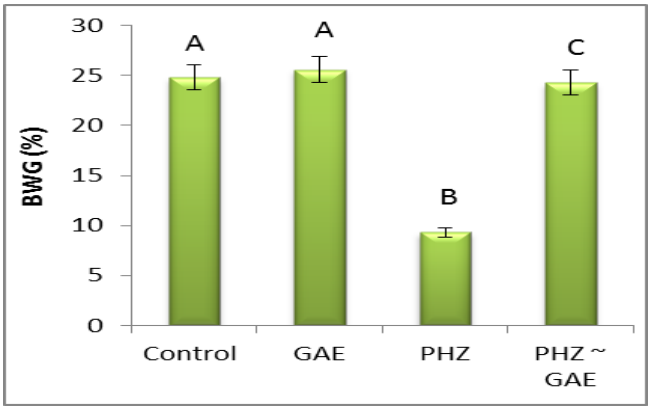

Figure (2): The body weight gain of experimental rats

Figure (2) Displayed the proportion of body weight gain (BWG) of control, GAE, PHZ, and PHZ~GAE treated rats. Means with superscript different symbols were differ significantly at $p \leq 0.05$ by one-way ANOVA followed by Duncan's post hoc test.

4- Hematological parameters of experimental rats

Intoxication of animals with PHZ resulted in detectable hematological disorders which were monitored from the marked drop in RBCs, PCV, and Hb concentration. TLC, lymphocytes, and PLT were significantly elevated. Favorably, treatment of PHZ-intoxicated rats with GAE markedly improved these hematological measurements **(Table 2).**

Table (2): Hematological parameters of experimental rats

Groups / Parameters	Control	GAE	PHZ	PZH~GAE
RBCs (10^6/ccm)	6.07 ± 0.35^{a}	6.0 ± 0.17^{a}	2.0 ± 0.17^{c}	5.33 ± 0.10^{b}
PCV (%)	45.2 ± 0.80^{a}	44.9 ± 0.17^{a}	16.8 ± 1.11^{b}	46.2 ± 2.3^{a}
MCV (f.l)	75 ± 3.0^{c}	75.2 ± 0.8^{c}	84.8 ± 1.75^{a}	86.5 ± 2.6^{b}
MCH (pg)	23.5 ± 1.2^{b}	24.5 ± 0.34^{b}	41.9 ± 1.0^{a}	24.6 ± 0.17^{b}
MCHC (g/dl)	31.3 ± 0.23^{b}	33.4 ± 0.43^{b}	49.5 ± 0.27^{a}	28.4 ± 0.69^{b}
HB (g/dl)	14.1 ± 0.14^{a}	$14.2\pm.23^{a}$	8.3 ± 0.53^{b}	13.1 ± 0.34^{a}
TLC (10^3/ccm)	14.5 ± 0.19^{c}	13.5 ± 0.55^{c}	235.4 ± 4.6^{a}	25.16 ± 0.12^{b}
LYM (%)	60.4 ± 2.9^{b}	58.9 ± 4.1^{b}	80.0 ± 1.67^{a}	54.8 ± 0.43^{b}
MO (%)	8.0 ± 0.17^{a}	7.5 ± 0.66^{a}	7.95 ± 0.31^{a}	7.35 ± 1.5^{a}
GR (%)	32.0 ± 3.14^{b}	33.4 ± 3.4^{b}	12.05 ± 1.3^{c}	40.9 ± 0.23^{a}
PLT (10^3/ccm)	1123 ± 90.9^{bc}	1102 ± 69.5^{c}	1663 ± 136^{a}	1144 ± 81.6^{b}

- Data are displayed as mean ± standard error
- Means with superscript different symbols differed significantly at $p\leq0.05$ by one-way ANOVA followed by Duncan's post hoc test.
- PHZ (Phenylhydrazine), GAE (*Ginkgo biloba* aqueous extract).

5- Serum biochemical markers of experimental rats

The results of the current investigation showed that PHZ administration resulted in a significant increase in the levels of serum total cholesterol, LDL-cholesterol and triglyceride, AST, ALT , urea, creatinine, glucose, LDH, CK-Total, CK-MB, iron and ferritin matched with a marked drop in LDL-cholesterol level and TIBC compared with those of the control values. Meanwhile, these highly disturbed levels of biochemical markers were improved significantly post-treatment with GAE; this improvement was noticed to be the highest regarding the GAE-treated rats **(Table 3).**

Table (3): Serum biochemical markers of experimental rats

Parameters \ Groups	Control	GAE	PHZ	PHZ~GAE
ALT (U/L)	37.2 ± 3.4^{b}	35.1 ± 1.7^{b}	123.9 ± 19.1^{a}	34.4 ± 0.433^{b}
AST (U/L)	81.1 ± 10.4^{b}	75.7 ± 095^{b}	281 ± 21.6^{a}	86.6 ± 3.2^{b}
Urea (mg/dl)	35.4 ± 1.7^{bc}	32.7 ± 0.14^{c}	99 ± 1.2^{a}	47.8 ± 1.5^{b}
Creatinine (mg/dl)	0.77 ± 0.04^{b}	0.67 ± 0.02^{b}	1.5 ± 0.02^{a}	0.64 ± 0.045^{b}
Glucose (mg/dl)	103.7 ± 0.23^{c}	103 ± 1.5^{c}	128.7 ± 4.1^{a}	109 ± 2.8^{b}
Cholesterol (mg/dl)	153.5 ± 2.5^{b}	149 ± 4.6^{b}	323 ± 10.3^{a}	142 ± 16.4^{bc}
Triglycerides (mg/dl)	127 ± 2.8^{c}	121 ± 0.57^{c}	350 ± 59^{a}	149 ± 8.08^{b}
HDL- Cholesterol (mg/dl)	43 ± 0.57^{a}	43.5 ± 0.28^{a}	31.5 ± 0.28^{b}	42.0 ± 0.57^{a}
LDL- Cholesterol (mg/dl)	85.1 ± 3.7^{b}	82.3 ± 4.4^{b}	221.5 ± 1.2^{a}	76.05 ± 16.1^{b}
VLDL- Cholesterol (mg/dl)	25.4 ± 0.57^{b}	23.2 ± 0.11^{b}	70.0 ± 11.8^{a}	29.8 ± 1.6^{b}
CK-MB (U/L)	1404 ± 48^{b}	1193 ± 27.1^{b}	2590 ± 247^{a}	1195 ± 115^{b}
CK-Total (U/L)	1050 ± 54.2^{b}	938 ± 53.9^{b}	1940 ± 219^{a}	948 ± 77.3^{b}
LDH (U/L)	575.3 ± 46.7^{c}	575.6 ± 11.8^{c}	7908 ± 155^{a}	2288 ± 380^{b}
Iron (µg/dl)	211 ± 7.7^{b}	202.6 ± 2.3^{b}	478.3 ± 4.9^{a}	220.3 ± 11.5^{b}
TIBC (mg/dl)	478.3 ± 10.3^{a}	499.6 ± 3.4^{a}	466.3 ± 6.6^{b}	274 ± 22.7^{a}
Ferritin (ng/ml)	209 ± 7.5^{b}	199 ± 11.8^{b}	377.6 ± 4.3^{a}	196 ± 3.1^{b}

- Data are displayed as mean ± standard error
- Means with superscript different symbols differed significantly at $p \leq 0.05$ by one-way ANOVA followed by Duncan's post hoc test.
- PHZ (Phenylhydrazine).
- GAE (*Ginkgo biloba* aqueous extract*)*.

6- Cardiac oxidative stress markers of experimental rats

Data in Table (4) show noticeable alterations in values of the oxidative (MDA) and antioxidant (GSH and CAT) markers in the PHZ group as compared with the control group as the cardiac MDA levels elevated significantly, while the cardiac GSH level and CAT activity

showed a significant decrease. Favorably, post-treatment of PHZ intoxicated animals GAE resulted in substantial improvements in the antioxidant indicators (GSH and CAT) coupled with a significant decrease in the cardiac oxidative ones (MDA); this amelioration was very clear and promising post-treatment with GAE.

Table (4): Cardiac oxidative stress markers of experimental rats

Parameters \ Groups	Control	GAE	PHZ	PHZ~GAE
MDA (nmol/g tissue)	77 ± 1.8^{c}	73.9 ± 0.55^{c}	144.7 ± 4.3^{a}	100 ± 1.9^{b}
GSH (mg/g tissue)	317 ± 8.6^{a}	320 ± 10.1^{a}	139.8 ± 4.6^{c}	266.2 ± 28.3^{b}
CAT (U/g tissue)	39.0 ± 4.7^{a}	42.8 ± 1.05^{a}	15.5 ± 1.7^{c}	33.0 ± 2.02^{b}

- Data are displayed as mean ± standard error
- Means with superscript different symbols differed significantly at $p \leq 0.05$ by one-way ANOVA followed by Duncan's post hoc test.
- PHZ (Phenylhydrazine).
- GAE (*Ginkgo biloba* aqueous extract*)*.

7- Serum pro-inflammatory markers of experimental rats

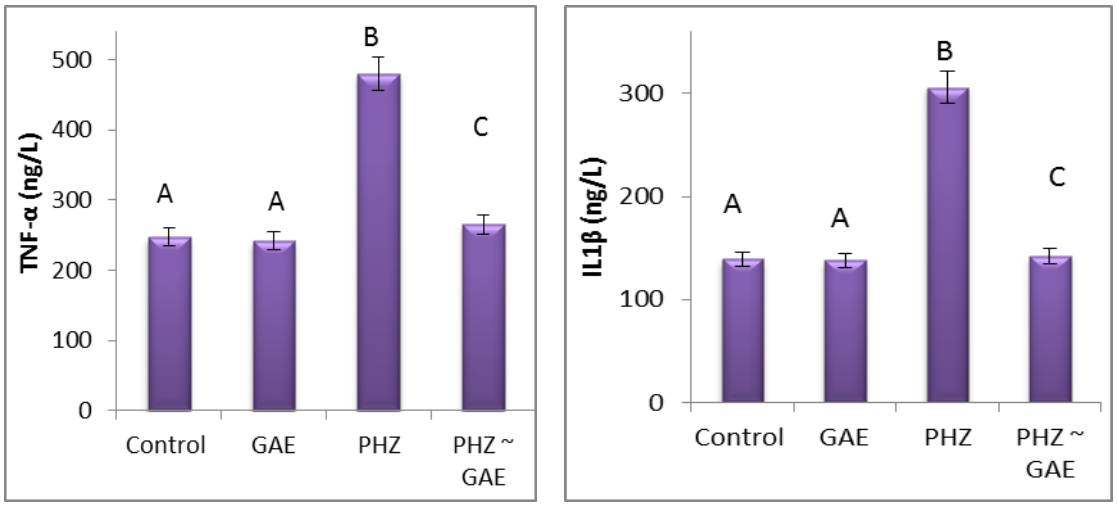

Figure (3): Serum pro-inflammatory markers of experimental rats

Data are displayed as mean ± standard error. Means with superscript different symbols differed significantly at $p \leq 0.05$ by one-way ANOVA followed by Duncan's post hoc test. PHZ (Phenylhydrazine). GAE (*Ginkgo biloba* aqueous extract*)*.

8- Hepatic histopathology investigation

The morphological assessment of hearts across various experimental groups revealed a spectrum of alterations, ranging from absence of injury (Control and GAE groups) to mild lesions (PHZ+GAE group*)* to severe damage (PHZ). The control group showed normal histological architecture characterized by branched striated cardiac

myocytes with acidophilic cytoplasm and centrally-located, vesicular, and oval nuclei; similarly, the striated cardiac fibers in the GAE group were intact and densely packed, indicating preservation of normal myocardial architecture. In contrast, the PHZ-intoxicate group displayed diverse myocardial alterations, including cytoplasmic degeneration, distorted striations of cardiac muscle, irregular spacing within interstitial regions, focal necrosis in small clusters of myocardial fibers, focal cellular infiltration, noticeable expansion in interstitial spaces, and extravasted RBCs, and suggesting cardiotoxicity. Favorably, post-treatment of PHZ intoxicated rats with GAE predominantly displayed nearly normal myocardial architecture with mild degenerative alterations, including intracellular edema and mild cytoplasmic vacuolization, without any inflammatory response compared to control groups, implying a discernible ameliorative effect of the toxin (Figure 4 A-F).

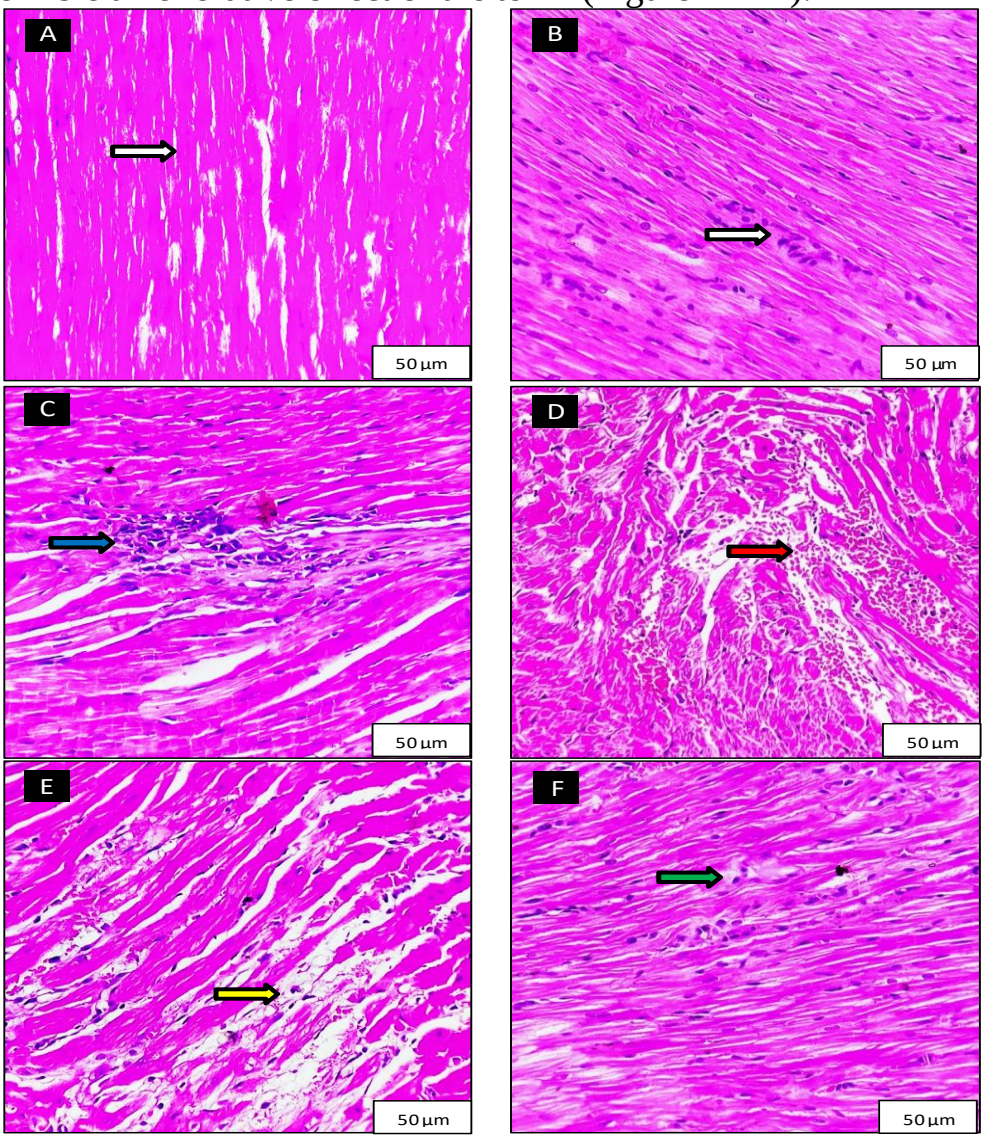

Figure (4) illustrates histopathological findings of the heart of the study animals' groups. Control group (A) shows normal cardiac histological architecture with branched striated cardiac myocytes (white arrows); GAE group (B) displays an intact myocardial structure with densely packed fibers; PHZ-intoxicated group (C-E) reveals severe myocardial damage characterized by cytoplasmic degeneration (blue arrow), distorted striations, and focal necrosis (yellow arrows), cell infiltration (blue arrows), and red extra-vASTed RBCs evident (red arrows); PHZ+GAE group (F) shows only mild degenerative changes (green arrow) (H&E x200).

Discussion

Data in Table (1) showed that moisture, ash, crude fat, protein, crude fiber, and total carbohydrate contents were identified in *Ginkgo biloba* leaves. All results were calculated as (g/100g on dry weight). Carbohydrates, calculated by difference and caloric value were calculated using Atwater factor (4 x protein, 9 x fat and 4 x carbohydrates) in grams. The most abundant macronutrients (58.38 g/100 g dw). Otherwise, protein and fat were the macronutrients present in lower amounts, (12 and 15.94g/100g dw) respectively. The levels of ash, crude fiber, and energy were (5.68, 8, 12.15 %, and 424.98 kcal), respectively. Our data were in agreement with **El-Khateeb, (2020)** who showed that *Ginkgo biloba* contained 9.15% moisture, 7.38% ash, 14.71% protein, and 22.54 % crude fiber. Our data also were in agreement with **Pereira et al. (2015)** who revealed the nutritional profile of G. biloba, the ash, crude fat, protein, crude fiber, and total carbohydrate recorded (12.91, 4.42, 15.32, 67.36, 370.44 g/100 g dw), respectively. However, our data were in disagreement with **Pereira et al. (2013)** who studied the chemical composition of *Ginkgo biloba* on a dried weight basis and they found carbohydrates, (72.98 g/100 g dw). Proteins and ash were 12.27 and 10.01 g/100 g dw, respectively. However, some differences in the composition may be due to environmental stress, climatic conditions, geographical, cultivation, and harvesting practices.

Using rat models, the current study investigated the protective impact of *Ginkgo biloba* against phenylhydrazine-induced oxidative stress, cardiac, and hematological damage. In animal experimental models, phenolhydrazine (PHZ) is frequently employed to cause anemia and oxidative stress **(Onyeabo et al., 2017; Lee et al., 2014).** It oxidizes hemoglobin, the oxygen-carrying protein in red blood cells, resulting in hemolysis, the death of red blood cells. Red blood cells' ability to carry oxygen is decreased when PHZ in the bloodstream combines with hemoglobin to create unstable intermediates like methemoglobin, a type

of hemoglobin that is unable to connect to oxygen **(Shukla et al., 2012; Berger, 2007).** Reactive oxygen species (ROS) are also produced during the breakdown of unstable intermediates, and these can oxidatively damage red blood cells and their membranes. In the end, this damage causes red blood cells to burst and releases hemoglobin into the blood, which causes hemolysis. These free radicals can start a redox cycle, wherein they react with oxygen to produce ROS such as hydrogen peroxide and superoxide anion **(Adwas et al., 2019).** These ROS can oxidatively damage lipids, proteins, and DNA, among other macromolecules found in cells, resulting in malfunction and damage **(Chinko et al., 2023; Wang et al., 2000).** Depletion of endogenous antioxidant enzymes involved in scavenging reactive oxygen species (ROS) is another consequence of PHZ-induced oxidative stress **(Banerjee et al., 2020; Paul et al., 2014).**

Serum levels of LDH, CK-MB, AST, and ALT activities were significantly elevated in rats treated with PHZ; these findings are in line with previous research indicating that PHZ-induced oxidative stress can lead to lipid peroxidation and the subsequent release of these enzymes into serum **(Chinko et al., 2023).** In spite of this, rats given GAE showed a significant recovery in LDH, CK-MB, AST, and ALT activities. This suggests that GAE suppresses lipid peroxidation and membrane disruption, stabilizing myocyte membranes and decreasing leakiness in cardiomyocytes. Additionally, histological examination of the cardiac tissue in normal control animals revealed an intact, united cell membrane free of edema, inflammation, and inflammatory cell infiltration; in contrast, coagulative myonecrosis, inflammation, and inflammatory cell infiltration were revealed in the rats' cardiotoxicity PHZ. Conversely, rats administered GAE exhibited edema, reduced permeability of inflammatory cells, and condensed myonecrosis. GAE appears non-toxic to cardiomyocytes when paired with hemodynamic and biochemical recovery as well as histological salvage, most likely because the endogenous antioxidant defense against PHZ has been restored.

The current study's findings demonstrated a large decrease in LDL cholesterol along with a significant increase in triglycerides, total cholesterol, and LDL cholesterol. This alteration in the lipid profile suggested that PHZ may raise the risk of atherosclerosis and lower the rate of cholesterol catabolism. Lipid profiles were effectively restored in PHZ-treated rats when GAE was administered, as was seen in the GAE group. These outcomes might indicate that the liver's fat metabolism has improved as a result of taking the tested supplements.

The injection of PHZ resulted in a significant decrease in cardiac GSH and CAT levels and an increase in cardiac MDA levels relative to

the control group. These findings are consistent with those of **Chinko et al. (2023) and Asogwa et al., (2024).** The equilibrium between oxidation and antioxidant defense is upset when PHZ causes an excessive amount of ROS to be produced. Lipid peroxidation (LPO), which is the end result of this imbalance, causes oxidative damage to proteins and DNA **(Banerjee et al., 2020; Paul et al., 2014).** The primary byproduct of polyunsaturated fatty acid peroxidation is MDA; whose existence denotes tissue or cell damage. Animals exposed to PHZ had higher levels of MDA, which suggests that PHZ caused cellular and tissue damage. Nonetheless, the antioxidant activity of GAE to lessen cellular and tissue damage is responsible for the much lower MDA after GAE intake **(Hai et al., 2024 and Essawy et al., 2024).** Similarly, the strong antioxidant activity of GAE is supported by the rise in the antioxidant enzymes glutathione and cALT ase. Free radicals and dangerous oxygen-derived species including hydrogen peroxide, hydroxyl radicals, and singlet oxygen can be scavenged by antioxidants; their capacity to prevent or mitigate cellular damage and the deleterious effects of oxidative stress-induced illnesses **(Hai et al., 2024).** According to the available data, GAE increased the activity of antioxidant enzymes, perhaps shielding the Wistar rats from oxidative damage brought on by PHZ.

Among other inflammatory pathways, PHZ can cause cardiotoxicity, cardiomyopathy, and congestive heart failure by upregulating TNF-α and IL-1β. Prior research has demonstrated that rats treated with PHZ have higher levels of TNF-α and IL-1β **(Aloke et al., 2021; Asogwa et al., 2024).** Among the processes by which phenylhydrazine causes anemia have been described as the induction of oxidative stress on erythrocytes and membrane lipid oxidation **(Banerjee et al., 2020).** Biological systems react physiologically to stressors to aid in defense and survival. Cytokines have different effects on biological processes and have been related to the pathophysiology of numerous diseases, including immunoinflammatory disorders **(Musaya et al., 2015)**. According to **Zangeneh et al. (2017),** our study also found that administering PHZ increases the amounts of proinflammatory cytokines and generates oxidative stress. Previously, **Ershler, (2003)** had documented elevated concentrations of proinflammatory cytokines such as TNF-alpha and IL-1β. Erythropoiesis regulation is associated with cytokines. Furthermore, it has been demonstrated that IL-1β and tumor necrosis factor (TNF) negatively impact erythropoiesis **(Ershler, 2003 2017).** Similar to the findings of **Zangeneh et al. (2017),** the concentrations of TNF and IL-1β were significantly higher in our study after PHZ injection when compared to the negative control. However, when compared to the negative control, which was similar to the findings of earlier authors, treatment with GAE

dramatically decreased their levels close to the normal control **(Hai et al., 2024).** The presence of various flavonoids and terpenoids in the extract may be the cause of this action.

Conclusion

Our findings show that *Ginkgo biloba* aqueous extract (GAE) can act as an antioxidant to assist the body in overcoming the symptoms of phenylhydrazine-induced hemato-cardiotoxicity; It was successful in reducing oxidative stress, improving, and returning the structure and function of the heart to a state that is nearly similar to health. Consequently, co-treating with *Ginkgo biloba* aqueous extract can prevent hemato-cardiotoxicity caused by phenylhydrazine.

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