10.21608/avmj.2025.369222.1636 Assiut University web-site: www.aun.edu.eg

A STUDY ON SAFETY MARGIN OF *BOUGAINVILLEA GLABRA* FLAVONOIDS RICH FRACTION IN RATS

MANAL M. MOHAMED [1*], WALAA M.S. AHMED [1], OLFAT SHEHATA [1], HAYAM S. AHMED [2], FATMA KHALIL [3], EMAD A. MAHDI [4] AND HAMDY H. KAMEL [1]

[1] Department of Clinical Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

[2] Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt

[3] Animal and Poultry Management and Wealth Development, Faculty of Veterinary Medicine, Beni-Suef 62511, Egypt

[4] Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

Received: 7 April 2025; **Accepted:** 28 May 2025

ABSTRACT

Bougainvillea glabra (BG) is an ornamental plant with valuable pharmacological activities. The current experiment aimed to evaluate the safety limit of *Bougainvillea glabra* leaves flavonoids-rich fraction in Wistar rats. Female rats were equally divided into 3 groups. One group served as a normal control, and the other groups were treated with 2 and 3 g/kg b.w of the fraction, respectively, as a single oral dose. Weight gain was measured weekly, and the rats were observed throughout the experimental period. After 14 days, behavioral activities were examined. Hematological and biochemical measurements, along with histopathological examination of the liver and kidney were performed. The results revealed that the BG fraction could be classed as category 5 following the OECD guidelines. The fraction at 2 g/kg b.w dose did not cause mortality or significant alteration in body weight gain, hematological, and biochemical parameters. Bougainvillea fraction at both doses improved working memory; however, the 2 g/kg b.w dose only had a significant anxiolytic effect. There was a significant decrease in Hb, PCV and absolute lymphopenia accompanied by neutrophilia in rats received a dose of 3 g/kg b.w, as well as a significant increase in ALT, AST, creatinine and urea. Histopathological examination of hepatic and renal tissues of this group also showed adverse effects. In conclusion, *Bougainvillea glabra* flavonoids rich fraction at 2 g/kg b.w dose was well tolerated by rats and caused no adverse effects, while the 3 g/kg b.w dose had a toxic effect on body weight, some hematological parameters, liver and kidney.

Key words: *Bougainvillea glabra*, flavonoids, toxicity, rats.

INTRODUCTION

Herbal medicines are widely used in management of many diseases. This increasing interest may be due to its minimal side effects, easy access, and lower cost compared to synthetic drugs. Although medicinal plants are often safe, their application has limitations, such as unknown chemical compounds and toxicity data. Therefore, a scientific assay is required to determine a safe dose for pharmaceutical use (Sarkar *et al.,* 2024).

Corresponding author: MANAL M. MOHAMED
E-mail address: Manal.Mustafa@vet.bsu.edu.eg
Present address: Department of Clinical Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

Flavonoids are an important category of phytochemicals found in abundance in vegetables, fruits, flowers and legumes. They are classified into variant groups according to the level of oxidation, conjugation and hydroxylation. Although they have a wide spectrum of pharmacological activities, higher doses were found to produce different adverse effects (Mehjabin *et al.,* 2024).

Bougainvillea, a member of the family *Nyctinaginacea*, is characterized by its vibrant bracts and adaptability in warm climate regions. *Bougainvillea glabra* (BG), paper flower, is the most prominent plant in this genus. This species is native to Brazil, Peru and Argentina but also cultivated in the Pacific islands, the Mediterranean, the Caribbean Islands, Southeast Asia and Australia as an ornamental plant (Ramesh *et al.,* 2024).

Phytochemical analysis of BG showed the presence of phenolics (quercetin, coumaric and ferulic acid), flavonoids, betacyanins, alkaloids and tannins (Giri *et al.,* 2023 and Elumalai *et al.,* 2012).

Bougainvillea glabra active components perform a variety of reported activities, such as antimicrobial, antioxidant, neuroprotective, cardioprotective, antidiabetic, anthelmintic, wound healing, anticancer and immunomodulatory activeities. Leaves of Bougainvillea glabra are traditionally used in India as a remedy against various gastrointestinal disorders (Saleem *et al.,* 2021; Tegeli *et al.,* 2019).

Various pharmacological uses are reported for BG; however, only a handful of studies have been conducted in vivo toxicological data of BG. These studies carried out on whole BG plant extract (Krishna and Sundararajan, 2018 and Rajyalakshmi *et al.,* 2024) or isolated essential oils (Ogunwande *et al.,* 2019), while experiments for safety and toxicity of different fractions are lacking (Saleem *et al.,* 2021).

Hence, the present experiment aimed to investigate the safety limit of *Bougainvillea glabra* leaves flavonoids rich fraction as described by the Organization for Economic Cooperation and Development guidelines (OECD-423).

MATERIALS AND METHODS

1-Animals

Thirty adult female Wistar rats weighing 150-170g were obtained from VACSERA, Ministry of Health, Egypt. They were kept in cages with free access to a standard diet and water. Rats were adapted for one week before beginning the study. The experimental protocol was conducted according to the Institutional Animal Care and Use Committee of Beni-Suef University (BSU-IACUC) with approval no. 022-314

2- Chemicals

Hemoglobin kit was obtained from BioMED Company, Egypt. Alanine aminotransferase, aspartate aminotransferase, urea and creatinine kits were purchased from Vitro Scient Company, Egypt.

3- Plant material

Fresh *Bougainvillea glabra* leaves were collected in April 2024 from Menoufia city, Egypt. The botanical identity of the plant was confirmed by the Department of Medical and Aromatic Plants, Faculty of Agriculture, Beni-Suef University, Egypt; Research Institute of Medicinal and Aromatic Plants (RIMAP), Beni-Suef University, Egypt. A voucher specimen (BUPD-25-03) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Egypt.

4-Preparation of extract

The extraction method was performed following Ahmed *et al.* (2016), with slight modifications where the leaves of *Bougainvillea glabra* (4 kg) were removed from the stalk, washed, dried in shade (1.25

kg) then ground with an electric grinder into a fine powder. The powdered plant was macerated in 80% aqueous ethanol (3 × 1500 mL, at room temperature (RT)), and then the extract was filtered and evaporated under vacuum. The residue was then suspended in distilled water and defatted with n-hexane (4x750 mL, at RT). The suspended residue was dried under reduced pressure to yield a solid residue that is rich in phenolic compounds. The dried extract was kept in a tightly closed container till further analysis.

5-Liquid chromatography-mass spectrometry (LC–MS) analysis of BG defatted extract

The analysis of the fraction sample was carried out through the use of liquid chromatography–electrospray ionization–tandem mass spectrometry with an ExionLC AC system for separation and SCIEX Triple Quad 5500+ system equipped with an electrospray ionization (ESI) for detection. An Ascentis® Express 90 Å C18 Column (2.1×150 mm, 2.7 µm) was used for the separation.

The mobile phases used for the separation were comprised of 2 eluents (A: 5 mM ammonium formate pH 8 and B: acetonitrile (LC grade)). The mobile phase gradient was automated as follows: 5% B at 0:1 minute, 5-100% B from 1:20 minutes, 100% B from 20:25 minutes, 5% at 25.01, 5% from 25.01:30 minutes. The flow rate was 0.3 ml/min with an injection volume of 5 µl. The electrospray source was applied in negative ionization mode with a scan (EMS-IDA-EPI) ranged from 100: 1000 Da for MS1 using the following parameters: curtain gas: 25 psi; IonSpray voltage: -4500; source temperature: 500°C; ion source gas 1 & 2 were 45 psi and from 50: 1000 Da for MS2 with a declustering potential: -80; collision energy: -35 (Ali *et al.,* 2024).

6- Experimental design

Based on previous studies performed on toxicity of isolated essential oils and whole extract of *Bougainvillea glabra*, this study was performed according to the limit test of OECD 423 guidelines using *Bougainvillea glabra leaves* flavonoids rich fraction at the limit dose (2g/kg b.w) and a higher dose (3g/kg b.w). Rats were randomly divided into 3 groups of 10 animals per group. The first one was considered a normal control (CN) and treated once orally with saline. The second (BG I) and the third (BG II) groups were treated with BG flavonoids-rich fraction dissolved in saline orally by gavage at a dose of 2 and 3g/kg b.w, respectively. All rats were weighed before dosing at day 0 and then weekly for two weeks.

7-Clinical observations

After treatment, each rat was observed individually throughout the first thirty minutes, sporadically over the first 24 hours, especially the first four hours, and then every day for the experiment period of 14 days. Clinical observations, including morbidity and mortalities, signs of toxicity and behavior patterns were recorded.

7.1-Neurobehavioral measurements using behavioral tests

7.1.1-Measuring anxiety-like behaviors (elevated cross maze test (EPM))

The elevated cross maze is a wooden maze with two opposing arms and a central platform (10 cm2) between them. The open arm is (50 × 10 cm) and the closed arm is (50 × 10 cm) with a sidewall height of 30 cm. The maze was positioned 50 cm above the ground. The rats were placed at the end of the open arm individually, facing the central platform, and left to explore the maze for 10 min. A videotaped camera was used to record the frequency and total amount of time animals spent in open and closed arms (Komada *et al.,* 2008). Following each rat, 70% ethyl alcohol was used to clean the apparatus floor.

7.1.2-Measuring working memory (Y-maze test)

The maze is a Y-shaped apparatus for determining spatial short-term working memory (Nasri *et al.,* 2012) by measuring spontaneous alternative behavior percent (SAP) in its arms according to the methodology of Wall *et al.* (2004) and Rasoulijazi *et al.* (2007). After each rat, 70% ethyl alcohol was used to clean the maze floor.

Sample collection

At the end of the experiment, blood specimens were collected into EDTA-containing tubes as an anticoagulant for hematological parameters and into plain tubes, which were centrifuged for 10 min at 3000 rpm to separate the serum. The serum was kept at -20°C until used for biochemical parameters.

The rats were subjected to deep anesthesia using isoflurane, and then cervical dislocation was done. Gross pathological examination for each animal was estimated. Liver and kidney specimens were preserved in 10% formalin to be examined histopathologically.

7-Hematological and biochemical analysis

Packed cell volume (PCV) % was estimated according to Bull *et al.* (2000). Hemoglobin (Hb) was determined as previously reported by Drabkins and Austin (1932) using commercial kits. Total erythrocytic (RBCs) and leucocytic (WBCs) counts were performed using a hemocytometer as explained by Feldman *et al.* (2000). Blood smears were fixed and stained with Giemsa stain for differential leucocytic count following Jain (1993).

Liver enzyme activities (ALT, AST) were estimated following Reitman and Frankel (1957). Creatinine and urea were determined using commercial kits as previously reported by Bowers and Wong (1980) and Patton and Crouch (1977), respectively.

Histopathological examination

The livers and kidneys were collected immediately after sacrificing rats and preserved in 10% formalin. Then they were processed and stained by hematoxylin and eosin (H&E) as described by Bancroft and Layton (2013). Histopathological lesions of liver (mononuclear cell infiltrations, vacuolation, necrosis, fibrous connective tissue and activation of Kupffer cells) and kidney (vacuolation and hyaline droplet) were scored for each microscopic field using a score checklist as follow: 0 = none, 1 = mild, 2 = moderate, 3 = severe (Ibrahim *et al.,* 2010).

Statistical analysis

All results were presented as mean ± standard error (SE) and were analyzed by one-way analysis of variance (ANOVA) accompanied by Tukey's post hoc test. Kruskal-Wallis (non-parametric) test was performed to determine SAP Percentage. The statistical significance was expressed at a value of $P<0.05$. To conduct statistical analyses, IBM SPSS version 26 was used.

RESULTS

1-LC-MS analysis:

LC-MS investigation of the defatted fraction of BG leaves enabled the annotation of fourteen constituents representing several chemical classes: flavonoids (eight metabolites), phenolic (two metabolites), terpenes, sterols and alkaloids (four metabolites) as shown in Table (1) and Figure (1).

2-Clinical observation and body weight

The rats in group BG I did not show any abnormal clinical signs or mortalities, or significant change in body weight gain in comparison with the control one. While in group BG II two female rats were found died after treatment by 3 hours and there was a significant ($P<0.01$) reduction in body weight gain in week II when compared with normal control and BG I groups (Table 2).

Table 1: Identified compounds by LC-MS analysis of the defatted BG leaves extract.

Peak NO.	RT (Min)	Observed m/z [M-H]$^-$	Proposed compound	Compound class	Molecular formula	Relative area (%)
1.	2.32	136.995	p-Salicylic acid	Phenolic compound	$C_7H_6O_3$	0.23
2.	3.48	594.934	Neoeriocitrin	Flavonoid	$C_{27}H_{32}O_{15}$	12.30
3.	4.78	162.975	p-Coumaric acid	Phenolic compound	$C_9H_8O_3$	2.00
4.	7.74	754.9111	Kaempferol 3-(2G-glucosylrutinoside)	Flavonoid	$C_{33}H_{40}O_{20}$	11.95
5.	8.43	431.027	Isovitexin	Flavonoid	$C_{21}H_{20}O_{10}$	2.91
6.	8.47	738.935	Robinin	Flavonoid	$C_{33}H_{40}O_{19}$	37.09
7.	8.57	608.929	Robinetin 3-rutinoside	Flavonoid	$C_{27}H_{30}O_{16}$	12.66
8.	8.65	431.0217	Vitexin	Flavonoid	$C_{21}H_{20}O_{10}$	3.09
9.	10.39	174.022	Calystegin B2	Alkaloid	$C_7H_{13}NO_4$	3.36
10.	10.68	409.065	Squalene	Terpenes	$C_{30}H_{50}$	0.38
11.	11.25	417.058	Oleoside dimethyl ester	Terpenes	$C_{18}H_{26}O_{11}$	0.40
12.	12.38	723.138	Alliospiroside C	Steroid	$C_{38}H_{60}O_{13}$	3.73
13.	13.58	284.967	Luteolin	Flavonoid	$C_{15}H_{10}O_6$	2.81
14.	25.90	269.166	Apigenin	Flavonoid	$C_{15}H_{10}O_5$	0.99

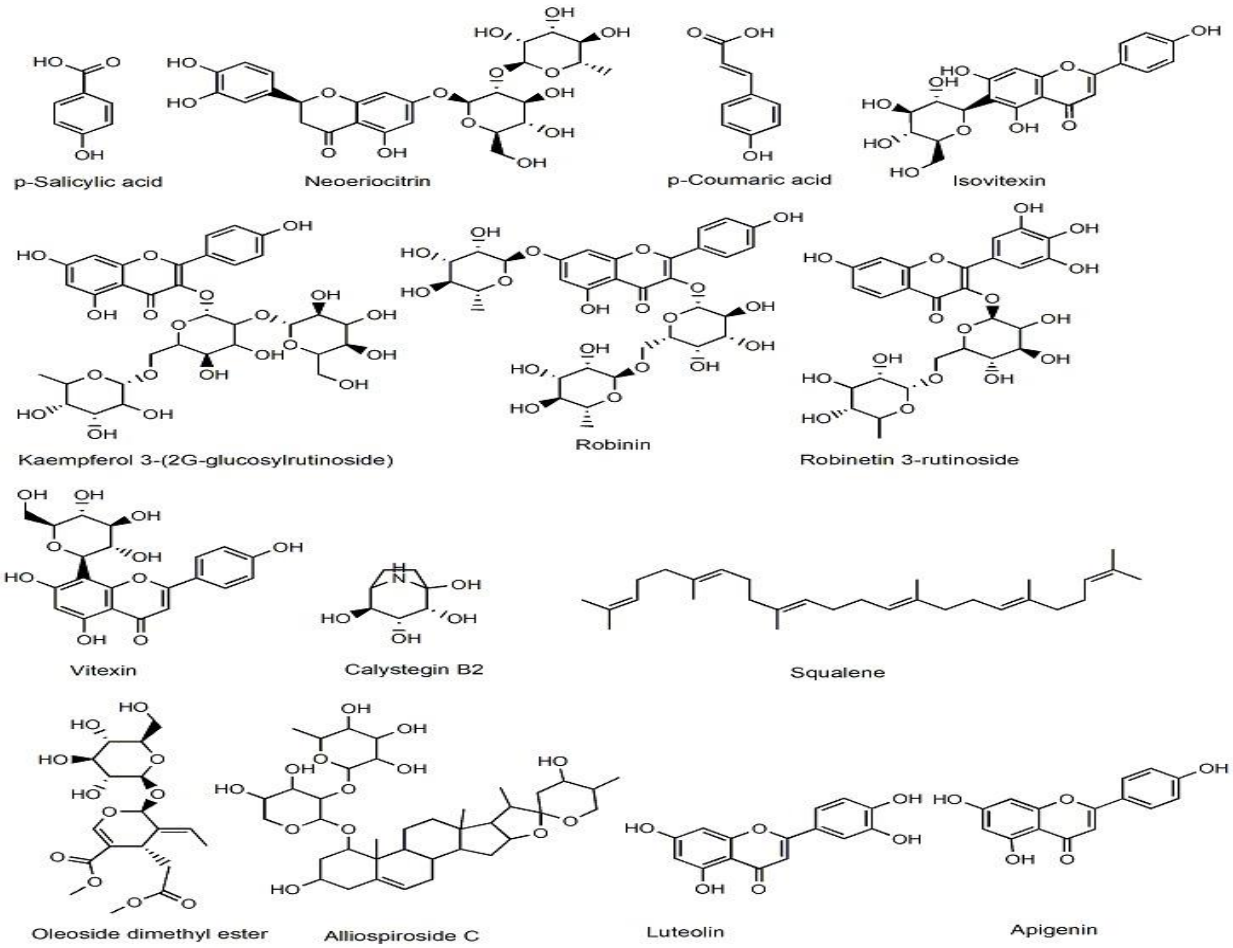

Figure 1: Chemical structures of the compounds identified based on LC–MS from BG leaves defatted extract.

Table 2: Effect of single oral treatment of BG flavonoids rich fraction on body weight gain.

Group / B.W gain (gm)	CN	BG I	BG II
Week I	15.67 ±1.45	15.00 ±1.15	12.33 ±1.56
Week II	21.67 ±1.20	24.33 ±0.67	15.67 ±0.67[a,b]

Data showed as mean ± SE (n=5) with different superscript letters (significant difference at *p<0.01*): (a) letter is statistically differing from control value; (b) letter is statistically differing from BG I group.

3-Neurobehavioural measurements

Measuring anxiety-like behaviors (elevated cross maze test (EPM))

Figure (2) illustrates that BG I significantly (*P<0.05*) prolonged the time /sec. that rats spent in the open arms in comparison to control rats. On the other hand, BG I significantly (*P<0.05*) decreased the time /sec. the rats spent in the closed arms, compared to the control.

Measuring working memory (Y-maze test)

Figure (3) shows that BG I and BG II induced a significant (*P<0.05*) improvement of SAP (short working memory) in relation to the control group.

Hematological and biochemical parameters

The current data showed that there was no statistical significance in erythrogram and leucogram between the control group and group BG I. In contrast, rats administered BG fraction at a dose of 3g/kg had a significant (*P<0.05*) decrease in the PCV, Hb and lymphopenia with neutrophilia when compared with control and BG I groups. However, there was no significant difference in monocytes and eosinophils (Table 3).

As shown in Table (3), there was a significant (*P<0.05*) increase in ALT, AST, Urea and creatinine in group BG II, compared to the control and BG I groups.

Table 3: Effect of oral administration of BG flavonoids rich fraction on hematological and biochemical parameters in different groups.

Parameters / Group	CN	BG I	BG II
Hematological parameters			
RBCs (×10^6/µl)	6.95 ±0.05	6.55 ±0.25	6.05 ±0.0.33
PCV %	41.25 ±0.75	40.75 ±0.47	38.50 ±0.29[a,b]
Hb (g/dl)	10.75 ±0.28	10.33 ±0.13	9.43 ±0.28[a]
MCV (fL)	59.39 ±0.84	62.51 ±2.70	64.19 ±3.72
MCH (pg)	15.46 ±0.31	15.84 ±0.63	15.70 ±0.78
MCHC %	26.08 ±0.78	25.35 ±0.17	24.53 ±0.85
WBCs (×10^3/µl)	14.00 ±0.26	14.51 ±0.25	14.90 ±0.40
Lymphocyte (×10^3/µl)	10.13 ±0.24	10.35 ±0.14	9.28 ±0.07[a,b]
Neutrophil (×10^3/µl)	2.94 ±0.03	3.15 ±0.09	4.23 ±0.24[a,b]
Monocyte (×10^3/µl)	0.51 ±0.03	0.58 ±0.01	0.75 ±0.10
Eosinophil (×10^3/µl)	0.42 ±0.09	0.43 ±0.00	0.64 ±0.05
Biochemical parameters			
ALT (U/L)	15.87 ±0.32	16.83 ±0.35	20.06 ±0.20[a,b]
AST (U/L)	48.96 ±1.45	49.32 ±0.75	56.04 ±0.63[a,b]
Urea (mg/dl)	45.08 ±0.14	47.54 ±0.90	50.85 ±0.58[a,b]
Creatinine (mg/dl)	0.68 ±0.02	0.67 ±0.03	1.00 ±0.06[a,b]

Data expressed as mean ± SE (n=5) with different superscript letters (significant difference at *p<0.01*): (a) letter is statistically differing from control value; (b) letter is statistically differing from BG I group.

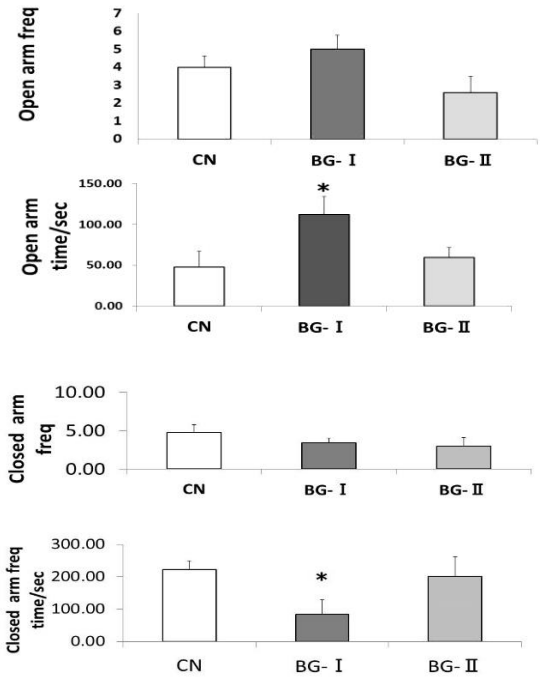

Figure 2: Effect of single oral treatment of BG flavonoids rich fraction on anxiety-like behavior in elevated plus maze.

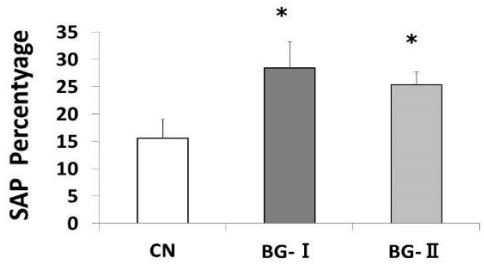

Figure 3: Effect of single oral treatment of BG flavonoid rich fraction on working memory of rats on y-maze.

Histopathological results

Figure (4) illustrates the histopathological results of liver and kidney tissues stained with H&E in all examined groups. Hepatic tissue sections (Figure 4 A) from normal control rats presented normal radiating hepatic cords and central veins. The sainted sections of most rats' livers in BG 2g/kg group showed moderate intracellular vacuolations (Figure 4 B). While rats treated with BG 3g/kg revealed mononuclear cell infiltrations in a focal manner as nodules within the portal triads, multifocal necrosis, activation of Kupffer cells, and vacuolation of hepatic cells. There was a proliferation of fibrous connective tissue with few intralobular lymphocytic infiltrations (Figure 4 C).

The stained renal tissues from the normal control group revealed normal histological structure of renal tubules and glomeruli (Figure 4 D). As well as stained renal sections of most rats in BG 2g/kg group appeared normal, while others showed mild vacuolation (Figure 4 E). However, rats received BG 3g/kg presented features of nephrosis in the form of intracellular vacuolation, cloudy swelling and hyaline droplet degeneration and casts (Figure 4 F). Table (4) summarizes the histopathological lesions of the liver and kidney using the scoring system.

Table 4: Effect of oral administration of BG flavonoids rich fraction on lesions scoring of liver and kidney tissues in different groups.

Lesions \ Group	CN	BG I	BG II
Liver tissues			
Mononuclear cell infiltrations	0.00 ±0.00	0.16 ±0.02	2.85 ±0.08[a,b]
Vacuolation	0.00 ±0.00	1.73 ±0.06[a]	2.79 ±0.08[a,b]
Necrosis	0.00 ±0.00	0.00 ±0.00	2.23 ±0.03[a,b]
Fibrous connective tissue	0.00 ±0.00	0.00 ±0.00	1.99 ±0.08[a,b]
Activation of Kupffer cells	0.00 ±0.00	0.16 ±0.02	2.05 ±0.06[a,b]
Kidney tissues			
Vacuolation	0.00 ±0.00	0.13 ±0.02[a]	2.82 ±0.02[a,b]
Hyaline droplet	0.00 ±0.00	0.10 ±0.01	2.34 ±0.04[a,b]

Data expressed as mean ± SE (n=5) with different superscript letters (significant difference at $p<0.01$): (a) letter is statistically differing from control value; (b) letter is statistically differing from BG I group.

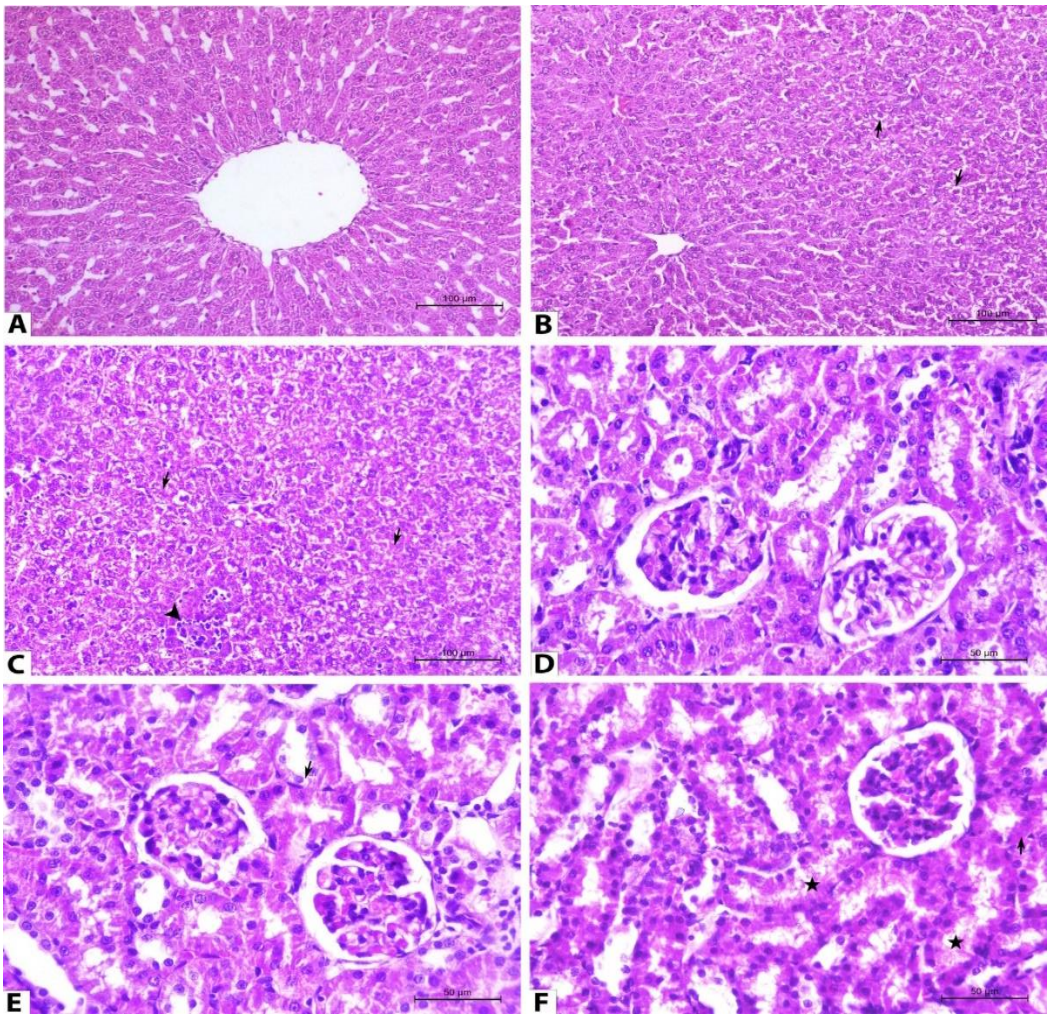

Figure 4: Representative photomicrograph of hepatic and renal tissue sections in each examined group. (A) Normal control rats revealed normal hepatic architecture (central vein and hepatic cords). (B) BG 2g/kg treated rats liver revealed mild intracellular vacuolation **(arrow)**. (C)While rats received BG 3g/kg showed mononuclear infiltration **(head arrow)** and multifocal necrosis **(arrow)**. (H&E, x200) (D) Kidney tissues from normal control rats presented normal histological structure of renal glomeruli and renal tubules. (E) Rats in BG 2g/kg group appeared normal with a few vacuolated cells **(arrow)**. (F) Rats treated with BG 3g/kg showed features of cloudy swelling **(arrow)** and hyaline droplets and cast **(star)**. (H&E, x400)

DISCUSSION

Medicinal plants are increasingly being used as an alternative to conventional medicines. Thus, screening plants to determine their toxicity is an important first step. Data obtained from these studies may be used to classify test plants according to their toxic effects. Toxicological studies typically use oral administration as the most effective method (Sarkar *et al.,* 2024).

LC-MS analysis of BG defatted fraction showed the presence of flavonoids, phenols, terpenes, sterols and alkaloid. Reviewing the literature, vitexin, isovitexin, apigenin, luteolin were stated to be isolated from the leaves of BG using column chromatography (Ahmed 2014). Furthermore, negative mode UHPLC-MS analysis of the aerial parts reported the tentative identification of flavonoid compounds (Saleem *et al.,* 2019) that matched with our results. These bioactive compounds are responsible for BG various pharmacological actions as well as some toxicity.

Changing in body weight is a sensitive indicator to evaluate negative effects of chemicals and plants. Toxic drugs may

cause irregularities in body weight, such as a decrease in body weight represents severe toxicity and a reduction in body weight gain indicates a mild form of toxicity (Olayode *et al.,* 2020).

The present data showed that there was no mortality or significant change in body weight gain in rats treated with BG flavonoids rich fraction at dose of 2 g/kg b.w, suggesting that fraction at limit dose (2g/kg b.w) is safe and had no adverse effect on normal body weight gain of rats as agreed with Krishna and Sundararajan (2018) and Rajyalakshmi *et al.* (2024).

The OECD 423 guide could not calculate the precise LD_{50}; however, it is a stepwise protocol. Thus, from the results of the present study, the flavonoids-rich fraction of *Bougainvillea glabra* can be put in category 5 (LD_{50} > 2000 mg/kg b.w) and fall in lower toxicity chemicals according to the globally harmonized classification system based on the OECD guide.

Administration of the fraction at a dose of 3 g/kg b.w in group BG II resulted in mortality of 20% of female rats and a significant decrease in body weight gain, compared to the control. A similar change in body gain was produced by Chicococca alba root extract (Gazda *et al.,* 2006) and Stryphnodendron adstringens bark (Rebecca *et al.,* 2002). The alteration in body weight gain may be due to reduced Hb in this group that leads to reduced metabolic rate and energy production as a result of impaired oxygen supply to several tissues (Ahmad *et al.,* 1995 and Auvinen *et al.,* 2021).

The observed data indicated that both doses of BG fraction improved memory, that parallel with Basnet *et al.* (2020) and Choudhari *et al.* (2021). In an early study by Abdel-Salam *et al.* (2021), they found that *Bougainvillea spectabilis* normalized memory impairment in AlCl3-treated rats as a result of decreased amyloid Aβ, oxidative stress and brain inflammation (IL-6).

In addition, rats treated with a 2g /kg b.w dose only had anxiolytic activity in EPM that agreed with Choudhari *et al.* (2021) and Ali *et al.* (2021). The reported improvement in memory and anxiolytic activity may be owing to BG effect on neurotransmitters (rise in catecholamine level) and antioxidant function (Choudhari *et al.,* 2021). Oxidative damage is typically associated with behavioral problems (Lamtai *et al.,* 2020). Flavonoids have a marked antioxidant and antidepressant effect (Yi *et al.,* 2008).

The hematopoietic system is a sensitive and vital index to evaluate physiological and pathological conditions in toxicity studies (Mukinda and Eagles, 2010). In the current study, all hematological parameters showed no statistical differences in BG I group in comparison to control group.

On the other hand, BG II group showed a significant reduction in Hb and PCV. The observed reduction in Hb may be as a result of flavonoids ability to decrease intestinal absorption of heme iron (Mehjabin *et al.,* 2024). The observed lymphopenia may be due to infiltration of lymphocyte in hepatic tissues as indicated in histopathological examination (SAWERUS *et al.,* 2025) while neutrophilia may be consequence to acute reaction (Grant and Lett-Brown, 1986).

The liver and kidney are responsible for vital functions, including metabolic processes, making them frequent targets for toxic compounds. Evaluation of health status of hepatic and renal tissues requires multiple blood biomarkers such as ALT, AST, creatinine and urea. Serum activities of ALT and AST are significantly increased in toxic environment. ALT, abundant enzyme in the cytoplasm of hepatic cells is more specific to evaluate liver cell damage. Urea is the first indicator of acute renal dysfunction following renal injury while

creatinine is the most reliable marker, increasing only when there is significant loss of renal function (Olayode *et al.,* 2020).

In our study, there was no significant alteration in liver and kidney function tests in rats received the fraction at dose of 2g/kg b.w. However, significant elevation in ALT, AST, creatinine and urea levels observed at dose of 3 g/kg b.w. Histopathological findings, correlated with this alteration, indicated liver toxicity in the form of multifocal necrosis and mononuclear cell infiltrations. Features of nephrosis are observed in renal tissues.
This alteration in liver and kidney function may be due to a high dose of flavonoids. Flavonoids have antioxidant properties through regenerating cell membrane antioxidants, scavenging ROS and chelating transition metal ions such as Fe+ and Cu+; however, they have pro-oxidant activity at higher doses (Decker, 1997 and Rietjens *et al.,* 2002). Higher doses of flavonoids may cause anemia, hepato-toxicity, nephrotoxicity, reproductive toxicity, gastrointestinal disturbance and hormonal imbalance (Mehjabin *et al.,* 2024 and Tang and Zhang, 2022).

CONCLUSION

The current study showed that *Bougainvillea glabra* flavonoids rich fraction is well tolerated in Wistar rats after administration of a single acute oral dose. In addition, it had memory improvement with anxiolytic effects. Thus, it could be classified as category 5 by the OECD. However, when treated with higher doses, hematological, biochemical and histological alterations were observed, indicating that fraction, when used at higher doses, does not represent a high level of safety.

ACKNOWLEDGMENT

The authors want to deliver their sincere appreciation to Dr. Ahmed M. Ayyat (Department of Medical and Aromatic Plant, Faculty of Agriculture, Beni-Suef University; Research Institute of Medicinal and Aromatic Plants, Beni-Suef University) for plant identification.

REFERENCES

Abdel-Salam, O.M.; El-Shamarka, M.E.S.; Youness, E.R. and Shaffie, N. (2021): Inhibition of aluminum chloride-induced amyloid Aβ peptide accumulation and brain neurodegeneration by Bougainvillea spectabilis flower decoction. Iranian journal of basic medical sciences, 24(10), 1437. https://doi.org/10.22038/IJBMS.2021.58246.12940.

Ahmad, F.; Ali, S.S. and Shakoori, A.R. (1995): Sublethal effects of Danitol (Fenpropathrin), a synthetic pyrethroid, on Chinese grass carp, Ctenopharyngodon idella. FOLIA BIOLOGICA-KRAKOW-, 43, 151-160.

Ahmed, A.H. (2014): New flavone from the aerial parts of Bougainvillea glabra. IJCER Online, 73, 2250-3005.

Ahmed, H.; Moawad, A.; Owis, A.; AbouZid, S. and Ahmed, O. (2016): Flavonoids of Calligonum polygonoides and their cytotoxicity. Pharmaceutical Biology, 54(10), 2119-2126. https://doi.org/10.3109/13880209.2016.1146778

Ali, A.M.; Makboul, H.E. and Moselhy, M.A. (2024): LC-ESI-MS/MS Analysis of Bioactive Metabolites from Streptomyces rochei with Antimicrobial, Antioxidant, and Cytotoxic Properties. Egyptian Journal of Chemistry, 67(13), 1947-1956. https://dx.doi.org/10.21608/ejchem.2024.322646.10482

Ali, S.M.; Shamim, S.; Younus, I.; Anwer, L. and Khaliq, S.A. (2021): Anxiolytic, antidepressant and inhibitory effect on MAO isoenzymes by Bougainvillea glabra flower extract in rats. Pakistan Journal of Pharmaceutical Sciences, 34, 1963-1968.

Auvinen, J.; Tapio, J.; Karhunen, V.; Kettunen, J.; Serpi, R.; Dimova, E.Y. and Koivunen, P. (2021): Systematic evaluation of the

association between hemoglobin levels and metabolic profile implicates beneficial effects of hypoxia. Science Advances, 7(29), eabi4822. https://doi.org/10.1126/ sciadv.abi4822

Bancroft, J.D. and Layton, C. (2013): The hematoxylin and eosin. In: Suvarna SK, Layton C, Bancroft JD (eds) Theory practice of histological techniques, 7th edn. Churchill Livingstone of El Sevier, Philadelphia, pp 179–220. http://dx.doi.org/10.1016/b978-0-7020-4226-3.00010-x.

Basnet, M.; HS, Y. and Swamy, S. (2020): Evaluation of Neuroprotective activity of Bougainvillea glabra leaves extract on experimentally induced Neurotoxicity in Albino rats. Research Journal of Pharmacy and Technology, 13(8), 3825-3832. http://dx.doi.org/10.5958/0974-360X.2020.00677.

Bowers, L.D. and Wong, E.T. (1980): Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical chemistry 26(5), 555-561.https://doi.org/10.1093/clinchem/26.5.555.

Bull, B.S.; Koepke, J.A.; Simson, E. and Van Assendelft, O.W. (2000): Procedure for determining packed cell volume by the microhematocrit method; approved standard. NCCLS Document H7-A3, 20(18), 1-8.

Choudhari, P.V.; Kanse, V.G.; Venkatachalam, A. and Pal, P.R. (2021): Antidepressant and nootropic activity of aqueous extract of Bougainvillea glabra. World Journal of Advanced Research and Reviews, 10(1), 334-339. https://doi.org/10.30574/wjarr.2021.10.1.0163.

Decker, E.A. (1997): Phenolics: prooxidants or antioxidants?. Nutrition reviews, 55(11), 396-398. https://doi.org/10.1111/j.1753-4887.1997.tb01580.x.

*Drabkin, D.L. and Austin, J.H. (1932):*Spectrophotometric studies: I. Spectrophotometric constants for common hemoglobin derivatives in uman, dog, and rabbit blood. J. Biol. Chem. 98(2), 719-733. https://doi.org/10.1016/S0021-9258(18)76122-X.

Elumalai, A.; Eswaraiah, M.C.; Lahari, K.M. and Shaik, H.A. (2012): In-vivo screening of Bougainvillea glabra leaves for its analgesic, antipyretic and anti-inflammatory activities. Asian Journal of Research in Pharmaceutical Science, 2(3), 85-87.

Feldman, B.F.; Zinkl, J.G. and Jain, V.C. (2000): In: Schalm's Veterinary hematology. 5th Ed. Lippincott Williams and Wilkins, Canada, pp. 1145-1146.

Gazda, V.E.; Gomes-Carneiro, M.R.; Barbi, N.S. and Paumgartten, F.J. (2006): Toxicological evaluation of an ethanolic extract from Chiococca alba roots. Journal of ethnopharmacology, 105(1-2), 187-195. https://doi.org/10.1016/j.jep.2005.10.017

Giri, S.S.; Kim, S.G.; Woo, K.J.; Jung, W.J.; Lee, S.B.; Lee, Y.M. and Park, S.C. (2023): Effects of Bougainvillea glabra leaf on growth, skin mucosal immune responses, and disease resistance in common carp Cyprinus carpio. Fish & Shellfish Immunology, 132, 108514. https://doi.org/10.1016/j.fsi.2022.108514.

Grant, J.A. and Lett-Brown, M.A. (1986): Bronchial asthma, leukocyte synthesis of slow-reacting substance of anaphylaxis, and other evidence for activation of blood cells in asthma. The Journal of Allergy and Clinical Immunology, 77(3), 407-410. https://doi.org/10.1016/0091-6749(86)90174-0.

Ibrahim, M.Y.; Abdul, A.B.; Ibrahim, T.A.T.; Abdelwahab, S.I.; Elhassan, M.M. and Syam, M.M. (2010): Evaluation of acute toxicity and the effect of single injected doses of zerumbone on the kidney and liver functions in Sprague Dawley rats. African Journal of Biotechnology, 9(28), 4442-4450.

Jain, N.C. (1993): In: Essentials of Veterinary Haematology. Lea and Febiger, Philadelphia (Pa), USA, pp. 76–250.

Komada, M.; Takao, K. and Miyakawa, T. (2008): Elevated plus maze for mice. J. Vis. Exp. (22), e1088. DOI:10.3791/1088.

Krishna, R.G. and Sundararajan, R. (2018): Cardioprotective and antioxidant effectsof Bougainvillea glabra against isoproterenol induced myocardial necrosis in albinorats. International Journal of Phytomedicine, 10(1), 45-57. DOI:10.5138/09750185.2200.

Lamtai, M.; Zghari, O.; Ouakki, S.; Marmouzi, I.; Mesfioui, A.; El Hessni, A. and Ouichou, A. (2020): Chronic copper exposure leads to hippocampus oxidative stress and impaired learning and memory in male and female rats. Toxicological Research, 36, 359-366. https://doi.org/10.1007/s43188-020-00043-4.

Mehjabin, S.; Akanda, M.K.M.; Hoque, N.; Hasan, A.N.; Parvez, G.M. and Mosaddik, A. (2024): Flavonoid Intake and Risk of Toxicity in Chronic Metabolic Disease. Role of Flavonoids in Chronic Metabolic Diseases: From Bench to Clinic, 511-534. https://doi.org/10.1002/9781394238071.ch14.

Mukinda, J.T. and Eagles, P.F. (2010): Acute and sub-chronic oral toxicity profiles of the aqueous extract of Polygala fruticosa in female mice and rats. Journal of Ethnopharmacology, 128(1), 236-240. https://doi.org/10.1016/j.jep.2010.01.022.

Nasri, S.; Roghani, M.; Baluchnejadmojarad, T.; Balvardi, M. and Rabani, T. (2012): Chronic cyanidin-3-glucoside administration improves short-term spatial recognition memory but not passive avoidance learning and memory in streptozotocin-diabetic rats. Phytother. Res. 26(8), 1205-1210. https://doi.org/10.1002/ptr.3702.

OECD, O. (2001): 423-Guidelines for the Testing of Chemicals Acute Oral Toxicity-Fixed Dose Procedure. Animals. 1–14.

Ogunwande, I.A.; Avoseh, O.N.; Olasunkanmi, K.N.; Lawal, O.A.; Ascrizzi, R. and Flamini, G. (2019): Chemical composition, anti-nociceptive and anti-inflammatory activities of essential oil of Bougainvillea glabra. Journal of ethnopharmacology, 232, 188-192. https://doi.org/10.1016/j.jep.2018.12.017.

Olayode, O.A.; Daniyan, M.O. and Olayiwola, G. (2020): Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. Journal of traditional and complementary medicine, 10(6), 544-554. https://doi.org/10.1016/j.jtcme.2019.05.001.

Patton, C.J. and Crouch, S.R. (1977): Enzymatic determination of urea. Anal. Chem, 49(82), 464-469.

Rajyalakshmi, B.; Rajani, V.; Raju, C.N. and Bhongiri, B. (2024): The Phytochemical Investigation and Pharmacological Evaluation of Hepatoprotective Activity of Bougainvillea glabra in Rats. Current Trends in Biotechnology and Pharmacy, 18(1), 1574-1580. https://doi.org/10.5530/ctbp.2024.1.5.

Ramesh, M.; Ravikanth, D.; Selvan, M.T.; Sahayaraj, A.F. and Saravanakumar, A. (2024): Extraction and characterization of Bougainvillea glabra fibers: a study on chemical, physical, mechanical and morphological properties. International Journal of Biological Macromolecules, 275, 133787. https://doi.org/10.1016/j.ijbiomac.2024.133787.

Rasoulijazi, H.; Joghataei, M.T.; Noubakht, M. and Roughani, M. (2007): The beneficial effect of (-)-epigallocatechin-3-gallate in an experimental model of Alzheimer's disease in rat: A behavioral analysis. Iran. Biomed. J. 11, 237–243.

Rebecca, M.A.; Ishii-Iwamoto, E.L.; Grespan, R.; Cuman, R.K.N.; Caparroz-Assef, S.M.; de Mello, J.C.P. and Bersani-Amado, C.A. (2002): Toxicological studies on Stryphnodendron adstringens. Journal of ethnopharmacology, 83(1-2), 101-104. https://doi.org/10.1016/ S0378-8741(02)00219-2.

Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28(1), 56-63. https://doi.org/10.1093/ajcp/28.1.56.

Rietjens, I.M.; Boersma, M.G.; de Haan, L.; Spenkelink, B.; Awad, H.M.; Cnubben, N.H. and Koeman, J.H. (2002): The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environmental toxicology and pharmacology, 11(3-4), 321-333. https://doi.org/10.1016/ S1382-6689(02)00003-0.

Saleem, H.; Usman, A.; Mahomoodally, M.F. and Ahemad, N. (2021): Bougainvillea glabra (choisy): A comprehensive review

on botany, traditional uses, phytochemistry, pharmacology and toxicity. Journal of ethnopharmacology, 266, 113356. https://doi.org/10.1016/j.jep.2020.113356.

Saleem, H.; Zengin, G.; Ahmad, I.; Lee, J.T.B.; Htar, T.T.; Mahomoodally, F.M. and Ahemad, N. (2019): Multidirectional insights into the biochemical and toxicological properties of Bougainvillea glabra (Choisy.) aerial parts: A functional approach for bioactive compounds. Journal of pharmaceutical and biomedical analysis, 170, 132-138. https://doi.org/10.1016/j.jpba.2019.03.027

Sarkar, S.; Modak, D.; Roy, S.K.; Biswas, A.; Islam, M.; Baishya, R. and Bhattacharjee, S. (2024): In silico, in vitro, and in vivo acute and sub-acute toxicity profiling of whole plant methanol extract of Equisetum diffusum D. Don from the sub-Himalayan West Bengal, India, having ethnobotanical uses. BMC Complementary Medicine and Therapies, 24(1), 324. https://doi.org/10.1186/s12906-024-04606-y.

Sawerus, M.G.; Ahmed, W.; Shehata, O.; El Amir, D.A.L.I.A.; Khalil, F.; Mahdi, E.A. and Kamel, H.H. (2025): Acute safety study of salvia officinalis terpenoids rich fraction in wistar rats. Assiut Veterinary Medical Journal, 71(184), 386-400. https://dx.doi.org/10.21608/avmj.2025.333302.1459.

Tang, Z. and Zhang, Q. (2022): The potential toxic side effects of flavonoids. Biocell, 46(2), 357. DOI:10.32604/biocell.2022.015958.

Tegeli, V.S.; Shinde, P.S.; Shiranal, P.A.; Shilwant, S.V.; Surwase, P.R.; Shendage, G.S. and Shirawar, M.M. (2019): A comparative phytochemical analysis of Bougainvillea glabra and Catharanthus roseus. J Pharmaco gPhytochem, 8(3), 2965-2968.

Wall, P.M.; Blanchard, R.J.; Markham, C.; Yang, M. and Blanchard, D.C. (2004): Infralimbic D1 receptor agonist effects on spontaneous novelty exploration and anxiety-like defensive responding in CD-1 mice. Behav. Brain. Res., 152(1), 67-79. https://doi.org/10.1016/j.bbr.2003.09.035.

Yi, L.T.; Li, J.M.; Li, Y.C.; Pan, Y.; Xu, Q. and Kong, L.D. (2008): Antidepressant-like behavioral and neurochemical effects of the citrus-associated chemical apigenin. Life sciences, 82(13-14), 741-751. https://doi.org/10.1016/j.lfs.2008.01.007.

دراسة عن الحد الآمن للجزء الغني ب فلافونويدات من نبات جهنمية جلابرا في الجرذان

منال مصطفى عبد الفتاح محمد ، ولاء محمد سيد أحمد ، الفت شحاته مجودة فرج الله ،
هيام صلاح حامد أحمد ، فاطمة حنفى سيد خليل ، عماد أحمد مهدى ، حمدى حلمى كامل أحمد

Email: Manal.Mustafa@vet.bsu.edu.eg Assiut University web-site: www.aun.edu.eg

الجهنمية جلابرا هو نبات زينة ذو خصائص دوائية قيمة. تهدف التجربة الحالية إلى تقدير حد الأمان للجزء الغني ب فلافونويدات من أوراق نبات الجهنمية جلابرا فى الجرذان. تم تقسيم إناث الجرذان بالتساوي إلى ثلاث مجموعات. مجموعة طبيعية ضابطة والمجموعات الأخري عولجت ب ٢ و ٣ جم/كجم من وزن الجسم على التوالي من هذا الجزء كجرعة فموية واحدة. تم تقييم زيادة الوزن أسبوعيًا و تم ملاحظة الجرذان طوال فترة التجربة. وبعد ١٤ يوم، تم إجراء الاختبارات السلوكية ثم إجراء فحوصات الدم والكمياء الحيوية بالإضافة إلى الفحص النسيجي للكبد والكلية. أظهرت النتائج أن جزء الجهنمية جلابرا يمكن أن يصنف ضمن الفئة ٥ وفقًا لإرشادات منظمة التعاون الاقتصادى والتنمية. لم تسبب الجرعة ٢ جم/كجم من وزن الجسم أي وفيات أو تغيرات ملحوظة فى وزن الجسم أو المعايير اختبارات الدم أو التحليلات البيوكميائية. كلا الجرعتين حسنوا الذاكرة العاملة إلا أن جرعة ٢ جم/كجم من وزن الجسم فقط كان لها تأثير مزيل للقلق ملحوظ. كان هناك انخفاض ملحوظ فى الهيموغلوبين و حجم خلايا الدم الحمراء، ونقص مطلق فى الخلايا الليمفاوية مصحوب بزيادة في خلايا الدم البيضاء المتعادلة فى الفئران التى تلقت ٣ جم/كجم من وزن الجسم، بالإضافة إلى زيادة ملحوظة فى إنزيمات الكبد والكرياتينين واليوريا. كما أظهر الفحص النسيجي للكبد والكلية لهذه المجموعة آثارًا جانبية. لذلك يبدو أن الجرعة ٢ جم/كجم من وزن الجسم من الجزء الغنى ب فلافونويدات من نبات الجهنمية جلابرا يمكن تحملها جيدًا من قبل الجرذان مع عدم حدوث أى آثار جانبية ، بينما الجرعة ٣ جم/كجم من وزن الجسم كان لها تأثير سام على وزن الجسم وبعض المعايير الدموية والكبد والكلى.