Advances in Bioresearch
Adv. Biores., Vol 14 (2) May 2023: 64-71

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.3.6471

Advances in Bioresearch

ORIGINAL ARTICLE

In-vivo pharmacological evaluation of *Barleria grandiflora* leaves for Hepatoprotective activity

Revendra Parganiha[1]*, Gaurav Tiwari[1], Neeraj Sharma[1]
[1]Patel college of Pharmacy, MPU, Bhopal.
Corresponding author's Email: parganiharevendra@gmail.com

ABSTRACT
Medicinal plants have been utilized as cure of diseases for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. The development of plant-based herbal medicine is based on the traditional knowledge that depends on the alternative system of medicine like Ayurveda. Barleria grandiflora commonly known as Dev- koranti or Shwet kesharia. The Literature survey reveals that not much of work has been reported towards the biological activities of this plant. The aim of this study is to determine the hepatoprotective activity of leaf extracts of Barleria grandiflora.
Keywords: *Barleria grandiflora, Hepatoprotective, Carbon tetrachloride, Hepatotoxicity.*

Received 10.01.2023 Revised 10.03.2023 Accepted 24.04.2023

How to cite this article:
Revendra P, Gaurav T, Neeraj S. *In-vivo* pharmacological evaluation of *Barleria grandiflora* leaves for Hepatoprotective activity. Adv. Biores. Vol 14 [3] May 2023. 64-71

INTRODUCTION

The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals. In more recent history, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century [1, 2]. Drug discovery from plants require some knowledge which may use traditionally; or plants having side or toxic effects or drug discovery and development from therapeutic effects-based plants [3, 4]. After determining the efficacy of natural medicines, the active ingredients could be found by modern scientific methods [5]. Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods [6]. As such, many pharmaceutical companies have eliminated or scaled down their natural product research [7]. The Liver is a key organ in the human body, regulating homeostasis and is a frequent target for a number of toxicants [8, 9]. In spite of tremendous scientific advancement in the field of hepatology during recent years, liver problems are on the rise [10]. Regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments. The most common liver diseases are various types of acute (sudden) hepatitis (inflammation), chronic (long duration) hepatitis, fatty liver, cirrhosis (scarring), and cancer.[11] Viruses, drugs, and alcohol, as well as metabolic, immune (defence) system, and genetic (hereditary) abnormalities are the common causes of these liver diseases [12]. It is necessary to identify alternative pharmaceuticals for the treatment of hepatic diseases, with the aim of these agents being more effective and less toxic [13]. The use of some plants and the consumption of different fruits have played fundamental roles in human health care [14]. Liver involved in multiple functions and it also has great power of regeneration; these makes estimation of the presence or absence of hepatic dysfunction is complicated [15]. The plants, fruits, and compounds described could offer novel alternatives to the limited therapeutic options that exist for the treatment of liver diseases thus; these foods should be considered in future studies. In general, some phytochemical with hepatoprotective activity, the principal mechanisms of action of which were related to their antioxidant potential, a characteristic that should motivate and promote the search for effective protective agents, which must be evaluated later in pre-clinical and clinical assays to determine their safety and their chemopreventive capacity [16]. In present study the various extracts (hydro alcoholic, ethanol, aqueous) of *Barleria*

grandiflora was evaluated for its hepatoprotective activity on carbon tetrachloride induced hepatotoxic Albino rats.

MATERIAL AND METHODS

Fresh leaves of *Barleria grandiflora* were collected from the local area of Raipur (Chhattisgarh), India. The Plant Herbarium was submitted for identification and authentication at the Government Ayurvedic College Raipur Chhattisgarh India. The collected samples were washed with water and air-dried. The dried samples were then ground into powder. The powdered and the whole dried samples were stored in tightly closed containers along with silica.

Extraction process : Leaves of *Barleria grandiflora* were defatted with petroleum ether then extracted sequentially with aqueous, alcoholic and hydro alcoholic solvents using continuous hot extraction method i.e. Soxhlet extraction.

AQBG: Aqueous extract of *Barleria grandiflora*

EABG: Ethanol extract of *Barleria grandiflora*

HAEBG: Hydro alcoholic extract of *Barleria grandiflora*

Pharmacological screening:

Animals: Animals Wistar rats weighing between 180-200 gm were selected for in vivo activity of extracts. All animals were housed in air-conditioned rooms with 10-15 air circulation cycles per hour. Standard pelleted basal diet and purified water were provided ad libitum to the animals. All the animals were acclimatized to the laboratory conditions before they were used in the experiments [17]. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC Approval No.: PCP/CPCSEA/2021/06) and ethical norms were strictly followed during all experimental procedures.

Hepatoprotective effect of extracts of the *Barleria grandiflora* leaves: The hepatoprotective activity of the extracts of *Barleria grandiflora* leaves were carried out using model of CCl4 induced hepatotoxicity in experimental animals (Wistar rats). Animals of group I were treated with 1 ml/kg bw of saline (0.85%) intragastrically and olive oil (3 ml/kg bw) intraperitoneally twice a week for four weeks. Rats of group II to IX were treated with CCl4 (30% in olive oil) at a dose of 3 ml/kg bw intraperitoneally twice a week for four weeks. Animals of group II received only CCl4 treatment. Animals of group III serve as standard treated with silymarin suspension (10 mg/kg bw).[18] However, animals of group IV to IX received extract of *Barleria grandiflora* leaves at a dose of 100-200 mg/kg bw by using oral feeding tube, respectively, in addition to CCl4 treatment, twice a week for four weeks. All the test samples, along with standard drugs were given orally except carbon tetrachloride. After 24 h of the last treatment, all the animals were weighted, sacrificed, collected the blood while liver was removed, weighted and perfuse in ice-cold saline solution. Liver samples were treated with liquid nitrogen and stored at −70 °C for further studies [19, 20].

Preparation and administration of test compound: The preparation of test extract *Barleria grandiflora* was done freshly prior to dosing. Aqueous extract (AQBG) ethanol extract (EABG) and hydroalcoholic extract (HAEBG), of *Barleria grandiflora* leaves were prepared. The animals were dosed approximately at the same time each day. The dosage volume administered to individual rat was adjusted according to its recently recorded body weight. All the animals were treated with test drugs (leaves extracts of *Barleria grandiflora*) and standard drug, (Silymarin) for 7 days. After this, the food supply was withdrawn and drinking water was provided ad libitum. All the animals were observed twice daily (morning and evening) for morbidity and mortality, throughout the acclimatization and period of study. After test item administration, individual animals were observed for abnormal clinical signs, due to treatment, throughout the study period. The clinical observations included changes in skin and fur, in the eyes and mucosal membrane in the respiratory, circulatory, central nervous and autonomous system and behaviour. Clinical signs were graded as follows 0 = No clinical signs, + = Mild, ++= Moderate, +++= Marked, ++++= Severe.

Body weight: Body weight was recorded on day 0 (prior to dosing) and day 8. Change in body weight (%) was calculated on day 8 based on previous body weight.

Biochemical Analysis: Using rat capillaries, blood was drawn from retro-orbital plexus and was transferred to the in heparinised eppendorf tubes. It was then centrifuged at 3000 RPM for 10 minutes to separate plasma, which was collected using clean pipette. After collection, plasma samples were subjected to biochemical analysis. Biochemical analysis was performed on blood of animals fasted overnight. The major reason for this is the increased variability that would inevitably result from feeding and would mask more complicated effects and make interpretation difficult.[21]

The biochemical parameters from the samples of blood plasma were evaluated using automated analyzer (ERBA Diagnostic Mannheim GmbH; Model: CHEM-7). The parameters Amino transaminases, which

included, Aspartate Aminotransaminase (AST) and Alanine Aminotransaminase (ALT) were analyzed.[22, 23]

Liver weight: Liver weight of all group animals was recorded after dissection. Liver weights of all groups animal were calculated. The absolute weight of liver of each rat was recorded after the terminal sacrifice. Liver tissues of all animals were collected in normal saline before weighing. Fat was trimmed and the weighing of wet tissue was done using electronic weighing balance.

Histopathology Evaluations: Histopathology of liver tissues of all animals was performed to evaluate the alteration incurred due to treatment of CCl4 and reversal effect of this by various extracts of *Barleria grandiflora* as well as by a known hepatoprotective compound silymarin, at microscopic level.

RESULT AND DISCUSSION

Hepatoprotective effect of extracts of the *Barleria grandiflora* leaves: The results of body weight, biochemical analysis, and organ weight, were presented as the mean ± SD of four rats per group. Graph Pad Prism 8.01 software was used for Statistical Analysis. Descriptive statistics and comparisons between groups were analyzed using one way analysis of variance (one way ANOVA).

Clinical signs and mortality: Administration of CCl4 and different extracts of *Barleria grandiflora* showed no mortality or morbidity in the animals during the period of study. Cage side observations did not show any observable clinical signs related to the compound toxicity. No tremors, convulsions, salivation, diarrhoea, lethargy, or unusual behaviours were observed in extract treated animals throughout the study period.

Effect of extracts of the *Barleria grandiflora* leaves on body weight: The body weights of the animals at day 0 and day 8 of the experiment were recorded. Treatment of CCl4 caused significant reduction ($P<0.01$) in body weight. The initial (day 0) to day 8 body weight of animals (Mean ±SD) from various experimental groups of animal were recorded. The gain in body weight has been calculated. The graphs of change in body weight were plotted. The weight gain in healthy control rats has been 32.89±0.64 g of the initial body weight. In the rats treated with the carbon tetrachloride the weight gain was lesser than all groups. Treatment with *Barleria grandiflora* extracts apparently took the animals towards normalcy since the weight gain in animals that of control animals. In the animals that received silymarin a hepatoprotective compound, resulted in a weight gain that was greater than control as well as disease control. In case of test samples the maximum weight gain was found in the rats of group V that received 200 mg/kg of the hydro alcoholic extract of *Barleria grandiflora*. There was no statistical significant difference between the weight gains of the groups of animals that received the standard for 8 days of the control.

Biochemical Analysis: Effect of extracts of *Barleria grandiflora* leaves on the activity levels of AST in blood plasma. The levels of AST activity in the blood plasma of animals in different experimental groups were performed. In the animals from normal control group, the level of AST was 101.41±3.53IU/L while that of the disease control was 205.16±7.03 IU/L, which was significant increase of the value of normal control. In animals that received the dose of 200 mg/kg hydroalcoholic extract of *Barleria grandiflora* leaves, the levels of AST was found to be 131.08±8.20IU/L, while it was found 140.60±2.85IU/L, when received at dose of 100 mg/kg. All extract of *Barleria grandiflora* leaves control AST level in dose dependent manner. Hydroalcoholic extract showed maximum control than ethanol and aqueous extract respectively.

Effect of extracts of *Barleria grandiflora* leaves on the activity level of ALT in blood plasma: The result of activity levels of ALT in blood plasma in animals of various experimental groups is displayed in Table 1 and is also depicted in Figure 3. The level of activity of ALT in normal control group was 59.97±2.71 IU/L and the level of ALT in disease control was 120.44±6.76 IU/L, which is 2 times more from the value of normal control group. The ALT level in the groups of standard drug treated was 68.72±8.65. In the test samples, ALT level in groups which received the dose of 200 mg/kg of the hydroalcoholic extract of *Barleria grandiflora* leaves showed maximum recovery, with the ALT activity 75.69±4.24. The ethanol extract was found just as effective as the hydroalcoholic extract in dose of 200 mg/kg but the aqueous extract was a little less effective.

Effect of extracts of *Barleria grandiflora* leaves on the level of ALP in blood plasma: The level of ALP activity in the control group was found to be 234.66±28.10 IU/L. Animals of disease control group, the level of ALP was much higher, recorded as 404.98±9.26 IU/L. The difference in the normal control and disease groups was quite significant. The hydroalcoholic extracts of *Barleria grandiflora* leaves showed reduced levels of ALP activity, 314.74±10.98 (100 mg/kg) and 299.82±09.28 (200 mg/kg). Ethanol extract of *Barleria grandiflora* leaves also found effective in control the level of ALP of that in the animals from the group of normal control. Administration of standard (Silymarin) at a dose of 10mg/kg , the level

of ALP activity was found to be 276.14±11.58 IU/L. The effect of ethanol extract of *Barleria grandiflora* leaves is also close to hydroalcoholic extract. Aqueous extract of *Barleria grandiflora* leaves control the ALP level less effectively than others.

Effect of extracts of the *Barleria grandiflora* leaves on Bilirubin concentration in blood plasma: The results of the analysis of bilirubin content in the blood plasma of the various groups of experimental animals were observed. In the normal control animals the concentration of bilirubin was 0.33±0.018 mg/dL and in the disease induced group was 0.68±0.040 mg/dL. Animals that received hydroalcoholic extract of *Barleria grandiflora* leaves at the dose of 200 mg/kg, the concentration of bilirubin was 0.42±0.012. which showed maximum level of recovery. The aqueous extract at 200 mg/kg showed the least recovery, the bilirubin content of blood plasma being 0.51±0.019. Standard treated animal showed bilirubin content of 0.40±0.015 mg/dL. This too suggests that the ethanol extract of *Barleria grandiflora* leaves at the dose of 200 mg/kg are also able to relieve the symptoms of hepatotoxicity.

Effect of extracts of *Barleria grandiflora* leaves on absolute weight of Liver: The absolute weights of animals in various experimental groups were noted. The absolute weight of liver in the animals of control group was 7.963±0.23 g and of the disease group absolute weight of the liver was 8.831±0.52g. Group that received hydroalcoholic extract of *Barleria grandiflora* leaves a dose of 200 mg/kg liver weight was recorded as 8.219±0.09 g. While the absolute weight of liver was found in group that received ethanolic extract 200 mg/kg was 8.311±. The aqueous extract at 200 mg/kg showed the least recovery, the liver weight being 8.489±0.14g. In group received ethanol extract of *Barleria grandiflora* leaves the absolute weight of liver remained less than or close to the absolute weight of liver in the groups received aqueous extracts.

Liver weight: Treatment of CCl4 caused increased in liver weight comparatively to control group. The liver weight of treated group with extract was restored with treatment of leaves extracts of *Barleria grandiflora*. Hydroalcoholic extract was significantly ($P<0.01$) restored the liver weight of treated group.

Histopathology: Group I histological sections revealed normal histology. The central vein and hepatocytes surrounding it were observed. Disease control group rats showed minimal focal perivascular leukocytic infiltration, focal mild hepatocellular necrosis, hepatocytes with pyknotic nuclei, condensed cytoplasm and severe cytoplasmic vacuolation over a large area. Group III was treated with standard did not reveal any lesion of pathological significance. Group received a dose of 100 mg/kg of ethanol extracts of *Barleria grandiflora* leaves showed minimal focal mild cytoplasmic vacuolation. Group received aqueous extract of *Barleria grandiflora* leaves at a dose of 100 and 200mg/kg respectively showed multifocal cytoplasmic vacuolation and minimal focal hepatocellular necrosis. Group that received a dose of 200 mg/kg of hydroalcoholic extracts of *Barleria grandiflora* leaves revealed normal histology. Group III was administered with hepatoprotective compound silymarin did not reveal any lesion of pathological significance and displayed normal histology. Treatment of animals with hydroalcoholic extract of *Barleria grandiflora* leaves provide more protection than other extracts against CCl4 induced hepatotoxicity.

Table 1: Effect of extracts of the *Barleria grandiflora* leaves on levels of AST, ALT, ALP and bilirubin in blood plasma of animals.

Group	Treatment	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	BIL(mg/dL)
1	Normal control	101.41±3.53	59.97±2.71	234.66±28.10	0.33±0.018
2	Disease control(CCL4)	205.16±7.03	120.44±6.76	404.98±9.26	0.68±0.040
3	Standard (Silymarin)	115.93±10.75	68.72±8.65	276.14±11.58	0.40±0.015
4	HAEBG 100 mg/kg	140.60±2.85	92.34±4.39	314.74±10.98	0.47±0.009
5	HAEBG 200 mg/kg	131.08±8.20	86.34±8.19	299.82±09.28	0.42±0.012
6	EABG 100 mg/kg	155.44±5.86	84.34±7.35	332.47±10.76	0.49±0.017
7	EABG 200 mg/kg	143.08±10.65	80.69±4.24	318.69±11.69	0.46±0.010
8	AQBG 100 mg/kg	171.96±09.23	99.34±5.27	358.71±12.85	0.53±0.014
9	AQBG 200 mg/kg	163.96±13.78	83.96±7.78	346.48±11.83	0.51±0.019

Table 2: Effect of extracts of the *Barleria grandiflora* leaves on absolute weight of liver.

Group	Treatment	Liver weight(g)
1	Normal control	7.963±0.23
2	Disease control	8.831±0.52
3	Standard silymarin	8.049±0.15
4	HAEBG 100 mg/kg	8.339±0.04
5	HAEBG 200 mg/kg	8.219±0.09
6	EABG 100 mg/kg	8.419±0.31
7	EABG 200 mg/kg	8.311±0.19
8	AQBG 100 mg/kg	8.559±0.11
9	AQBG 200 mg/kg	8.489±0.14

Note: Values are mean ± SD. Statistical significance at 95% confidence interval (P ≤ 0.05)

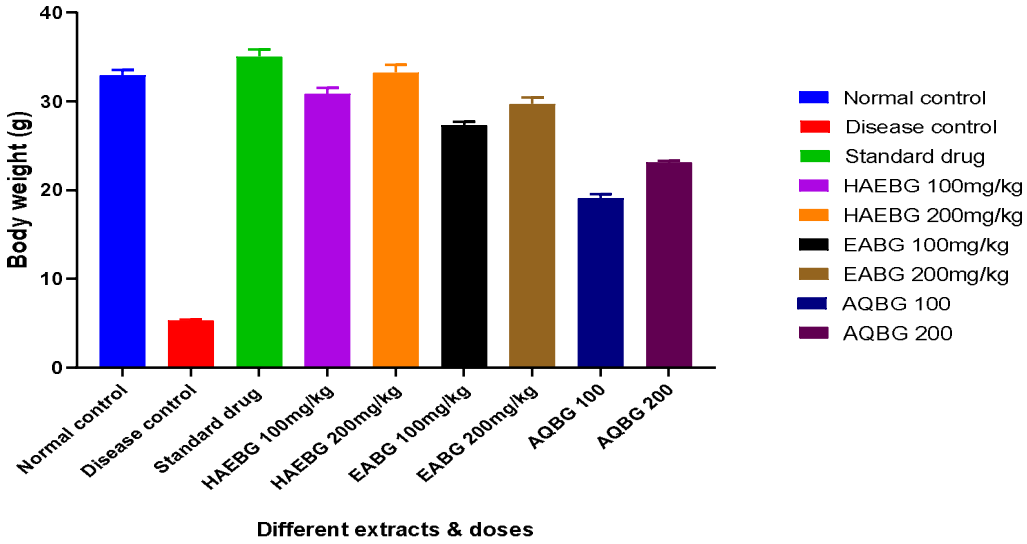


Figure 1: Effect of extracts of the *Barleria grandiflora* leaves on body weight.

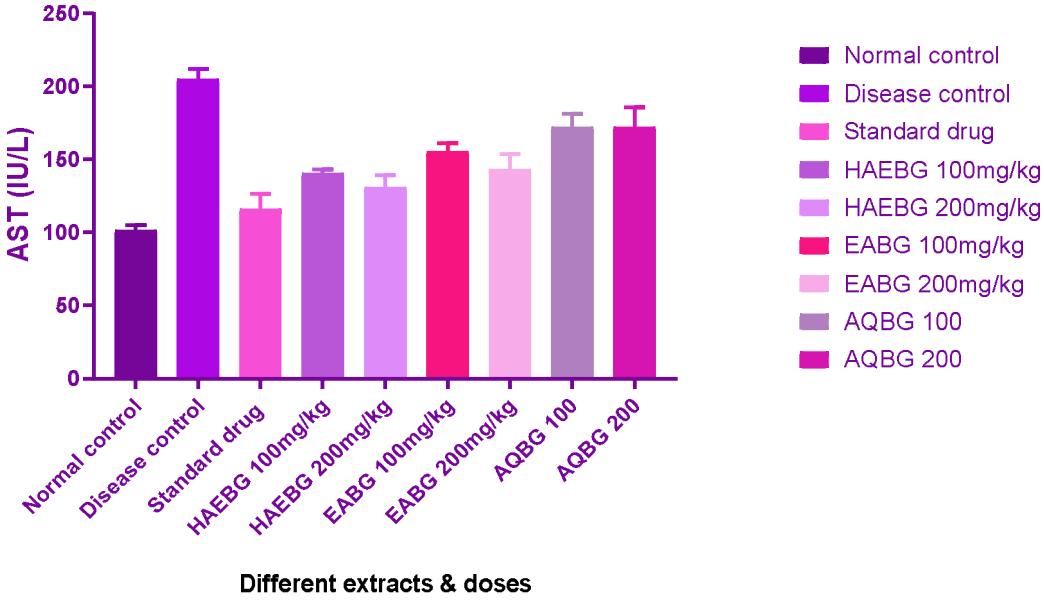


Figure 2: Effect of extracts of the *Barleria grandiflora* leaves on levels of AST in blood plasma of animals.

Note: Values are mean of 6 replicates ± SD.

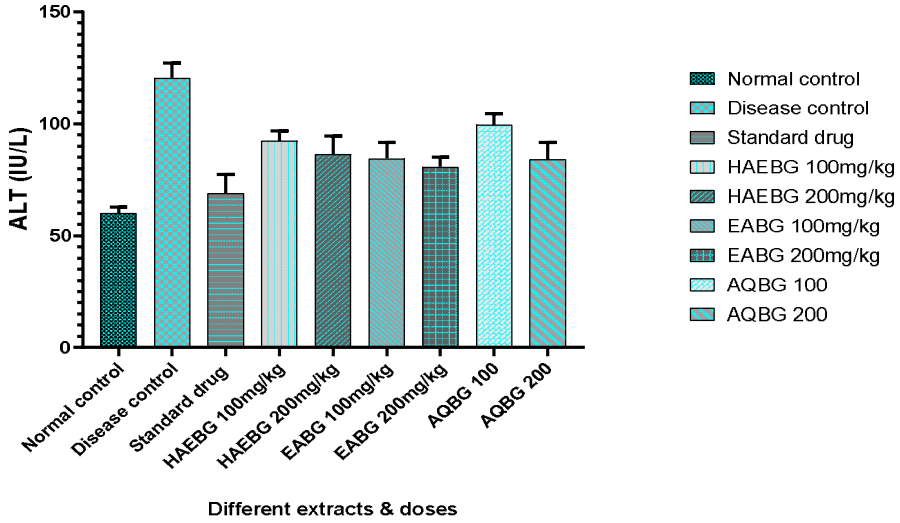


Figure 3: Effect of extracts of *Barleria grandiflora* leaves on the level of ALT in blood plasma of animals.

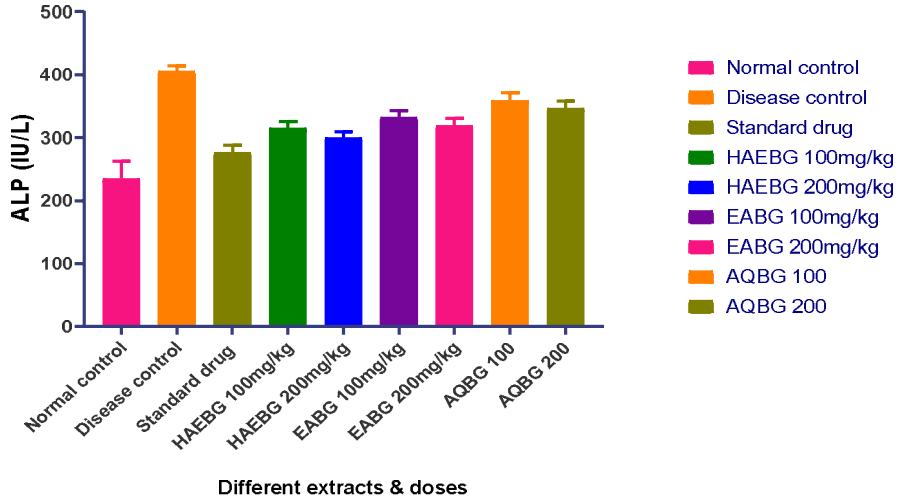


Figure 4: ALP levels in the blood plasma of animals treated with extracts of *Barleria grandiflora* leaves.

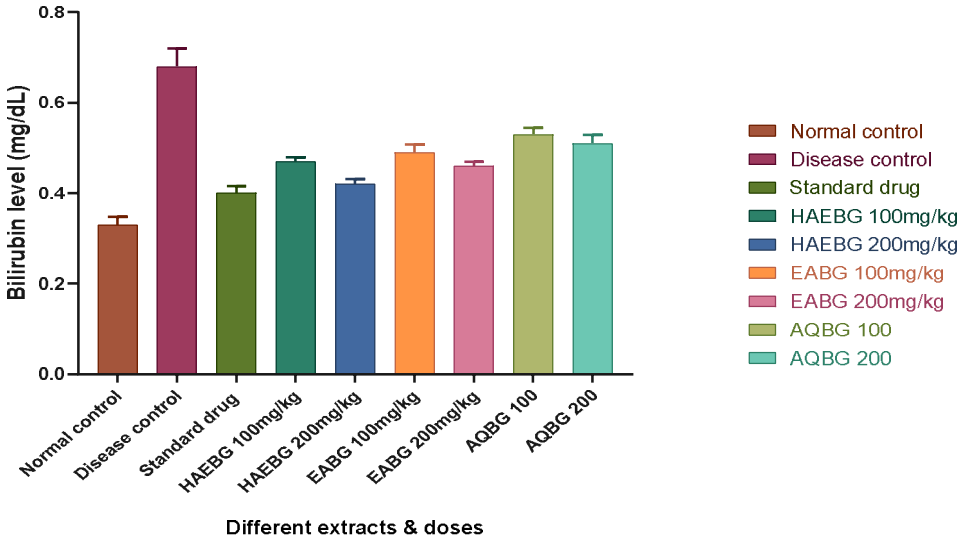


Figure 5: Bilirubin level of animals treated with extracts of *Barleria grandiflora* leaves.

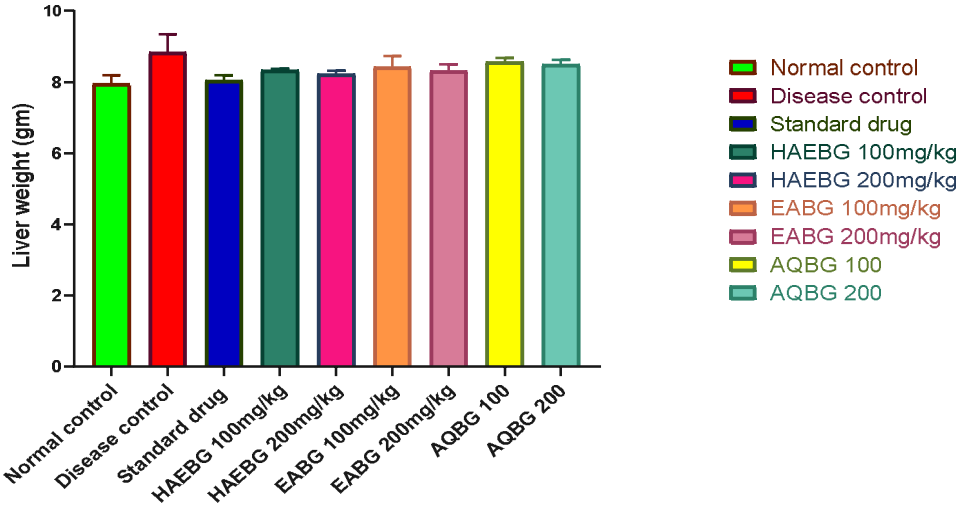
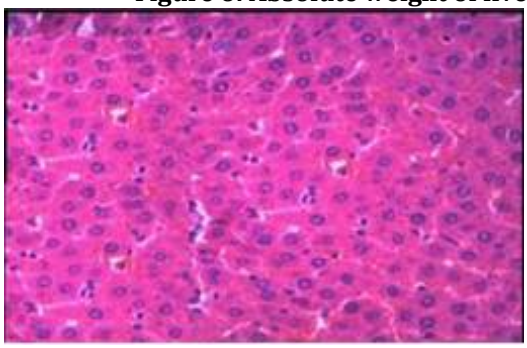
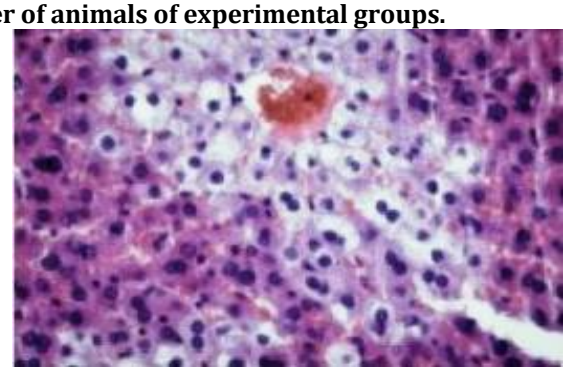


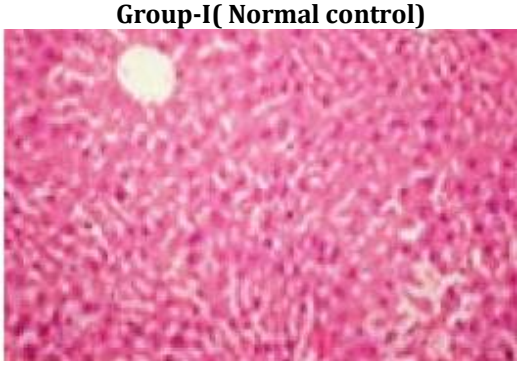
Figure 6: Absolute weight of liver of animals of experimental groups.



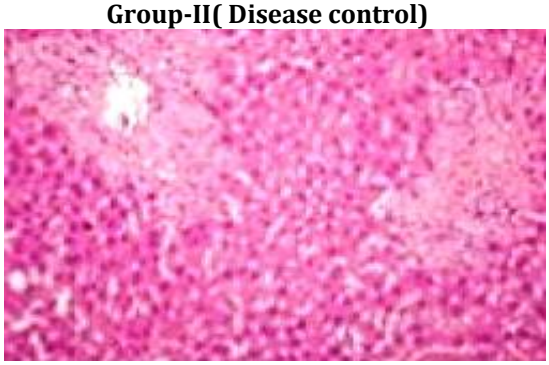
Group-I(Normal control)



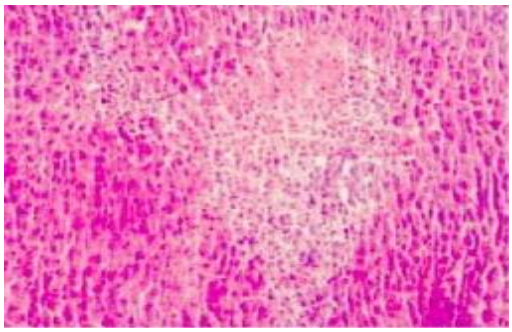
Group-II(Disease control)



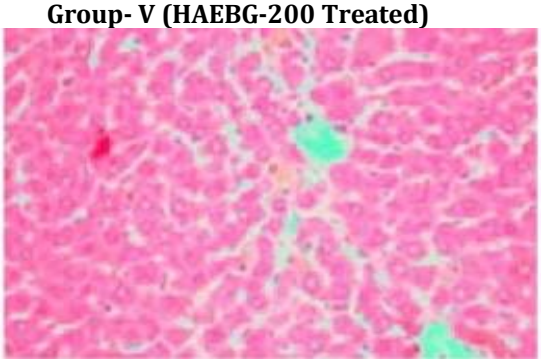
Group- III(Standard Treated)



Group- V (HAEBG-200 Treated)



Group- VII (EABG-200 Treated)



Group- IX (AQBG-200 Treated)

Figure7. Histopathology of liver of the animals treated with different extracts of *Barleria grandiflora* leaves.

REFERENCES

1. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5), 559.
2. Süntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*, 19(5), 1199-1209.
3. Pal, S. K., & Shukla, Y. (2003). Herbal medicine: current status and the future. *Asian pacific journal of cancer prevention*, 4(4), 281-288.
4. Baker, D. D., Chu, M., Oza, U., & Rajgarhia, V. (2007). The value of natural products to future pharmaceutical discovery. *Natural product reports*, 24(6), 1225-1244.
5. Nikam, P. H., Kareparamban, J., Jadhav, A., & Kadam, V. (2012). Future trends in standardization of herbal drugs. *Journal of applied pharmaceutical science*, (Issue), 38-44.
6. Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life sciences*, 78(5), 431-441.
7. Plumb, K. (2005). Continuous processing in the pharmaceutical industry: changing the mind set. *Chemical Engineering Research and Design*, 83(6), 730-738.
8. Dhiman, R. K., & Chawla, Y. K. (2005). Herbal medicines for liver diseases. Digestive diseases and sciences, 50(10), 1807.
9. Stickel, F., & Schuppan, D. (2007). Herbal medicine in the treatment of liver diseases. Digestive and liver disease, 39(4), 293-304.
10. Rustgi, V. K., Davis, G. L., Herrine, S. K., McCullough, A. J., Friedman, S. L., & Gores, G. J. (2008). Future trends in hepatology: challenges and opportunities. *Hepatology*, 48(2), 655-661.
11. Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. (2019). Burden of liver diseases in the world. *Journal of hepatology*, 70(1), 151-171.
12. Clark, J. M., Brancati, F. L., & Diehl, A. M. (2002). Nonalcoholic fatty liver disease. *Gastroenterology*, 122(6), 1649-1657.
13. Watkins, P. B., & Seeff, L. B. (2006). Drug-induced liver injury: summary of a single topic clinical research conference. *Hepatology*, 43(3), 618-631.
14. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, 10(5), 210-229.
15. Katoonizadeh, A., Laleman, W., Verslype, C., Wilmer, A., Maleux, G., Roskams, T., & Nevens, F. (2010). Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. *Gut*, 59(11), 1561-1569.
16. Fahmi, A., Hassanen, N., Abdur-Rahman, M., & Shams-Eldin, E. (2019). Phytochemicals, antioxidant activity and hepatoprotective effect of ginger (Zingiber officinale) on diethylnitrosamine toxicity in rats. *Biomarkers*, 24(5), 436-447.
17. Jalalpure, S. S., Patil, M. B., Prakash, N. S., Hemalata, K., & Manvi, F. V. (2003). Hepatoprotective activity of the fruits of Piper longum Linn. *Indian journal of pharmaceutical sciences*, 65(4), 363.
18. Mahli, A., Koch, A., Czech, B., Peterburs, P., Lechner, A., Haunschild, J. & Hellerbrand, C. (2015). Hepatoprotective effect of oral application of a silymarin extract in carbon tetrachloride-induced hepatotoxicity in rats. *Clinical Phytoscience*, 1(1), 1-8.
19. Singh, B., Chandan, B. K., Prabhakar, A., Taneja, S. C., Singh, J., & Qazi, G. N. (2005). Chemistry and hepatoprotective activity of an active fraction from Barleria prionitis Linn. in experimental animals. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 19(5), 391-404.
20. Khan, R. A., Khan, M. R., & Sahreen, S. (2012). CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC complementary and alternative medicine*, 12(1), 1-6.
21. Mukherjee, B. (2022). Pharmacokinetic Sample Collection and Processing. In *Pharmacokinetics: Basics to Applications* (pp. 175-184). Singapore: Springer Singapore.
22. Wolf, P. L. (1999). Biochemical diagnosis of liver disease. *Indian Journal of Clinical Biochemistry*, 14, 59-90.
23. Ye, X., Feng, Y., Tong, Y., Ng, K. M., Tsao, S., Lau, G. K., & Kobayashi, S. (2009). Hepatoprotective effects of Coptidis rhizoma aqueous extract on carbon tetrachloride-induced acute liver hepatotoxicity in rats. *Journal of Ethnopharmacology*, 124(1), 130-136.