PHARMACOLOGICAL EVALUATION OF COMMERCIALLY AVAILABLE POLYHERBAL FORMULATIONS FOR HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES

Rajendra O. Ganjiwale[a]*, Pramod G. Yeole[b] and Dilesh J. Singhavi[c]

(Received 08 December 2023) (Accepted 10 March 2024)

ABSTRACT

Polyherbal formulations, are frequently used to treat liver dysfunction, preserve the liver, and regenerate the liver. This research was carried out to determine whether or not polyherbal formulations could protect the livers of mice exposed to carbon tetrachloride -induced hepatotoxicity. 13 groups (n = 6) were generated at random from 78 male albino rats: Group I (serving as the normal control), Group II (containing only carbon tetrachloride), Group III (combining silymarin at a dosage of 100 mg kg^{-1} with carbon tetrachloride), and Groups IV–XIII (combining carbon tetrachloride with different brands of commercially available formulations). Serum levels of hepatic enzymes were analyzed to determine hepatic biochemistry and the extent of liver damage. Antioxidant activity analysis and histopathological analysis of the formulations were also conducted. The commercial formulations significantly ($P < 0.01$) lowered high liver biochemical markers, as validated by histological findings. Likewise, these formulations also displayed a strong antioxidant potential.

Keywords: Polyherbal formulations (PHF), hepatoprotective, scavenging, antioxidant, histopathological

INTRODUCTION

The liver is an organ accountable for body metabolic processes, playing a vital role in various essential functions. Hepatic problems are a result of the liver's exposure to a wide range of environmental toxins, including drugs. Liver damage can result in hepatic failure, ultimately leading to death[1]. Current medical treatments are insufficient and inefficient in addressing liver diseases, which is a global concern[2]. Effective measures for managing liver diseases are lacking. Recently, there has been increased interest worldwide in natural drugs obtained from plant and herbal sources, because of their better tolerance by humans and the minimal side effects associated with them. According to Indian medical traditions, herbal medications used to treat such conditions are both efficient and safe[3]. These medications are particularly useful for treating chronic illnesses that call for long-term treatment. To optimize the advantages of their combined strength, plant-based medications are frequently used in combination[4].

Antioxidant and hepatoprotective activities are just two of the many benefits associated with using plant-based natural compounds, including flavonoids, terpenoids, and sterols[5]. Numerous polyherbal formulations (PHFs) are commercially available that claim to have hepatoprotective activity. These formulations contain various plant extracts that are reported to have hepatoprotective and antioxidant activities. Therefore, these formulations may show positive hepatoprotective effect when coupled with allopathic drugs, either synergistically or additively. They hold promise as potential alternative therapies for treating a wide range of liver ailments in humans and other animals.

A wide range of ayurvedic formulations is available in the market. However, there is little scientific research on these ayurvedic formulations' hepatoprotective effects. Hence, this study examined the hepatoprotective and antioxidant properties of six marketed PHFs.

[a] Dept. of Pharmacognosy, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha - 442 001, Maharashtra, India
[b] Dr. Babasaheb Ambedkar Marathwada University, Aurangabad – 431 004, Maharashtra, India
[c] Dept. of Pharmaceutics, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha - 442 001, Maharashtra, India
*For Correspondence: E-mail: ro_ganjiwale@rediffmail.com

https://doi.org/10.53879/id.61.04.14460

MATERIALS AND METHODS

Materials

The commercially available formulations, comprising Brands: A (capsule), B (capsule), C (capsule), D (tablet), E (tablet), F (tablet), H (syrup), G (syrup), I (syrup), and J (syrup), were obtained from local drugstores. The diagnostic kits utilized for the assessment of biochemical parameters, including SGOT, ALP, SGPT, cholesterol, total protein, and bilirubin, were procured from Merck Limited, Mumbai. The samples of silymarin and carbon tetrachloride (CCl_4) were obtained from Loba Chemie Private Limited, Mumbai and Sigma Aldrich, Bangalore, India. All reagents and chemicals employed were of high purity for analytical purposes.

Experimental animals

The hepatoprotective investigation was carried out on albino Wistar rats, weight ranging between 150 and 180 g, after approval from the Institute of Pharmaceutical Education and Research, Wardha Institutional Animal Ethical Committee (Registration No. 535/02/a/CPSCA). The rats were not selected based on their sex. Standard animal housing for rats included a 12 h light/dark cycle and 25 °C to 27 °C temperature control. The animals were fed a regular food in clean, spacious polypropylene cages with constant water access. After 10 days of lab acclimatization, the animals were employed for research. The Institutional Animal Ethical Committee permitted the experimental protocols, and the study followed CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Assessment of hepatoprotective activity

Following groups of rats were made to evaluate the hepatoprotective activity:

1) Normal group: received normal saline for 7 days
2) Control group: given normal saline during first 6 days and CCl_4 on the seventh day
3) Standard group: received silymarin for 6 days and CCl_4 on the seventh day
4) Test group (PHF-treated group): received hepatoprotective PHFs for 6 days and carbon tetrachloride on the seventh day[6].

Observational screening and acute toxicity

The acute toxicities of the PHFs were determined according to OECD guideline No. 423, which uses the Acute Toxic Class Method. The potential for acute toxicity in rats was evaluated by administering dosages of 50, 100, 300, 1000, and 2000 mg kg^{-1} of body weight to groups of 3 rats each. The animals were continuously examined for 1 h, and then every hour for the next 4 h to monitor any gross behavioral changes, such as writhing, convulsions, gnawing, and pilorection and changes in general motor activity, pupil size, fecal output and feeding behavior. The response to tail pinching was also observed. The observations were extended up to 72 h to assess any occurrences of mortality.

Acute toxicity tests established a maximum non-lethal dosage of 2000 mg kg^{-1} body weight. Therefore, the effective dose was determined to be 200 mg kg^{-1} body weight, which is one tenth of this number[7,8].

Measurement of biochemical parameters

Measurements were made of biochemical parameters such as total protein, cholesterol, bilirubin, SGPT, SGOT, and ALP.

The following groups, each containing six rats of the same sex, were created.

Group I: Regular; got saline solution and standard rat meal.

Group II: Control, CCl_4-intoxicated (0.7 mL kg^{-1} body weight administered by intraperitoneal injection).

Group III: Administered 100 mg kg^{-1} of body weight of silymarin orally.

PHF Brand A was in Group IV. PHF Brand B was in Group V. PHF Brand C was in Group VI. PHF Brand D was in Group VII. PHF Brand E was in Group VIII. PHF Brand F was in Group IX. PHF Brand G was in Group X. PHF Brand H was in Group XI. PHF Brand I was in Group XII. PHF Brand J was in Group XIII.

Experimental protocol

As mentioned earlier, 13 groups, each consisting of 6 animals, were made.

Group I (Normal control): For 7 days, animals took normal saline orally as a treatment.

Group II (Toxic control): Following 6 days of infusion with normal saline, the animals were administered a single dose of CCl_4 by intraperitoneal injections on the 7th day.

Table I: Effect of polyherbal formulations on serum biochemical levels in rats

Group	SERUM LEVEL					
	SGOT IU dl^{-1}	SGPT IU dl^{-1}	ALP IU dl^{-1}	Total protein g dl^{-1}	Total cholesterol mg dl^{-1}	Total bilirubin mg dl^{-1}
I	165.2±29.46	109.0±40.47	116.0±16.4	4.907±1.916	212.2±77.80	1.558±1.717
II	335.9±85.15**	253.6±58.95**	259.3±32.37**	3.25 ±1.250**	260.0 ±21.49**	6.402±2.336**
III	156.3±36.82**	73.68±16.72**	120.8±28.06**	7.068 ±2.212**	198.9±75.96**	2.108±0.5181**
IV	184.9±46.73**	98.30±66.40**	143.2±32.99**	6.210±1.723**	222.6±51.06**	3.843±1.016**
V	191.7±57.25**	111.1±51.69**	150.8±61.94**	5.868±1.054**	231.5±71.24**	3.160±1.269**
VI	163.0±47.01**	88.55±37.84**	127.8±29.59**	6.803±2.459**	209.0±22.30**	2.447±1.118**
VII	170.7±40.92**	106.5±55.52**	163.3±75.94**	6.480±2.092**	237.8±32.47**	3.097±1.662**
VIII	210.9±84.9**	144.4±50.21**	164.0±62.31**	6.580±2.792**	224.7±17.72**	3.862±0.5322**
IX	209.7±65.41**	129.7±49.45**	141.4±54.31**	6.672±1.944**	237.2±68.05**	3.592±1.185**
X	218.2±56.48**	142.9±54.62**	140.3±56.93**	6.155±0.7119**	219.7±35.73**	3.802±1.272**
XI	180.2±48.34**	150.1±44.47**	140.5±43.43**	6.743±2.193**	239.6±31.72**	3.342±0.9793**
XII	221.3±44.92**	134.4±39.59**	156.3±38.85**	4 ± 1.408**	233.7±29.77**	3.388±1.408**
XIII	210.3±68.73**	123.9±47.75**	137.1±49.00**	5.337 ±2.445**	217.3±58.56**	4.277±1.021**

- *Values are given as mean ± S.D. of the six rats in each group.*
- *Control was compared with the normal, $p < 0.01$*.*
- *Experimental groups were compared with the control, $p < 0.01$**.*

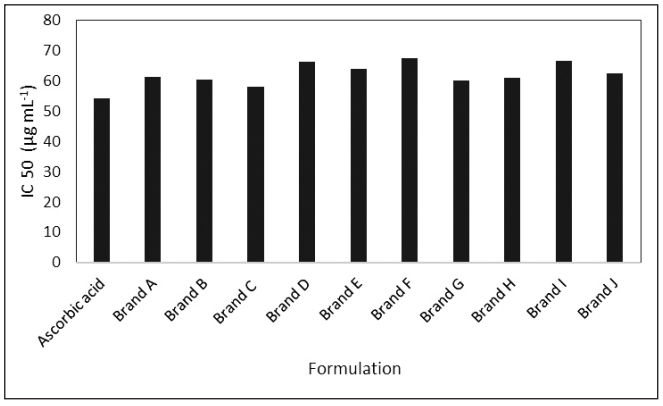

Fig. 1: Medium inhibitory concentrations of formulations for antioxidant activity assay by DPPH scavenging method

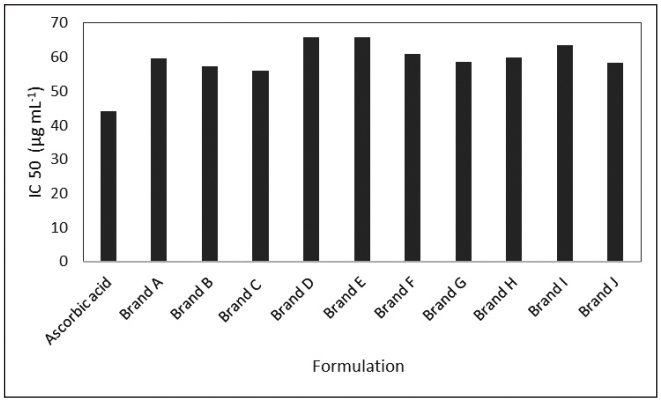

Fig. 2: Medium inhibitory concentrations of formulations for antioxidant activity assay by nitric oxide scavenging method

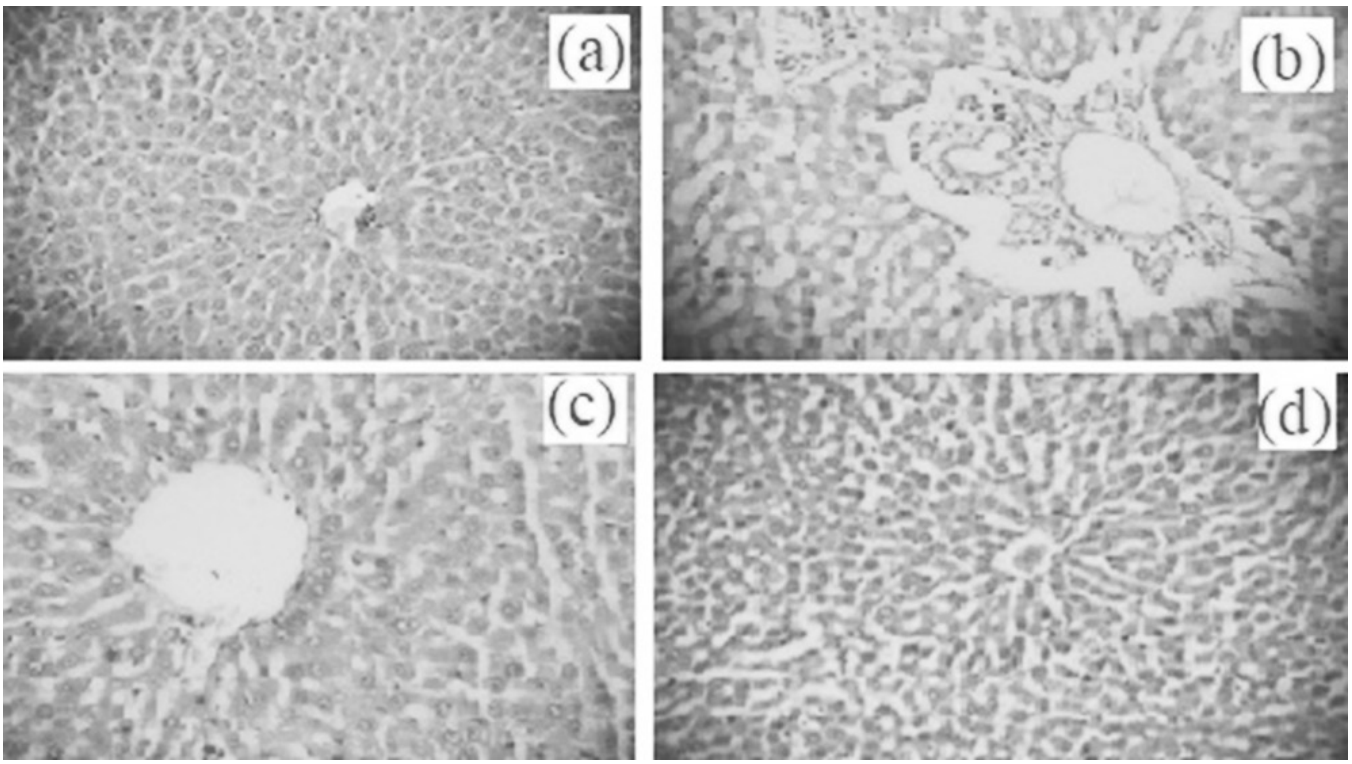

Fig. 3: Photomicrograph showing (a) normal hepatocytes arranged in cords around the central vein in a normal rat (H & E, ×4), (b) disarranged and edematous hepatic erosion with edema and inflammatory infiltrate around the central vein in CCl_4-treated rats (H & E, ×10), (c) reduced edema and infiltrate with comparative restoration of normal liver structure in formulation (Brand C, H & E, ×10), and (d) normal restoration of liver architecture in silymarin-treated rats (H & E ×10)

The dosage was diluted in olive oil (1:1) at a dosage of 1.5 mL kg^{-1} of body weight.

Group III (Standard drug control): The animals were given silymarin at a dosage of 25 mg kg^{-1} for six days. Then, on the 7th day, they were given an intraperitoneal injection of CCl_4 at a dose of 1.5 mL kg^{-1} body weight in a 1:1 ratio with olive oil.

Group IV (Formulation-treated group): Animals received PHF Brand A (200 mg kg^{-1} body weight) orally. The formulation was administered orally once daily for 6 days in a 1 % V/V Tween® 80 aqueous solution. On the 7th day, an intraperitoneal injection of 1.5 mL kg^{-1} body weight of CCl_4 in a 1:1 ratio with olive oil was administered to the animals.

Group V (Formulation-treated group): Animals received PHF Brand B (200 mg kg^{-1} body weight), orally. A 1 % V/V Tween® 80 aqueous solution was utilized to disseminate the formulation once daily for 6 days. The rats were injected intraperitoneally with CCl_4 (1.5 % by weight of body weight in olive oil, 1:1 ratio) on day 7.

Group VI (Formulation-treated group): Every animal was given an oral dose of PHF Brand C (200 mg kg^{-1} body weight). Every day for 6 days, the formulation was diluted in a 1 % V/V Tween® 80 aqueous solution. On the seventh day, the animals were administered an intraperitoneal injection of CCl_4 in a 1:1 ratio with olive oil, at a dosage of 1.5 mL kg^{-1} body weight.

Group VII (Formulation-treated group): Animals received PHF Brand D (200 mg kg^{-1} body weight), orally. For six days, the formulation was administered once day in a 1 % V/V Tween® 80 aqueous solution. On the seventh day, the animals were administered an intraperitoneal injection of 1.5 mL kg^{-1} body weight of CCl_4 in a 1:1 ratio with olive oil.

Group VIII: Formulation-treated group got oral PHF Brand E (200 mg kg^{-1} body weight). Daily administration of the formulation in 1 % V/V Tween® 80 aqueous solution was done for 6 days. On day seven, mice received a single intraperitoneal injection of CCl_4 (1.5 mL kg^{-1} body weight in olive oil, 1:1 ratio).

Group IX: Formulation-treated group received PHF Brand F (200 mg kg^{-1} body weight), orally. The formulation was administered daily in 1 % V/V Tween® 80 aqueous solution for six days. On the seventh day, the animals got a single dose of CCl_4 (Administered 1.5 mL kg^{-1} of body weight using a 1:1 proportion of olive oil) through intraperitoneal injections.

Group X: Formulation-treated group – received PHF Brand G (2 ml kg^{-1} body weight), orally. The formulation was administered daily in 1 % V/V Tween® 80 aqueous solution for six days. On the seventh day, the animals got a single dose of CCl_4 (Administered 1.5 mL kg^{-1} of body weight using a 1:1 proportion of olive oil) through intraperitoneal injections.

Group XI: Formulation-treated group – received PHF Brand H (2 mL kg^{-1} body weight), orally. The formulation was administered daily in 1 % V/V Tween® 80 aqueous solution for 6 days. On the 7^{th} day, the animals got a single dose of CCl_4 (Administered 1.5 mL kg^{-1} of body weight using a 1:1 proportion of olive oil) through intraperitoneal injections.

Group XII: Formulation-treated group – received PHF Brand I (2 mg kg^{-1} body weight), orally. The formulation was administered daily in 1 % V/V Tween® 80 aqueous solution for six days. On the 7th day, the animals got a single dose of CCl_4 (Administered 1.5 mL kg^{-1} of body weight using a 1:1 proportion of olive oil) through intraperitoneal injections.

Group XIII: Formulation-treated group – received PHF Brand J (2 mL kg^{-1} body weight) orally. The formulation was administered daily in 1 % V/V Tween® 80 aqueous solution for six days. On the 7^{th} day, the animals got a single dose of CCl_4 (Administered 1.5 mL kg^{-1} of body weight using a 1:1 proportion of olive oil) through intraperitoneal injections.

Assessment of liver function

Unconsciousness was induced in rodents in all groups with ether. The retro-orbital plexus was punctured to obtain blood samples from all rodent groups. The samples coagulated after 45 minutes at room temperature. The serum was spun at 2500 rpm and 30°C for 15 minutes in a Remi centrifuge. SGPT, ALP, SGOT, LDH, direct bilirubin (DB), and total bilirubin (TB) were instantly measured using a microplate reader (Power wave XS) and colorimetric methods[9].

DPPH radical scavenging

By employing the stable radical DPPH, the radical-scavenging or hydrogen-donating capabilities of different formulations and with ascorbic acid as standard were evaluated in terms of their ability to scavenge free radicals.

A methanol solution containing 0.1 mM DPPH was formulated, and 1 mL of this solution was mixed with 3 mL of each formulation's solution (varying concentrations between 10 and 100 g mL^{-1}). At a wavelength of 517 nm, the absorbance was measured after 30 minutes. The comparison standard consisted of a DPPH solution that was dissolved in methanol. Scavenging effect was determined by the following formula.

$$\text{Scavenging effect (\%)} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \qquad (1)$$

Significantly higher levels of free radical scavenging activity are indicated by a lower absorbance value. The IC_{50} value represented each formulation's antioxidant activity. The IC_{50} value is a substance concentration (in g mL^{-1}) required to neutralize 50 % of the DPPH radicals[10, 11].

Nitric oxide scavenging

Using the Griess reagent, nitric oxide was produced from sodium nitroprusside and quantified. Sodium nitroprusside creates nitric oxide on its own in a physiologically pH-balanced aqueous solution. This nitric oxide then reacts with oxygen to make nitrite ions. The Griess reagent can be used to determine the concentration of these ions. Nitric oxide-scavenging compounds compete with oxygen, resulting in a decrease in nitric oxide production.

There were several different formulation concentrations that were created in methanol, including 20, 40, 60, 80, and 100 g mL^{-1}. These solutions were individually mixed with sodium nitroprusside solution (5 mM) in phosphate buffered saline. The next step was to incubate the mixture at 25° C for 3 h. The Griess reagent, which contains 2 % orthophosphoric acid, 1 % sulphanilamide, and 0.1 % naphthyl ethylene diamine dihydrochloride, was mixed with 1.5 mL of each solution after 3 h. The resultant chromophore's absorbance was examined at the wavelength of 564 nm. Ascorbic acid was utilized as standard. Using the same reaction mixture without

the formulation extract but with an equivalent amount of ethanol, a control was established. Following formula was utilized for scavenging activity determination

$$\text{Percent inhibition} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Histopathological studies

After the rats were euthanized, their livers were carefully removed, rinsed with saline, and then preserved in a 10 % formalin solution. Sections measuring 4-6 µm in thickness were created by processing and embedding small pieces of liver tissue in paraffin wax. The next step was to use hematoxylin and eosin staining on these slices[12]. Due to legal restrictions on animal euthanization, the Institutional Animal Committee granted permission for a histopathological study of only one formulation. The formulation that was found to be most significantly effective was chosen for the histopathological study. Some of the samples of liver tissue were delivered to the Pathology Department of Jawaharlal Nehru Medical College, located at Sawangi (Meghe), Wardha for analysis.

RESULTS

Acute toxicity study

Base on acute toxicity testing, 2000 mg kg^{-1} of animal body weight was determined as formulations' maximum lethal dose. The effective dose chosen for administration in the succeeding studies was 1/10th of the highest non-lethal dose, or 200 mg kg^{-1} of body weight.

Biochemical parameters measurement

The results indicated that Group II, which was treated with CCl_4 without any pretreatment, exhibited higher levels of SGOT (335.9 ± 85.15 IU dl^{-1}), SGPT (253.6 ± 58.95 IU dl^{-1}), ALP (259.3 ± 32.37 IU dl^{-1}), and cholesterol (260 ± 21.29 mg dl^{-1}) compared with the normal control group (SGOT, 165.2 ± 29.46 IU dl^{-1}; SGPT, 109.0 ± 40.37 IU dl^{-1}; ALP, 116.0 ± 16.4 IU dl^{-1}; cholesterol, 212.2 ± 77.80 mg dl^{-1}) (Table I).

In comparison to Group II, all groups given different brand formulations showed a substantial decrease in SGOT, ALP, SGPT, and cholesterol level. The PHFs reduced these biochemical parameters less compared with silymarin, but were still comparable with the normal group.

DPPH activity

Formulation brands i.e. A, B, C, D, E, F, G, H, I, and J each had an IC_{50} value of 61.47, 60.49, 58.23, 66.31, 64.07, 67.52, 60.13, 61.06, 66.60, and 62.48 g mL^{-1}, respectively. The relative antioxidant abilities of the formulations are illustrated in Fig. 1.

All of the formulations' IC_{50} values were found to be much greater than those of ascorbic acid. Scavenging effects of the samples of the different brands followed this sequence: F > I > D > E > J > A > H > B > G > C > ascorbic acid.

Nitric oxide scavenging

Formulation brands i.e. A, B, C, D, E, F, G, H, I, and J each had an IC_{50} value of 59.74, 57.31, 56.01, 65.86, 65.91, 61.02, 58.58, 60.02, 63.49, and 58.39 g mL^{-1}, respectively. The relative antioxidant potentials of the different brands are shown in Fig. 2.

All of the formulations' IC_{50} values were found to be much greater than those of ascorbic acid. The samples' nitric oxide radical scavenging activity went in the order of: Brand E, Brand D, Brand I, Brand F, Brand H, Brand A, Brand G, Brand G, Brand J, Brand C and ascorbic acid.

Histopathological studies

The observations of the histopathological evaluations are shown in Fig. 3. The photomicrograph of the liver slice from the normal control group displayed the typical pattern of hepatocytes forming cords around the central vein. In contrast, the liver section from Group II, the control group treated with CCl_4 alone, had disrupted and edematous hepatic tissue, with edema and inflammatory infiltrates around the central vein.

In the case of Group III, treated with a PHF, there was a noticeable reduction in the edema and infiltrates, along with a comparative restoration of the normal liver structure in the liver section. Additionally, liver tissue from rats given silymarin treatment displayed a complete and normal restoration of the liver architecture.

DISCUSSION

The objective of the current investigation was to examine the liver histology and hepatic enzyme levels in rats for evaluation of the overall impact of the chosen pharmaceuticals employed to produce the experimental PHF. The liver contains a significant amount of drug-metabolizing enzymes, primarily found in the hepatic

microsomes[13]. The hepatotoxicity induced by carbon tetrachloride in rats is a suitable model for examination of the effectiveness of hepatoprotective drugs[14]. The breakdown of CCl_4 produces CCl_3 free radicals, which can damage liver cells' structure and functionality[15]. The levels of liver enzymes (ALP, SGPT, and SGOT), and cholesterol rise as a result of this disruption.

Hepatic enzymes (ALP, SGPT, and SGOT) and cholesterol levels were also significantly increased in Group II, which was treated with CCl_4. The elevated levels of liver enzymes and cholesterol were reversed after PHFs were given.

In the acute toxicity study, no signs of lethality or morbidity were observed in rats administered various doses up to 2000 mg kg^{-1}. This indicates that doses of the PHF up to 2000 mg kg^{-1} can be regarded safe, despite the fact that the median lethal dose is probably higher.

The oral administration of formulations from different brands to rats resulted in a reduction in the levels of liver enzymes. This might be due to the healing of liver cell damage induced by CCl_4 and the stabilization of plasma membranes. The results suggest that the formulations possess the capability to alleviate the biliary dysfunction induced by CCl_4 in the liver of rat. The compositions' hepatoprotective efficacy has been linked to phenolic, flavonoid, and tannin components, according to previous research.

Brand C exhibited the best hepatoprotective activity with a dosage of 200 mg kg^{-1} body weight, comparable to the gold standard drug silymarin. Other formulations also demonstrated significant hepatoprotective activity. The ability of antioxidants to donate hydrogen plays a role in scavenging free radicals, and the DPPH radical-scavenging property is essential for hepatoprotective effects. All formulations showed radical scavenging ability, as measured by the DPPH assay, which may be attributable to the presence of flavonoid, tannin and phenolic components.

In addition to its role in inflammation, nitric oxide (NO) has a part in a number of other physiological processes that are orchestrated by macrophages and endothelial cells. The formulations demonstrated effective inhibition of nitric oxide synthesis. These findings are consistent with the antioxidant and anti-inflammatory effects associated with tannins, phenolic compounds, and flavonoids.

The histopathological study revealed that the CCl_4-treated control group exhibited a loss of normal liver architecture, characterized by disarranged and edematous hepatic cords, with inflammation and edema around the central vein, accompanied by centrilobular necrosis. However, the groups treated with the test formulation and the standard drug, silymarin, showed significant recovery from CCl_4-induced liver damage. These histological findings are consistent with the results of the liver assessment tests.

CONCLUSION

In mouse model of hepatotoxicity caused by CCl_4, studying the protective effects of PHFs was the primary focus of this investigation. The investigation focused on assessing hepatic enzyme levels and liver histopathology in rats to understand the potential benefits of the formulations. The formulations' efficiency in preventing liver damage brought on by free radicals was assessed using the CCl_4-induced hepatotoxicity model.

The results clearly indicated that the administration of PHFs led to a significant reduction in the hepatic enzymes level such as ALP, SGPT, and SGOT, as well as of cholesterol. This reduction demonstrated the ability of the formulations to counteract the damaging effects of CCl_4-induced free radicals, and to stabilize the integrity of hepatic cell structures. Moreover, the formulations were found to effectively stabilize biliary dysfunction in the rat liver, which can be attributed to the presence of phenolic, flavonoid, and tannin traces, that are responsible for their hepatoprotective and antioxidant properties.

The acute toxicity assessment demonstrated the safety of the formulations up to doses of 2000 mg kg^{-1}, indicating a high median lethal dosage. Additionally, the formulations exhibited significant radical scavenging ability, likely due to their antioxidant components. Furthermore, the formulations displayed inhibition of nitric oxide synthesis, suggesting potential anti-inflammatory properties.

Histopathological examination provided visual confirmation of the positive effects of the PHFs. In contrast to the control group, the treatment groups recovered significantly from CCl_4-induced liver injury, as evidenced by an improvement in hepatic architecture. These findings aligned well with the results of the liver assessment tests, further reinforcing the conclusion about the hepatoprotective potential of the formulations.

In summary, the results of the study support the efficacy of the tested PHFs, with Brand C standing out as particularly effective, even comparable with the silymarin as standard drug. The presence of antioxidant and anti-inflammatory compounds, such as phenolic compounds, tannins, and flavonoids, likely played a significant role in the observed hepatoprotective effects. The study provides promising insights into the possible therapeutic applications of the experimental PHF in liver protection and suggests its potential for further clinical investigation.

ACKNOWLEDGEMENT

The authors are grateful Dr. Arvind Bhake, professor and head of the department at the Datta Meghe Institute of Medical Sciences in Sawangi, (Meghe), Wardha for providing facilities for histopathological studies

REFERENCES

1. Kalra A., Yetiskul E., Wehrle C.J. and Tuma F. Physiology, Liver. 2023 May 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30571059.
2. Sharma A. and Nagalli S. Chronic Liver Disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023
3. Ekor M.: The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, **Front. Pharmacol**., 2014, 4, 177.
4. Patil M.B., Mastiholimath V.S., Gadad A.P. and Dhumansure R.H.: Development and evaluation of hepatoprotective polyherbal formulation containing some indigenous medicinal plants. **Indian J. Pharm. Sci**., 2008, 70(2), 265.
5. Ali S.A., Sharief N.H. and Mohamed Y.S.: Hepatoprotective activity of some medicinal plants in Sudan. **Evid-Based Complem. Altern. Med.,** 2019, 18, 2019.
6. Abdelhafez O.H., Fawzy M.A., Fahim J.R., Desoukey S.Y., Krischke M., Mueller M.J. and Abdelmohsen U.R.: Hepatoprotective potential of *Malvaviscus arboreus* against carbon tetrachloride-induced liver injury in rats. **PLoS One**, 2018, 13(8), e0202362.
7. OECD. Test no. 420: acute oral toxicity-fixed dose procedure, OECD Guidelines for the Testing of Chemicals, OECD, Paris, 2001.
8. Sholikhah E.N., Mustofa M., Nugrahaningsih D.A., Yuliani F.., Purwono S., Sugiyono S., Widyarini S., Ngatidjan N., Jumina J., Santosa D. and Koketsu M.: Acute and subchronic oral toxicity study of polyherbal formulation containing *Allium sativum* L., *Terminalia bellirica* (Gaertn.) Roxb., *Curcuma aeruginosa* Roxb., and *Amomum compactum* Sol. ex. maton in rats, **BioMed. Res. Int**., 2020, Mar-PMID: 8609364
9. Tatiya A.U., Surana S.J., Sutar M.P. and Gamit N.H.: Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats, **Pharmacognosy Res.**, 2012, 4(1), 50.
10. Loganayaki N., Siddhuraju P. and Manian S.: Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L., **J. Food Sci. Technol.**, 2013, 50(4), 687-695.
11. Aryal S., Baniya M.K., Danekhu K., Kunwar P., Gurung R. and Koirala N.: Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal, **Plants**, 2019, 8(4), 96.
12. Deng Y., Yang Y., Ma Y., Fan K., Yang W. and Yin G.: Nano-hydroxyapatite reinforced polyphenylene sulfide biocomposite with superior cytocompatibility and *in vivo* osteogenesis as a novel orthopedic implant. **RSC Advances**., 2017, 7(1), 559–573.
13. Sheweita S.A.: Drug-metabolizing enzymes mechanisms and functions, **Curr. Drug Metab.**, 2000, 1(2), 107–132.
14. Ouassou H., Bouhrim M., Daoudi N.E., Mekhfi H., Ziyyat A., Legssyer A., Aziz M. and Bnouham M.: Evaluation of hepatoprotective activity of *Caralluma europaea* stem extract against CCl_4-induced hepatic damage in Wistar rats, **Adv. Pharmacol. Sci**., 2021, Article ID 8883040.
15. Weber L.W., Boll M. and Stampfl A.: Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, **Crit. Rev. Toxicol**., 2003, 33(2), 105–136.