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Research article

Hepatoprotective activity of polyherbal tablet containing *Andrographis paniculata* and *Tephrosia purpurea* extracts against paracetamol-induced toxicity in rats

Sanjay Lohar[1,*], Sunil Jalalpure[2], Kalpana Patil[1,*], Popat Kumbhar[1] and Rutuja Patil[3]

[1] Department of Pharmacognosy, Tatyasaheb Kore College of Pharmacy, Warana University, Warananagar, 416113, Maharashtra, India
[2] Department of Pharmacognosy, KLE College of Pharmacy Belagavi-590 010
[3] Department of Pharmacognosy and Phytochemistry, Annasaheb Dange College of B. Pharmacy, Ashta, Sangli, 416301, India

* **Correspondence:** Email: kalpanaspatil@tkcpwarana.ac.in; Tel: +91 9449024899.

Abstract: Hepatic diseases are a serious concern across the globe. The death of liver cells is a significant cause of liver diseases. Phytochemicals from *Andrographis paniculata* and *Tephrosia purpurea* are reported to have hepatoprotective potential. The oral delivery of extracts containing phytochemicals using a tablet can produce a synergistic effect and enhance safety and patient compliance. This study aimed to extract phytochemicals from those plants and investigate their phytochemical potential, the pre- and post-compression attributes of the extracts' powder, their compression into tablets, and the in vivo performance of the tablets in paracetamol-induced hepatotoxicity in Wistar rats. Phytochemical screening of two extracts revealed the presence of flavonoids, phenols, carbohydrates, terpenoids, tannins, proteins, cholesterol, diterpenes, and glycosides. Evaluation of pre-compression properties revealed good flowability and suitability for compression into tablets. The post-compression quality control parameters were found to match the standard. The polyherbal tablet exhibited dose-dependent hepatoprotective activity in rats. The histological study revealed that the administration of 650 mg/kg body weight of paracetamol resulted in damage to the liver, as evidenced by increased levels of various enzymes, such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP), total cholesterol (TC), triglycerides (TG), and total proteins (TP). Liver enzyme levels were significantly ($p < 0.005$) decreased after treatment with the polyherbal tablet

formulations. Furthermore, histological analysis showed that the liver anatomy of the rats given polyherbal tablets was restored to near-normal architecture. The polyherbal tablet comprising *A. paniculata* and *T. purpurea* extracts can be a promising approach in treating hepatotoxicity.

Keywords: *Andrographis paniculata*; *Tephrosia purpurea*; hepatotoxicity; hepatoprotective activity; polyherbal tablet; *in vivo* activity

1. Introduction

The liver is one of the body's larger, more complicated organs. It is involved in the intermediate metabolism of proteins, lipids, and carbohydrates and the synthesis of plasma proteins [1]. The liver is the only organ with the remarkable property of self-regeneration, acting as a stem cell with homogeneity due to hepatocytes [2]. Many toxic substances, including alcohol, paracetamol, peroxidized oils, aflatoxins, carbon tetrachloride, and chlorinated hydrocarbons, together with autoimmune diseases, are the primary causes of hepatic failure [3]. Paracetamol-induced liver toxicity is commonly observed in individuals. In small doses, paracetamol converts into non-toxic N-acetyl-p-benzoquinone imine; at higher doses, it can react with proteins, causing liver damage [3].

Liver damage is thought to be primarily caused by oxidative stress brought on by free radicals, in an imbalance between antioxidant enzymes and reactive oxygen species (ROS). Following xenobiotic entry into the liver, endogenous antioxidants neutralize the free radical species generated by CYP450 enzymes. However, an increase in ROS impairs the protective antioxidant mechanism of the liver, causing oxidative stress-induced liver damage [4,5].

Free radicals harm the membrane's lipid bilayer, which sets off lipid peroxidation and a series of events that result in ROS. The prevalence of liver illness is rising globally, and because current medications have negative health effects, they are insufficient to treat liver diseases. Finding affordable, side-effect-free medicines to avoid liver problems is therefore imperative [4,5].

To find potential drugs, scientists are looking to traditional medicine. Ayurvedic medicine has long been employed in India to treat an assortment of human ailments, including liver disorders. Secondary metabolites (flavonoids, phenolic compounds, alkaloids, etc.) with pharmacological properties, such as hepatoprotective, anti-inflammatory, and antioxidant qualities, have been found in medicinal plants by several researchers in recent decades [4,5]. Additionally, these herbal remedies offer a variety of benefits, including affordability, long-lasting effects, and safety. However, unpleasant odor and taste can lower patient compliance, and poor stability is another significant downside of herbal components. Such challenges justify the delivery of these herbal-based therapeutics in the form of a solid dosage via the oral route. Oral delivery using a tablet confers a range of advantages, such as ease of administration and handling, and the ability to mask unpleasant tastes and smells of the plant extract. Additionally, enhanced stability and better patient compliance are noteworthy benefits of tablet dosage forms [6].

Andrographis paniculata, also referred to as the *King of the Bitters*, is a member of the Acanthaceae family that grows throughout tropical and subtropical Asia. *Tephrosia purpurea* is a perennial plant found all throughout India that belongs to the Fabaceae family. Phytochemical tests have revealed the presence of flavonoids, glycosides, rotenoids, isoflavones, flavanones, chalcones, flavonols, and sterols. These plants include phytochemicals that offer a variety of biological properties,

such as antibacterial, antidiabetic, antimalarial, and anti-inflammatory properties [7,8], also being reported to have hepatoprotective characteristics [9]. Existing studies have dealt with the delivery of individual phytochemicals from both plants through suitable oral solid dosage forms like tablets [10]. On the other hand, this research is focused on the delivery of both plant phytochemicals through a single dosage form. Thus, the novelty of the present research lies in the co-delivery of phytochemicals from both plants using a polyherbal tablet. The delivery of such polyherbal components through a solid dosage can enhance stability and patient compliance. Moreover, this polyherbal formulation can produce a synergistic effect and manage hepatic damage efficiently and safely. Therefore, the present research aims to develop a polyherbal tablet comprising phytochemicals from *A. paniculata* and *T. purpurea* and investigate their hepatoprotective activity using suitable animal models.

2. Materials and methods

2.1. Plant collection and authentication

In August, plants of *Andrographis paniculata* and *Tephrosia purpurea* were collected from Vantamuri, District Belagavi, and Sangli. Dr. Harsha Hegde, Scientist E, RMRC, ICMR, Belgaum, India, verified the authenticity of the plants, and the plant herbarium was deposited at RMRC, ICMR, Belgaum.

2.2. Chemicals

In this experiment, only analytical-grade materials were used. Paracetamol was purchased from John Baker Inc. (Colorado, USA), and silymarin was purchased from Biochemika. Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), triglycerides (TG), total proteins (TP), and total cholesterol were all measured using diagnostic kits manufactured by Elba Diagnostics Ltd. A typical orogastric cannula was used to provide oral medicine.

2.3. Animals

Wistar albino rats weighing 150–200 g was used. This study was approved by the Institutional Animal Ethic Committee (IAEC) under the ethical permission number KLECOP/CPCSEA-Reg.No.221/Po/Re/S/2000/CPCSEA, Res.28-12/10/2019.

2.4. Preparation of plant extract

Extraction of phytoconstituents from the whole *Andrographis paniculata* plant and leaves of *Tephrosia purpurea* was performed by sequential maceration with 70% alcohol and filtering; the filtrate was kept aside to efficiently extract phenols, flavonoids, glycosides, and tannins. Then, Soxhlet extraction was conducted using 95% alcohol. The filtrates of the two extraction methods were merged and concentrated on a rotary evaporator at 40 °C under vacuum to obtain a semisolid mass. The residue was treated with microcrystalline cellulose dissolved in alcohol, which acts as an

effective adsorbent to obtain a dry powder, used for several pharmacognostical and biological screening tests [11].

2.5. Phytochemical investigation

To identify the occurrence of flavonoids, phenols, terpenoids, carbohydrates, tannins, proteins, cholesterol, diterpenes, and glycosides, a preliminary phytochemical screening was performed on a hydro-alcoholic extract [12,13].

2.6. Characterization of the powder for micromeritics properties

The obtained extracts' powder was evaluated for diverse micromeritics properties.

2.6.1. Bulk density

A graduated cylinder was filled with a predetermined amount of the tablet mixture. The powder's weight and volume were recorded, and the bulk density was calculated using the following formula:

$$Bulk\ density = Mass\ of\ powder / Bulk\ volume\ of\ powder$$

2.6.2. Tapped density

A graduated cylinder was filled with a known weight of the mixture. At 2 s intervals, the graded cylinder was dropped from a height of 10 cm onto a hard surface, allowing it to fall under its own weight. After the volume did not significantly change after tapping, the tapped density was calculated using the following formula:

$$Tapped\ density = Mass\ of\ powder / Tapped\ volume\ of\ powder$$

2.6.3. Angle of repose

Flow parameters of the powder material were computed using the angle of repose, which was calculated using a fixed funnel method. Using the following formula, a 10 mm diameter, 2 cm height funnel was placed on a level, flat surface:

$$\tan\theta = Pile's\ height\ (h) / Average\ radius\ of\ the\ powder\ cone\ (r)$$

2.6.4. Compressibility index

The Carr's compressibility index for the powder material was calculated utilizing the bulk and tapped density.

$$Carr's\ index = Tap\ density - bulk\ density\ /\ Tap\ density \times 100$$

2.6.5. Hausner's ratio

The Hausner's ratio was assessed based on the tap and bulk density, as follows:

$$Hausner's\ ratio = Tap\ density / Bulk\ density$$

2.7 Extract-loaded tablet formulation

Using the excipients comprising carboxymethyl cellulose, lactose, sodium starch glycolate, magnesium stearate, talc, and PVP, polyherbal tablets containing extracts of *A. paniculata* and *T. purpurea* were made by the direct compression method in a 1:1 ratio [14–16]. Round, flat-faced tablets were crushed using an automated single-punch tablet press with a 9 mm die and a 10 kN compression force. Table 1 displays different polyherbal tablet compositions.

Table 1. Tablet formulations.

Ingredients	**Quantity per tablet (mg)**		
Plant extracts	100	200	400
Carboxymethylcellulose	100	100	100
Lactose	100	100	100
Sodium starch glycolate	05	05	05
Magnesium stearate	02	02	02
Talc	02	01	02
PVP	01	01	01
Total weight of the tablet	310	410	610

2.8. Evaluation of the developed polyherbal tablets [17]

2.8.1. Weight, thickness, and diameter uniformity

Twenty tablets were used to ensure tablet weight uniformity according to the Indian Pharmacopoeia. A caliper was used to measure each tablet's thickness and diameter. After 20 tablets from each batch were collected, the average thickness and diameter were calculated.

2.8.2. Hardness and friability

Twenty tablets of each formulation were evaluated using a Pfizer hardness tester and Electro Lab friability testing equipment to determine the tablets' levels of hardness and friability.

2.8.3. Disintegration time

Six tablets were put in tubes and covered with a plastic disk. The tubes were sealed, and disc pressure was applied to the tablets. The tablets were kept at 37 ℃ in aqueous medium, and the test tubes were free to oscillate between 29 and 32 times per minute. Tablet disintegration time was estimated as the time required for all tablets to go through 8–10 meshes.

2.9. In vivo hepatoprotective activity

Animals were maintained in a controlled environment with a 12/12 h light/dark cycle, at 23 ± 2℃, and 50% ± 5% humidity. Before the test, animals were allowed to adapt for seven days. Six groups comprising six rats in each group were created. Group I comprised the control and was administered distilled water orally. Groups II, III, IV, V, and VI were given paracetamol at a daily dose of 650 mg/kg body weight for 14 days in order to treat illness, hepatotoxicity, or liver damage. Group II was regarded as a disease group that was not given any medical attention. On the other hand, post-induction of hepatotoxicity, group III was provided treatment with silymarin 50 mg/kg body weight. Polyherbal tablets at extract doses of 100, 200, and 400 mg/kg body weight were given to groups IV, V, and VI [18,19]. Blood and serum samples were collected, and the following treatments were conducted to investigate different biochemical parameters. Additionally, the livers of the animals were subjected to a histological evaluation [20].

2.10. Biochemical parameters and histopathological evaluation

Non-heparinized tubes were used to collect blood samples at the end of the treatment period. The non-heparinized blood samples were centrifuged at 1000 rpm for 20 min. Serum samples were stored at −20℃ prior to analysis. Using specific diagnostic kits, separate sera were utilized to measure SGOT, SGPT, ALP, triglycerides (TG), total proteins (TP), and total cholesterol [21]. The isolated livers were also cleaned with phosphate buffer, dried using tissue paper, and then left in a 10% formalin fixing solution for 48 h. A microtome was used to cut 5-mm-thick slices, which were then stained with hematoxylin and eosin and inspected under a microscope to observe any alterations in liver histology [22].

2.11. Statistical analysis

Data are shown as mean ± SEM, n = 6. Graph Pad Prism 8 was used for statistical investigation, using one-way ANOVA and Tukey's multiple comparison tests. The findings were assumed to be statistically significant if $p < 0.05$.

3. Results

3.1. Extraction of phytochemicals

Successive extraction of *A. paniculata* and *T. purpurea* plants was carried out to completely extract the desired phytochemicals. Phenols, flavonoids, tannins, and glycosides are examples of thermolabile components that can be obtained during maceration and that have a strong hepatoprotective impact, as confirmed by phytochemical investigations. Thus, maceration was followed by the Soxhlet extraction method to recover residual phytoconstituents, such as cholesterol and terpenoids. As shown in Table 2, the phytochemical analysis of the extracts revealed a variety of phytochemicals, including flavonoids, polyphenols, carbohydrates, and cholesterol. The presence of cholesterol is observed in both extracts at low concentration, similarly to previous study reports [23]. Flavonoids and phenols were the most prevalent phytoconstituents in both plant extracts. Flavonoids

had a highly beneficial effect on *Andrographis paniculata* and *Tephrosia purpurea*, causing noticeable color changes.

Table 2. Phytochemical screenings of plant extracts.

Phytochemicaltest	***Andrographispaniculata* extract**	***Tephrosiapurpurea* extract**
Flavonoids	+ +	+ +
Polyphenols	+ +	+ +
Carbohydrates	+	+
Terpenoidsand steroids	+	+
Cholesterol	+	+
Diterpenes	+	+
Glycosides	+	+
Tannins	+	+

3.2. Micromeritics properties of the extract powders

Powder mixtures of each batch of tablet formulations with different extract concentrations were examined for micromeritics characteristics, such as Hausner ratio, bulk density, tap density, angle of repose, and compressibility index. The bulk density and tap density of the powder material were determined to be between 0.25 and 0.24 g/cm^3 and 0.31 and 0.35 g/cm^3, respectively. The angle of repose varied between 25° and 28°. The Hausner ratio and Carr's compressibility index of the powder materials varied between 1.11 and 1.14, and between 12.3 and 13.4, respectively.

3.3. Evaluation of tablets

Regarding the post-compression parameters of the developed formulation, tablet thickness and diameter ranged between 4.9 and 5.5 ± 0.11 mm and 10.14 and 12.2 ± 0.16 mm, respectively. Hardness was found to be 2.78–3.44 ± 0.19 kg/cm^2. Friability was 0.62%–0.65% ± 0.11%. The average variation in tablet weight was found to be 307–606.5 mg. The in vitro disintegration time was between 7.52 and 10.27 ± 1.31 min.

3.4. In vivo hepatoprotective activity

Compared with group I (vehicle control group), all groups injected with paracetamol showed significantly ($p < 0.05$) increased levels of various liver enzymes, including SGOT, SGPT, ALP, TG, TP, and total cholesterol. However, the levels of liver enzymes significantly decreased after treatment with silymarin (group III) and polyherbal tablets (groups IV, V, and VI) (Figure 1). Rats treated with 400 mg/kg tablets (group VI) showed a notable and similar hepatoprotective effect to those treated with the standard.

3.5. *Histological examination of liver*

The hepatocyte architecture and cellular morphology of the animals in the normal control group

were both intact (Figure 1A). The paracetamol-treated group displayed severe cytoplasmic vacuolization, centrilobular necrosis, hepatocyte ballooning, an injured central vein, and hepatic sinusoidal congestion, associated with the control group (Figure 1B). In contrast, animals administered silymarin exhibited minimal hepatocellular necrosis, maintained hepatocyte structure and lobular architecture, and displayed neither inflammatory cell infiltration nor lipid change (Figure 1C). Similarly, due to the exposure to paracetamol, the PHF-treated group that received dosages of 100, 200, and 400 mg/kg demonstrated a substantial healing from liver injury (reduced hepatotoxicity) (Figure 1D–F). Treatment with PHF tablets of 100 and 200 mg/kg lowered necrosis and ballooning of hepatocytes, signifying poor improvement. On the other hand, 400 mg/kg PHF treatment restored the sinusoidal gaps to normal with less vacuolization and necrosis (Figure 1F). The group that received 400 mg/kg PHF tablets also had a normal central vein and improved cellular architecture.

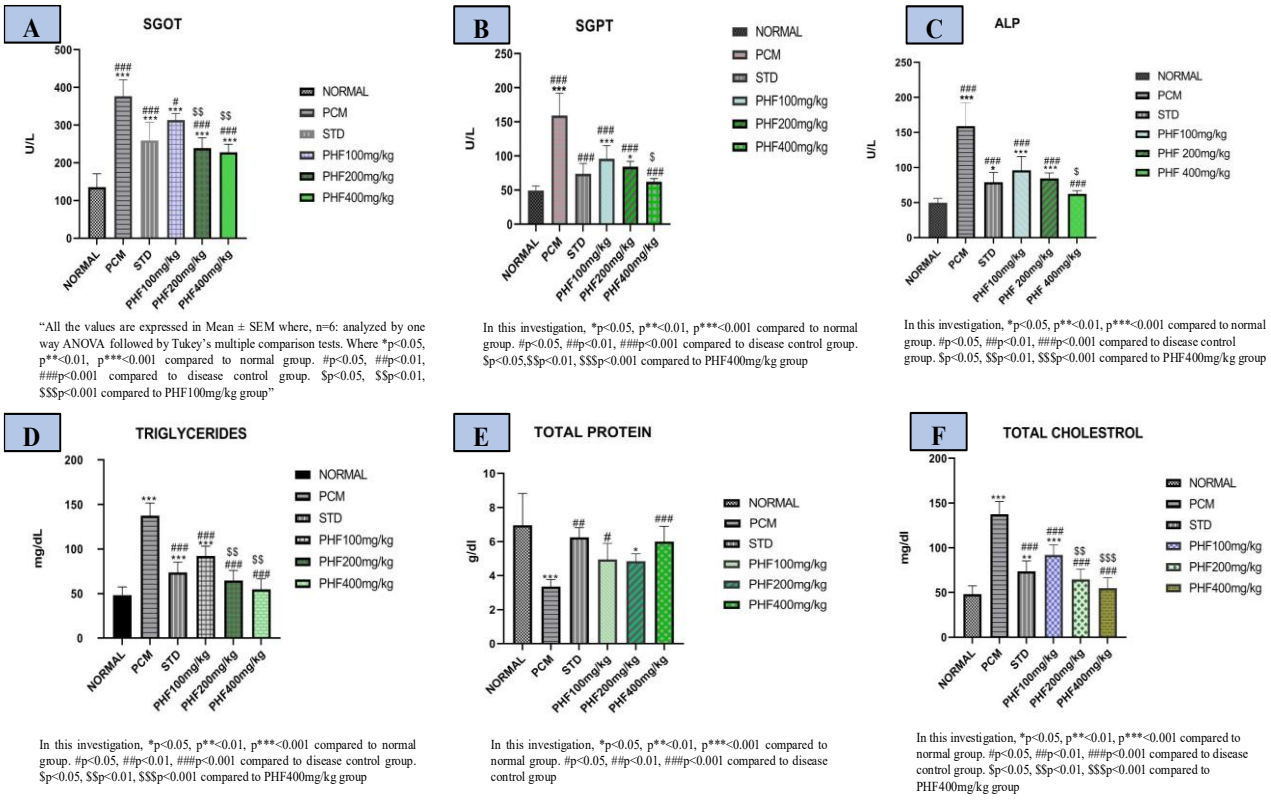

Figure 1. Biochemical parameters of different groups of animals treated with different formulations.

Figure 2A shows the typical morphology of hepatic lobules. The hepatic parenchyma, which has small sinusoids and noticeable nuclei, extends from the central veins. Figure 2B shows augmented sinusoidal space, ballooning deterioration, focal hepatocyte necrosis, and fatty modification in the paracetamol-treated group. Similar to the control group, the hepatocyte architecture of the silymarin-treatment group is depicted in Figure 2C. Hepatocyte recovery was gradual and dose-dependent (Figure 2D–F) when treated with PHF (100, 200, and 400 mg/kg), with a little amount of vacuolization and sinusoidal space.

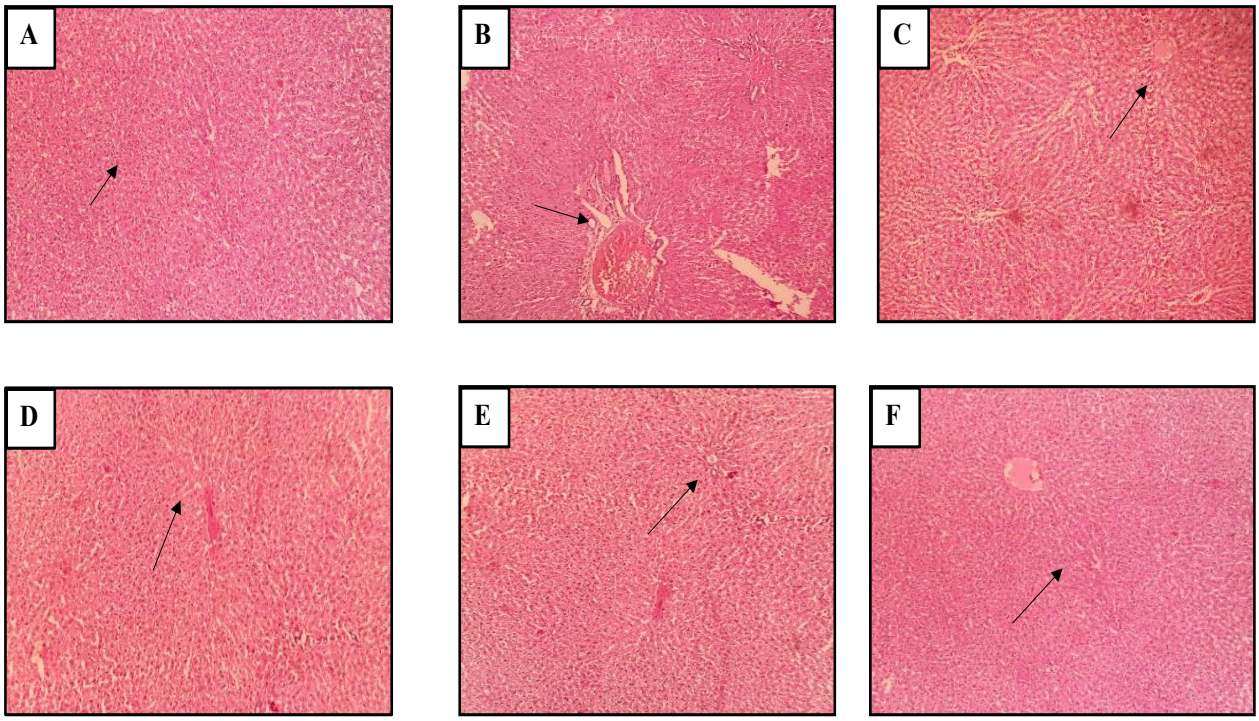

Figure 2. Protective effect of polyherbal tablet formulation against paracetamol resulting in histological variations in hepatic tissue (100×).

4. Discussion

The liver is a vital organ in the body that is in charge of metabolism. Various synthetic drugs used to treat diseases are associated with serious toxicity to the liver. Therefore, there is a dire need to identify some herbal remedies with significant hepatoprotective activity. *Andrographis paniculata* and *Tephrosia purpurea* contain phytochemicals with potential hepatoprotective activity. The components present in the *A. paniculata* and *T. purpurea* plants were extracted using maceration followed by the Soxhlet extraction process. Numerous phytochemicals, including flavonoids, phenols, sugars, tannins, cholesterol, diterpenes, and glycosides, were found in these extracts, according to phytochemical analysis. In both plants, flavonoids and phenols were the most prevalent types of chemicals, which are reported to be responsible for hepatoprotective activity [10]. A possible hepatoprotective mechanism of the phytoconstituents (flavonoids, terpenoids, phenols) is the inhibition of reactive oxygen species generation, preventing oxidative damage and liver protection. Moreover, the underlying hepatoprotective activity mechanism of cholesterol involves the reduction of bad cholesterol production, thereby safeguarding the liver [23].

Hausner ratio, Carr's index, angle of repose, bulk density, and tap density were the pre-compression variables assessed. The angle of repose, which varied from 25° to 28°, indicated that the powder material had good fluidity. Additionally, a Carr's index of 12.3–13.4 and a Hausner's ratio of 1.11–1.14 indicate that powder materials have good flowability. As a result, the powder materials showed pre-compression characteristics that indicate their appropriateness for tablet compression. The tablet's post-compression properties, such as thickness, diameter, hardness, friability, weight fluctuation, and in vitro disintegration, were then investigated. There was no discernible disparity in

the weight of the three strengths of tablets, indicating that the weight of the created tablet was consistent. Furthermore, the tablets' capacity to endure and preserve their integrity during transit was demonstrated by the friability study, which showed a weight loss percentage of less than 1%.

Paracetamol-induced hepatotoxicity is a commonly used model in the evaluation of diseases. As such, paracetamol was employed as a toxic agent in this investigation to assess the hepatoprotective potential of the polyherbal formulation against paracetamol-induced hepatotoxicity. Various biochemical measures, namely SGOT, SGPT, ALP, TG, TP, and TC, were elevated in groups treated with only paracetamol. In contrast, polyherbal tablets and silymarin-treated groups, in which hepatotoxicity was induced using paracetamol, demonstrated decreased levels of the enzymes and TC. This decreased level of TC following treatment with the polyherbal tablet could be attributed to the presence of good cholesterol in extracts of both plants [23]. The herbal tablet containing phytochemical extract (400 mg/kg) exhibited a more potent hepatoprotective activity than the herbal tablets containing 100 and 200 mg of extracts. This substantially improved hepatoprotective activity of polyherbal tablets containing phytochemical extract (400 mg/kg) could be attributed to the higher dose of phytochemicals. Moreover, a histopathological study showed adverse changes in the liver anatomy of the rat group treated with paracetamol, indicating liver toxicity by paracetamol. Notably, there was extensive interlobular necrosis, inflammatory lymphocyte infiltration, and lipid alterations, which showcase severe effects on the liver's anatomical functions. The silymarin-treated group, co-administered with paracetamol, showed normal lobular structure. Similarly, tablet formulation developed from the combined sources, at doses of 100, 200, and 400 mg, caused no significant anatomical alterations in the liver anatomy of animals. Similar to the control group, the liver's structure was found to remain intact. At a dose of 400 mg/kg, the polyherbal tablet formulation had liver shielding effects comparable to those of silymarin, suggesting substantial protection against the toxins generated by paracetamol. The hepatoprotective potential of both plants might be due to the presence of flavonoids, phenolics, and cholesterol. In conclusion, the polyherbal tablet containing extracts of *A. paniculata* and *T. purpurea* could be promising in managing liver toxicity efficiently and safely.

5. Conclusions

The current study examined the hepatoprotective properties of phytochemicals derived from *Andrographis paniculata* and *Tephrosia purpurea*. Using the hydro-alcoholic extraction method, the plant components were successfully extracted. These extracts contained flavonoids, phenols, carbohydrates, tannins, proteins, cholesterol, diterpenes, and glycosides, according to phytochemical examination. Good flowability and suitability for compression into tablets were shown by assessing the extract powder's pre-compression characteristics. Following compression, the tablets' characteristics were within the precise bounds specified by the standards. Rats given different dosages of three tablets containing extracts from medicinal plants demonstrated hepatoprotective effectiveness against paracetamol-induced liver injury. However, compared to tablets containing extracts of 100 and 200 mg, a polyherbal tablet comprising 400 mg of extract showed notable hepatoprotection. Additionally, its hepatoprotective effect was comparable with that of regular silymarin. Additionally, a histological liver study of rats given conventional silymarin and polyherbal tablets revealed no discernible changes in liver structure. Therefore, the current study demonstrates the usefulness of polyherbal tablet formulation as a hepatoprotective drug for future development and

supports its traditional use in controlling liver illnesses.

Author contributions

Supervising, KSP, SSJ, PSK; Writing—original draft preparation, SBL, RNP; Writing—review and editing, KSP, SSJ, PSK; methodology, SBL, KSP, SSJ, PSK, RNP; Software, SBL. All authors have read and agreed to the published version of the manuscript. All authors have read and approved the final version of the manuscript for publication.

Use of Generative-AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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