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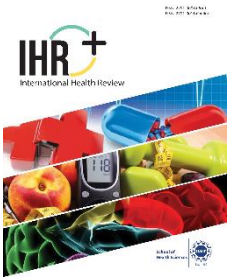

Title: **Evaluation of Hepatoprotective and Nephroprotective Impact of Phytomedicine using Experimental Animal Models**

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Evaluation of Hepatoprotective and Nephroprotective Impact of Phytomedicine using Experimental Animal Models

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ABSTRACT

Human beings have been using plants as medicines for centuries. The development of traditional medicinal systems utilizing plants as a form of medicine may be traced back only as far as historical documentation of their similarity. Immunosuppression and bone resorption is a major drawback in conventional chemotherapy. Modern medicines contain plant-derived components that are the source of immunomodulators. *Withania somnifera* and *Cnidium monnieri* are the plants which have been used for immunomodulatory and anti-osteoporotic activities. Medicinal plant samples were obtained from diverse parts of Pakistan. Following extraction, phytochemical analysis and antimicrobial potential were investigated, and the hepatoprotective effect was evaluated. An in vivo study was conducted on experimental animals by administering ethnomedicinal plant extracts in comparison to some allopathic drugs. Various blood samples were collected to analyze calcium level, vitamin-D, thyroid-stimulating hormone (TSH), parathyroid hormone (PTH), follicle stimulating hormone (FSH), and Testosterone for antiosteoporosis activity. The possible outcomes were positive and hepatoprotective as well as antimicrobial effect was present in Polyherbal preparation (PHP). During trails on Albino rats, the improvement in their body weight was recorded. PHP administration of plant extracts enhanced the parameters of blood and neutralized the effect

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of CCl4 intoxication. The proper administration of plant extracts showed a positive effect on hormones, particularly progesterone, testosterone, and oestradiol. In the future, these selected medicinal plants and their PHP might be preferred for the separation of lead bioactive compounds to overcome the extremely alleviating issues associated with infertility.

Keywords: antimicrobial, clinical trial, Cnidium monnieri, hepatoprotective, medicinal plants, neurprotective, Withania somnifera

1. INTRODUCTION

Reproduction is a biological process that produces new individual organisms which is vibrant for the endurance of all forms of life. Infertility is a reproductive issue that may be present in both parents, such as hormonal abnormalities and sexual disorder. The reproductive system acts on the pituitary gland which releases two hormones, that is, luteinizing hormone (LH) and folic stimulating hormones (FSH). These hormones regulate the egg production in women and perform the function in men [1]. The sex hormones, such as estrogen and progesterone are produced enzymatically in ovaries through steroidogenesis. These hormones regulate egg production in the female reproductive system. In males, luteinizing hormone (LH) and folic stimulating hormones (FSH), that are released from the pituitary gland play an important role in testis and plasma membrane receptors [2].

Infertility is commonly described as the inability to conceive after one year of sexual contact without the use of contraception. This syndrome is identified in women over the age of 35 after 6 months of inability to conceive. Using this criterion, there would be women, although a few, who fit the requirements for "infertility" but go on to conceive without medical assistance [3]. However, this term is utilized to ensure that people with fixable issues can get medical attention as soon as possible.

Infertility is more frequent than people realize. Contrary to what most of the people were led to believe in their high school sex education classes (if we even had access to such a thing), the average couple has only a 20% chance to get pregnant in any given month. Infertility affects 10-15% of couples throughout the course of a lifetime. When a woman is older than 35, this rises to 33% [4]. In approximately 45% of cases, the man is to blame, while in 30% of the cases, the woman is to blame, and in 20% of cases both couples may be the cause of the problem. According to World

Health Organization (WHO), infertility is the inability to achieve pregnancy due to irregular or unprotected sex within one year. Infertility is a serious problem and about 8-12% of the couples suffer from infertility worldwide. Age factor tends to effect the infertility considerably, approximately 1-6% of the couples are infertile in Australia and 3% of women are infertile due to the over age of 35 years [5].

Liver is the most important organ in the human body. It plays a critical role in regulating a wide range of bodily processes. There are several critical processes that depend on it including metabolism, secretion, and storage. It can synthesize helpful principles and detoxicate harmful compounds [6]. The global issue of illnesses associated with liver continues to be one of the key challenges to public health. They are mainly caused by substances, such as paracetamol (in high dosages), excessive alcohol intake, infections, and autoimmune diseases. Tripathi et al. [7] stated that the most hepatotoxic substances primarily cause lipid peroxidation and other oxidative damage to liver cells. Acetaminophen, a recently invented moderate analgesic and antipyretic medication, may result in significant liver necrosis in both people and lab animals when taken in high dosages [8]. Even though, alcoholic liver disease is one of the leading causes of end-stage liver disease globally, it is the second most frequent indication for liver transplantation in the US. The incidences of liver illnesses have grown in China as a result of excessive drinking and dietary changes, such as an increase in fat content [9]. It is now a significant risk factor for morbidity and death in addition to viral hepatitis. Alcoholic liver disease has many symptoms, starting with fatty liver and progressing to alcoholic hepatitis, fibrosis, and cirrhosis.

No effective medication is available to enhance liver functioning or which protects the liver from damage or helps regenerate hepatic cells, despite the enormous advances in modern medicine [10]. Therefore, it is important to look for new medicines for the treatment of liver diseases in order to substitute drugs of uncertain effectiveness and safety that are currently used. Medicinal plants play a crucial role in human healthcare. About 80% of the world's population depends on the use of conventional medicine which is primarily based on traditional medicines. Scientific research available on medicinal plants indicates that promising phytochemicals can be created for many health problems [11]. For instance, for anti-cancer activities, vinca alkaloids (vincristine, vinblastine, and

vindesine), extracted from *Catharanthusroseus*, *Vincarosea*, *Lochnerarosea*, and *Ammocallisrosea*, have been used.

There are a number of chemical constituents in liver-defensive plants, such as phenols, coumarins, monoterpenes, glycosides, alkaloids, and xanthenes. The literature related to natural products (crude plant extracts and chemically identified molecules) with hepatoprotective potential was reviewed in the current study. Such results provide greater resources and versatility to help researchers classify compounds with strong hepatoprotective potential [12]. Such protection from liver damage caused by oxidative processes of toxic chemicals can be given by traditional dietary antioxidants as well. It is probable that paniculata and P. amarus are active against Hepatitis B virus [13]. Most liver cells die or transform into a fibrotic condition in the event of serious liver damage. Consequently, in the current study, bioactive compounds/phytochemicals were extracted having the potential to protect the liver from cirrhosis and other types of diseases and their results were compared with standard allopathic drugs.

2. METHODOLOGY

2.1. Screening of Medicinal Plants

Table 1. Plants Selected for Current Study

Common Name	Scientific Name	Family Name	Parts Used
Shechuangzi,Osthole, Jashoshi and Cnidii Fructus	*Cnidium Monnieri*	Umbelliferae	Roots
Barrenwort, fairywings and bishop'shat	*Epimediumgrandiflorum*	Berberidaceae	Roots
Ashwagandha and Indian ginseng	*WithaniaSeminifera*	Nightshade	Leaves
Marapuama, raiz delmacho and potencywood	*Muira Puama*	Olacaceae	Roots

Preliminarily following plants were screened, such as *Withania somnifera* (common name asashwagandha and Indian ginseng), *Cnidium monnieri* (common name as Shechuangzi, Osthole, Jashoshi and Cnidii Fructus), *Muira Puama* (Bois de la Puissance Sexuelle, Bois dela Puissance, Marapuama, or Potency Wood), and *Epimedium grandiflorum* (common

nameis barrenwort). Medicinal plants were collected from various areas of Faisalabad. Valuable parts of selected plants were poised, desiccated, grounded into powder, and took out on methanolic solvent. Plants that were collected for research have been listed below along with their scientific, common, and family names.

2.2. Preparation of Bioactive Extracts

The samples of medicinal plants were collected from various regions of Pakistan. The materials of plants were washed with tape water to remove dust particles and other contaminants. Afterwards, the material was again washed with distal water and dried in shade. shade dried the plant sample and Grinding of plant material carried out with an electric grinder. Grinder is a machine or tool used for refining, improving, or smoothing the surface of something. Electrical grinder is also known as angle or disc grinder. Angle grinders can be powered by a petrol engine, electric motor, or compressed air, and plants' powder materials are extracted with different solvents using different extraction techniques. All medicinal plants' hydroalcoholic extracts were made using a modified protocol of the method outlined by Hudaib et al. [14]. To separate the desired natural products from raw material, extraction process is used which includes sublimation, distillation method, pressing, and solvent extraction according to their extraction principle. The extracts produced were freed of solvent under reduced pressure. The dose percentage of extracts (g/100g of dry plant) was calculated from the weighed concentrated extracts by using the equation given below:

Percentage Yield (%) = Dried extract weight/Dried plant material weight×100

2.3. Phytomedicine Preparation

Polyherbal preparation (PHP) is Phytomedicine made from the aqueous extract of test plants' powder of *Withania somnifera, Cnidium monnieri, Muira Puama,* and *Epimedium grandiflorum* by taking 10mg of each to form total of 40mg.

2.4. Antioxidant Potential of Plant Extracts

Different methods were applied to study the antioxidant activity of the above-selected therapeutic plants material.

2.5. Determination of Total Phenolic Contents (TPC)

The total phenolic contents of methanolic plants extract were resoluted by Folin-Ciocalteumethod using the protocol [15]. The Folin–Ciocalteu (F-C) reaction is an antioxidant-based assay that depends upon the electron transfer and it measures the antioxidant' reductive capacity. The Folin–Ciocalteu reagent (FCR) or Folin–Denis reagentor Folin's phenol reagent is also known as gallic acid equivalence method (GAE). Colorimetric in vitro assay of polyphenolic and phenolic antioxidants was analyzed by using GAE. It has been used for the determination of total phenol/polyphenol content biological samples. Standard curve was constructed by using gallic acid in different concentrations, that is, 50, 100, 150, 200, 250, and 300 mg/ml. About 5ml of Folin-Ciocalteu reagent and 4ml of 20% sodium carbonate was added to 1ml of standard solution and 0.001 g/ml of plant extract. After incubating for one hour, the absorbance of reaction mixture was observed at 765 nm. Plants' extracts underwent same procedure three times. The total phenolic contents of plant material were calculated as milligram (mg) Gallic acid per milliliter (ml) of plants methanolic extract. The formula given below determined Total phenolic amount as equivalent to Gallic acid.

$$\text{Total phenolics} = C \times V / M$$

As, Total phenolics = Total phenolic contents (mg of gallic acid/ground medicinal plantmaterial in grams). C = GAE concentration (mg/mL) revealed out from the standard curve.V = extract volume (ml). M = weight of plants extracts in plants.

2.6. Total Flavonoids Contents (TFC)

The total flavonoids' amount in plants extract was identified by following the method [16]. Shortly, 0.15 ml of 5% $NaNO_2$ solution was added to 2ml of distilled water and plant extract (0.5ml) and was incubated for 6 mins. Afterwards, 0.15ml of 10% Aluminum Chloride solution was added to the mixture along with 4% NaOH solution and was incubated for 6 minutes. Methanol was added to make a volume of up to 5 milliliters and was mixed well. The reaction mixture's optical density (OD) was observed at 510 nm after incubating for 15 minutes. The total flavonoid contents of plants extract were determined as microgram CE/gram of dried plants material from the Catechin standard curve.

2.7. Antimicrobial Activity of Plant Extract

Diffusion method was used to determine the antimicrobial activity of plant extracts (methanolic) against the selected strains of microbes.

2.8. Bacterial Strains

Salmonella, Pseudomonas, Bacillus aerius, Escherichia coli, Acinetobacter, and *Klebsiella pneumonia* were used for antimicrobial activity analysis. For this process, media was prepared by dissolving 28g of nutrient agar (Oxoid) in 1L of distilled water and mixed well for uniform distribution. Afterwards, the media was autoclaved at 121ºC for 15 minutes. Before pouring inoculum into sterile culture plates, 100 µL/100ml of bacterial inoculum was moved to the culture medium. The well borer was punched into semi-solid agar media and inoculum to form the wells. The specific wells that were labeled on petri plate were filled with 100 µl of plant extracts and positively controlled rifampicin. The cultured plates were incubated at 37ºC for 24 hrs. The growth of bacteria was inhibited by plant extract designated by the formation of clear regions around the wells.

2.9. Minimum Inhibitory Concentrations (MIC) of Plant Extracts

For this process, 96 wells of micro dilution plates were filled with 50µl of nutrient broth and were diluted by two-fold dilution method. Afterwards, all the wells were filled with 10µL of bacterial strains. After this, 10µl of resazurin (indicator) was added in all wells. At the end, plates were covered to evade the desiccation of bacteria and gestated at 37^{o}C for 24 hours.

2.10. Phytochemical Analysis

Numerous phytochemicals, such as flavonoids, alkaloids, saponins or tannins are identified in plant extracts through usual means. The plant extract was studied for the detection of cardiac glycosides, steroids, and triterpenoids [17].

2.11. Cytotoxicity Activity of Medicinal Plants

2.12. Preparation of Extract Solution

When 10 mg/ml of plant material was dissolved in 20% of Dimethyl sulfoxide, an extract solution was prepared.

2.13. Assay Procedure

The cytotoxic activity of methanolic plant extract was determined using a hemolytic test. After collecting 3ml of Heparinized blood in a sterile falcon tube with a capacity of 15 ml, blood was centrifuged for 5 minutes. The supernatant was removed and RBCS was washed 3 times with chilled (4^oC) phosphate buffer saline (5 ml) having pH 7.4. RBCs were counted by hemacytometer and were maintained at 180 µl of diluted cell suspension and 20 microliters of plant extract were transferred into eppendorf tube having capacity of 2 ml and were incubated (37^oC) for 35 minutes. The tubes were agitated throughout the incubation, cooled for 5 minutes by placing on ice, and centrifuged for 5 minutes at 3500 rpm. After centrifugation,100 microliter of supernatant was taken and diluted by adding 900 ml of chilled phosphate buffer saline (PBS). About 96 microwell plates were filled with 200 µl of mixture taken from each Eppendorf. 0.1% Triton X-100 was used as the positive control, while phosphate buffer saline was used as a negative control. The absorbance was measured at 576 nm with a Microwell Plate Reader (BioTek, Winooski, VT, USA). Each sample underwent three replications. By using the formula given below, percent hemolysis of RBCs by extract was identified:

Percent Hemolysis = At-An/Ac-An×100

Here:

At = the Absorption of test sample

An = Absorption of saline control,

Ac =Dimethyl sulfoxide control absorbance

2.14. Thrombolytic Activity of Plant Extracts by Clot Lysis Method

The thrombolytic activity of plant extracts in terms of invitro clot lysis was identified. About 5ml of distilled water was mixed with lyophilized streptokinase vial (1 500 000 IU) (commercially available) and was diluted appropriately. About 0.5ml of venous blood was collected from ten volunteers without any anticoagulant therapy, following the approved protocol of IRSC. Blood (0.5ml) was shifted to the microcentrifuge tubes (pre-weighed) and was incubated at 37°C for 45 minutes until the clot formed. After the clot formation, the fluid was removed from the tubes. To each tube (containing the pre-weighed clot) 10 mg/ml of methanolic plant extract (100 µL) was transferred. Distilled water was used as negative control, while streptokinase (100 microliters each) was used as positive

control. All the tubes were incubated at 37°C for 90 minutes to observe clot lysis. As incubation of 90 minutes led to clot formation, weight difference was calculated after pouring off the released fluid from tubes. Percent clot lysis was expressed as weight difference obtained in clot lysis.

Percent clot lysis = (weight of clot before lysis –weight of clot after lysis) / weight of clot before lysis × 100

The capacity of extracts to dissolve the clot was compared with that of standard and blank.

2.15. In Vivo Study on Animal Model

An in vivo study was conducted on Albino rats of both genders, adhering to the parameters provided by Govt. College University, Faisalabad. There were 2 main groups on masculine base, that is, group B for female rats and group A for male rats. Afterwards, each group was further sub-classified into six groups each for both male and female rats, comprising four animals as normal, positive, poisonous, control, and test groups. Plants' extract in different dose concentration was specified to control group animals.

All the animals classified into groups are as follows:

Group 1: Control group

Group 2: Positive control (Synthetic drug administered)

Group 3: Plant extract treated group (Minimum dose)

Group 4: Plant extract treated group (Intermediate dose)

Group 5: Plant extract treated group (Maximum dose)

Group 6: Without plant extract treated group (without any treatment)

All the animals in the test groups were treated by administering plant extract in different dose combinations.

2.16. Handling of Animals

Normal husbandry environment was provided to all the animals selected for the current study. The animals were given normal diet as well as 12 hours of light and dark cycle. Simple diet was given to normal control group of animals, CCl4 was injected to toxic group and was left untreated. While at the same time, CCl4 was also injected to positive control group but was

treated by injecting testosterone to males and giving estradiol valerate tablets to female rats. Three different doses of plants bioactive compound were administered to test the group animals injected with CCl4. Without normal control rats, all the rats were injected with CCl4. After inducing toxicity, known drug and plant extract were given to rats for treatment in positive test and control groups to estimate the biochemical and hormonal changes in response to given extracts. Positive control female and male rats were administered with testosterone and estradiol, respectively to compare the results between plants given rats test group and positive control group. The blood sample was collected in gel tubes for serum separation and in EDTA (as anticoagulant) coated tube for hematological study by venipuncture technique from all study subjects after a particular time of administering plant extracts. Until analysis, the nonanticoagulated blood sample would be permitted to centrifuged, clot, and frozen.

2.17. Statistical Analysis

The acquired data was reported as Mean±SD and analyzed statistically by using one-way ANOVA test [18] as well as the difference among groups pairwise research was assessed by Tukey's test and Fisher's test by using statistical software that was Minitab 17 (trial version).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

The analysis of phytochemicals (alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids) was performed from all the medicinal plants. The data obtained has been presented in Table 2 (qualitative) and Table 3 (quantitative) estimation. Results showed that flavonoids, glycosides, triterpenoids, and steroids were present in high concentration in all the four selected medicinal plants as compared to saponins in some plants in less amount. In another research, phytochemical screening and characterization of *P. emblica* indicated the occurrence of phenols, flavonoids, and tannins that revealed the beneficial impact of hydroalcoholic extract of the bark of *Phyllanthus emblica* (PEE) in an ethanol-induced hepatotoxicity in rat models [19].

Table 2. Qualitative Analysis of Phytochemicals

Plants/ Phytochemicals	Withaniasomnifera	Cnidiummonnieri	MuiraPuama (Ptychopetalu molacoides)	*Epimedium grandiflorum*
Alkaloids	+	-	++	++
Tannins	+	-	+	++
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Glycosides	+	+	++	++
Triterpenoids	+	+	+	+
Steroids	+	+	+	++

Significant amount of TPC (mg GAE/g) was observed in *Epimedium grandiflorum, that is*, 671 ± 10.63 as compared to other selected medicinal plants. TFC (µg CE/g) was present in higher concentration in *MuiraPuama* as 133 ± 6.13 and DPPH Scavenging activity (%) was found to be higher in *Epimedium grandiflorum as* 71.90 ± 4.21 as compared to other plants. The research conducted by Tungmunnithum et al. [20] validated the findings by analyzing the pharmacological and medical applications of flavonoids and other phenolic chemicals found in medicinal plants.

Table 3. Quantitative Analysis of Phytochemicals

Plants/ Phytochemicals	*Withaniasomnifera*	*Cnidiummonnieri*	*MuiraPuama (Ptychopetalu molacoides)*	*Epimedium grandiflorum*
TPC (mg GAE/g dryplants material)	179.51± 5.63	291.99 ± 8.43	587 ± 15.33	671 ± 10.63
TFC (µg CE/g dryplants material)	35.76± 0.53	77.1 ± 0.90	133 ± 6.13	169 ± 9.03
DPPH Scavenging activity (%)	47.41± 3.13	36 ± 4.16	63 ± 5.73	71.90 ± 4.21

3.2. Antimicrobial Activity

Total antimicrobial activity of medicinal plants was calculated against different microbial strains, such as *E. coli, Bacillus subtilis, Pasteurella multocida, and Staphylococcus aureus*. All the herbal medicines showed significant antimicrobial activities. The results were highly significant. The highest antimicrobial activity was determined against *E. coli* by *Epimedium grandifloruma* and lowest using *Withania somnifera*. All the data obtained from different medicinal plants against different microbial species has been presented in Table 4 (antibacterial) and Table 5 (antifungal). The antibacterial property of *P. emblica's* chemical components was reported in

research conducted by Sheoran et al. [21] who investigated the extract's antimicrobial ability against the proliferation of both Gram-negative bacteria (*K. pneumoniae*, *E. coli*) and Gram-positive (*S. aureus*, *B. subtilis*). In comparison with other areas and amoxyclav, their extract had the strongest antibacterial activity against *B. subtilis* (19.50.71 mm). While, inhibitory zones of *K. pneumoniae* (21.52.12), *E. coli* (17.50.71), and *S. aureus* (21.01.41 mm) were identified. Methanolic extracts of medicinal plant parts were found to have antibacterial activity against *S. aureus*, *B. subtilis*, *P. multocida*, and *E. coli* in the current study.

Table 4. Antibacterial Activity of Different Medicinal Plants

Plants/ Bacterial Strains	*Withaniasomnifera*	*Cnidiummonnieri*	*MuiraPuama (Ptychopetal umolacoides)*	*Epimedium grandiflorum*	Rifampicin
Antimicrobial activity (Inhibition zone in mm)					
E. coli	16 ± 1.1	17± 1.9	28 ± 2.1	29 ± 1.5	34 ± 2.0
B. subtilis	17 ± 1.7	18± 0.9	22 ± 2.3	25 ± 1.9	35 ± 2.1
P. multocida	11 ± 0.4	13 ± 1.0	20 ± 1.4	22 ± 1.8	32 ± 1.9
S. aureus	08 ± 0.35	12 ± 0.8	23 ± 1.6	31 ± 2.4	36 ± 2.7

Table 5. Antifungal Activity of Different Medicinal Plants

Plants/ Bacterial Strains	Withaniasomnifera	Cnidiummonnieri	MuiraPuama (Ptychopetal umolacoides)	*Epimedium grandiflorum*	Terbenafin
	Antifungal activity (Inhibition zone in mm)				
A. niger	6 ± 0.31	7 ± 0.42	11 ± 0.81	9 ± 0.61	28 ± 2.0
A. flavus	5 ± 0.11	8 ± 0.38	09 ± 0.41	12 ± 0.38	29 ± 1.3
F. solani	--	--	6 ± 0.31	8 ± 0.33	29 ± 2.1
A.alternaria	8	--	13 ± 0.55	11 ± 0.51	26 ± 2.2

3.3. Renal Function Test

Total RFT's status was also determined from male group named normal. The data obtained has been presented in Table 6 below. The Fisher LSD method was used to determine the grouping information using 95% confidential interval. Normal groups with normal diet, injected CCl4 low dose 20mg/kg body weight, injected testosterone per body weight, injected Laboob e Kabeer medicine per body weight, plant extract *Muira puama* low dose 100mg/kg body weight, *Muira pauma* high dose 200mg/kg body weight, plant extract *Epimedium grandiflorum* low dose 100mg/kg body weight, *Epimedium grandiflorum* high dose 200mg/kg body weight, injected PHP low dose 50mg/kg body weight, injected PHP high dose 150mg/kg body weight. Values are mean ± SE (Standard error) of means of the study groups. Rows/bars are sharing the same letters or without letters

or are non- significantly ($p>0.05$) different. The $p<0.05$ is considered significant, while $p<0.001$ indicates highly significant value. The use of PHP showed significant results as compared to 20% CCl4 treated group. Total RFT's status was also determined from male group named intoxicated. The data obtained has been presented in Table 6 below and bar graphs have been constructed by using Microsoft Excel 2016. Results showed that the level of creatinine was 0.808±0.038 in Laboob e Kabeer group as compared to 0.608±0.032 in phytomedicine group.

Table 6. Renal Function Tests' Analysis from Normal Groups

Tests	Control	Testosterone	Laboob e Kabeer	Phytomedicine
Urea	51.2±5.12	58±5.10	39.6±3.7	36.2±4.32
Creatinine	0.722±0.061	0.898±0.071	0.808±0.038	0.608±0.032
Uric acid	4.72±0.33	4.34±0.4	4.34±0.30	3.3±0.37

First of all, normal groups with normal diet, injected CCl4 low dose 20mg/kg body weight, injected testosterone per body weight, injected Laboob e Kabeer medicine per body weight, plant extract *Muira puama* low dose 100mg/kg body weight, *Muira pauma* high dose 200mg/kg body weight, plant extract *Epimedium grandiflorum* low dose 100mg/kg body weight, *Epimedium grandiflorum* high dose 200mg/kg body weight, injected PHP low dose 50mg/kg body weight, injected PHP high dose 150mg/kg body weight. Values are mean ± SE (Standard error) of means of the study groups. Rows/bars are sharing the same letters or without letters or are non- significantly ($p> 0.05$) different. The $p<0.05$ is considered significant, while $p< 0.001$ indicates highly significant value. Results showed that the level of urea was raised in control group, that is, 67.2±4.91, however, this level was restored in plant extracts' treated group, that is, 36.6±2.88. Likewise, the level of uric acid was restored in plants treated group, that is, 3.96±0.34 as compared to 6.78±0.26, observed in intoxicated group in which CCl4 was administrated. Anto et al. [22] investigated the oral chronic toxicity of ethanol extract of balakka fruit (*Phyllanthus emblica*) and determined that ethanol extract of *P. emblica* (EEPE) considerably reduced the amount of urea in female rat body which was consistent with the observations.

Table 7. Renal Function Tests of Intoxicated Group Against Phytomedicine

Tests	Control	CCL4	Testosterone+CCl4	Laboob e Kabeer+CCl4	PHP+CCl4
Urea	51.2±5.12	67.2±4.91	49.4±5.32	34.4±3.65	36.6±2.88
Creatinine	0.722±0.061	1.642±0.06	0.682±0.05	0.522±0.06	0.684±0.02
Uric acid	4.72±0.33	6.78±0.26	3.1±0.24	3.18±0.26	3.96±0.34

3.4. Liver Function Test

Total LFT's status was also determined from male group named normal. The Fisher LSD method was used to determine the grouping information using 95% confidential interval. Normal groups with normal diet, injected CCl4 low dose 20mg/kg body weight, injected testosterone per body weight, injected Laboob e Kabeer medicine per body weight, plant extract *Muira puama* low dose 100mg/kg body weight, *Muira pauma*high dose 200mg/kg body weight, plant extract *Epimedium grandiflorum* low dose 100mg/kg body weight, *Epimedium grandiflorum* high dose 200mg/kg body weight, injected PHP low dose 50mg/kg body weight, injected PHP high dose 150mg/kg body weight. Values are mean ± SE (Standard error) of means of the study groups. The $p<0.05$ is considered significant, while $p< 0.001$ indicates highly significant value. All LFT's showed the lowest p-value ($p<0.05$) which means that the result is highly significant. The use of PHP showed significant results as compared to 20% CCl4 treated group. Total LFT's were also determined from different male groups treated with the desired herbal medicines. The overall data has been presented in Table 8 in which results were highly significant as compared to allopathic drugs and control groups.

First of all normal groups with normal diet, injected CCl4 low dose 20mg/kg body weight, injected testosterone+CCl4 per body weight, injected Laboob e Kabeer+CCl4 medicine per body weight, plant extract *Muira puama*+CCl4low dose 100mg/kg body weight, *Muira pauma*+CCl4 high dose 200mg/kg body weight, plant extract *Epimedium grandiflorum*+CCl4low dose 100mg/kg body weight, *Epimedium grandiflorum*+CCl4 high dose 200mg/kg body weight, injected PHP+CCl4 low dose 50mg/kg body weight, injected PHP+CCl4 high dose 150mg/kg body weight. The Mean SE (Standard error) values were calculated for each group. Rows/bars are sharing the same letters or without letters or are non-significantly ($p> 0.05$) different. The $p<0.05$ is considered significant, while $p< 0.001$ indicates highly significant value. All LFT's showed the lowest p-value ($p< 0.001$) which means that the result is highly significant. The use of PHP showed highly significant results as compared with 20% CCl4

treated group. Naz and Abbas [23] investigated the hepatoprotective activity of *P. emblicus* and *fruits of silymarin* against cisplatin-induced hepatotoxicity and found that their fruit extract considerably lowered the levels of ALT and AST in rat models which agreed with the outcomes of the current study.

Table 8. Potential of Plants Extract on Liver Enzymes of Intoxicated Groups of Male Rats

Tests	Control	Intoxicated	Testosteron	Laboob e kabeer	MP	Epi	PHP
ALT	12.6 ±5.8	243.6 ±8.23	59.6 ±4.98	47 ±4.06	48.8 ±3.27	66.4 ±6.1	59.8 ±3.96
AST	261.4 ±9.02	270 ±12.6	110.4 ±7.33	137.2 ±8.70	152.6 ±7.4	118.4± 5.99	126.4 ±6.5
ALP	347.4 ±9.7	627.2 ±9.5	215.2 ±14.5	293 ±13.2	597.2 ±20.2	334.6± 14.13	362.8 ±11.2
LDH	1638.6 ±229.3	2448 ±186.5	1578.6 ±53.6	1668.4 ±68.8	1598.4 ±89.1	1093.4 ±84.4	854.6 ±52.8

3.5. Hepatoprotective Effect

Its antioxidant action, which boosts glutathione content and reduces lipid peroxidation and hydroperoxides in the liver and returns their activities to normal, is responsible for its hepatoprotective properties. Liver plays a crucial role in the body's metabolic processes, release of important hormones, storage of nutrients, and detoxification of harmful chemicals. However, the antioxidant process can prevent further damage to the liver from oxidative stress and free radicals [24]. The greatest place to get these antioxidants, which can also mediate hepatoprotective effect, is in plant extracts.

The histological examination of liver tissues revealed that CCl4 intoxication of rats significantly damaged the hepatic cells that showed hyperchromatism, hyperplasia, and proliferating hepatocyte as compared to normal control group and PHP treated liver of rat group testis in which the architecture of the liver was restored showing regeneration of normal hepatocytes (Figure 01). Figure 01 shows the normal architecture of liver tissue in which hepatocytes are in plate shape, sinusoidal are between these plates, and various degeneration regions are also shown. Figure 01 shows that certain small sinuses really contain inflammatory cells (leukocytes). Some leukocytes congregate in liver parenchyma and near blood vessels.

Histo-architecture was much better in sections from rats given hydroethanolic plant extract following CCl4 poisoning. Hepatocyte infiltration is greatly reduced and fibrosis in the liver is kept to a minimum in liver sections from the plant-treated group, demonstrating the hepato-protective action of PHP. Kerdput and Pradidarcheep [25] examined the potential of hua-khao-yen extract against hepatocellular carcinoma and reported that significant restoration was seen in regeneration of normal liver cells. Their results positively supported the findings of the current study.

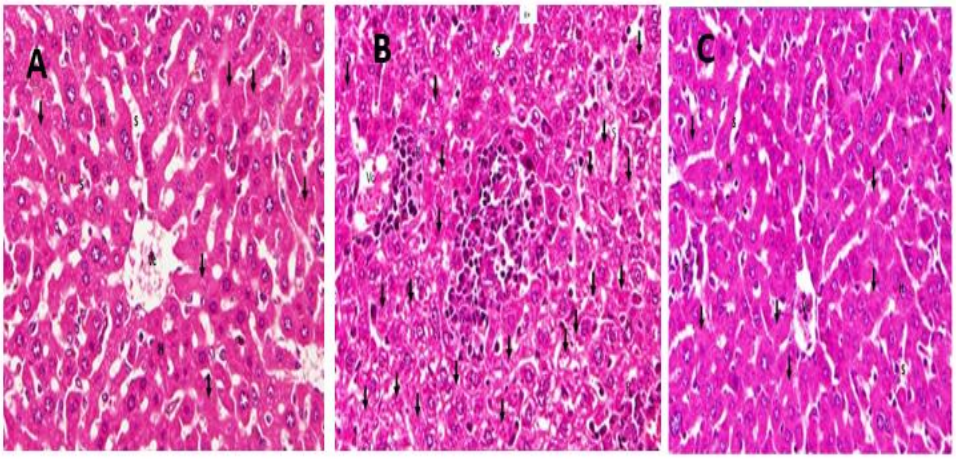

Figure 1 A. The 400x magnification of the histology of normal liver in which H (hepatocytes) are in plate shape, (S) sinusoidal are between these plates, arrows show the degenerative regions. Sinusoid contains erythrocytes and little leukocytes. Central vein (Vc). **B.** Histology of the liver tissue at a 400x magnification in CCl4 induction mice. The majority of hepatocytes show indications of degeneration (arrows) including lysis and vacuole cytoplasm or fuzzy nuclei. Hepatocytes are grouped in plates with sinusoids which seem thin and contain inflammatory cells (leukocytes) between the hepatocyte plates. Some leukocytes collect in groups around blood vessels and in the parenchyma of the liver (R). Vc, or the central vein. **C.** PHP was handled, Hepatocytes are organised in plates, and between each plate are sinusoids that contain inflammatory cells (leukocytes). Certain leukocytes gather around blood vessels and in the parenchyma and certain hepatocyte cells exhibit symptoms of senescence (arrow) including the central vein (Vc) and hepatic vein (Vh).

3.6. Conclusion

Medicinal herbs have been used by the mankind to treat a wide range of disorders since long. *Withania somnifera* and *Cnidium monnieri* have a wide range of therapeutic applications. These beneficial effects of *Epimedium* species are due to the presence of various phytoconstituents and about more than 260 compounds have been isolated; among them prenyl-flavonoids are the major constituents and also important chemotaxonomic markers. By checking the qualitative analysis of plants, it was analyzed that PHP was rich in phytochemicals, that is, phenolics, flavonoids, Tannins, and Saponins contents. ANOVA was applied and comparison test was used for data analysis. Clinical trials were conducted on rats as experimental models and all the rats except normal group/control group were intoxicated with CCl_4. Based on results, it can be stated that the polyherbal preparation of selected plants has significant antimicrobial potential as compared to standard drugs. Significant in vitro and in vivo antioxidant potential, and significant hepatoprotective capacity in male albino rats shown and therapeutic effect is directly proportional to doses of ethanolic extract administered. The results show that the hepatoprotective effect depends on the induction of drug dose. If low dose of drug is induced, the hepatoprotective effect as well as the rapturation of cells would not be present. Similarly, with the intermediate drug dose, the hepatoprotective effect was good as well as rapturation of cells was not present. If the high dose of drug is induced, the results would not be significant. High dose damaged the cells of liver. The hepatoprotective effect was minimal in order to solve these challenges, especially on a global scale. However, an additional research must be conducted to extract the new chemicals found in this medicinal plant.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

REFERENCES

1. Hardie J. Hormones and reproduction. In: Leather SR, ed. *Insect Reproduction*. CRC Press; 2018:95–108.

2. Hamilton KJ, Hewitt SC, Arao Y, Korach KS. Estrogen hormone biology. *Curr Top Dev Bio*. 2017;125:109–46. https://doi.org/10.1016/bs.ctdb.2016.12.005
3. Rooney KL, Domar AD. The relationship between stress and infertility. *Dialogues Clin Neurosci*. 2018;20(1):41–47. https://doi.org/10.31887/DCNS.2018.20.1/klrooney
4. Hanson B, Johnstone E, Dorais J, Silver B, Peterson CM, Hotaling J. Female infertility, infertility-associated diagnoses, and comorbidities: a review. *J Assist Reprod Genet*. 2017;34:167–77. https://doi.org/10.1007/s10815-016-0836-8
5. Stellar C, Garcia-Moreno C, Temmerman M, van der Poel S. A systematic review and narrative report of the relationship between infertility, subfertility, and intimate partner violence. *Int J Gynaecol Obstet*. 2016;133(1):3–8. https://doi.org/10.1016/j.ijgo.2015.08.012
6. Rishi G, Subramaniam VN. The liver in regulation of iron homeostasis. *Am J Physiol Gastrointest Liver Physiol*. 2017;313(3):G157–G165. https://doi.org/10.1152/ajpgi.00004.2017
7. Tripathi A, Debelius J, Brenner DA, et al. The gut–liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol*. 2018;15(7):397–411. https://doi.org/10.1038/s41575-018-0011-z
8. Andrade RJ, Chalasani N, Björnsson ES, et al. Drug-induced liver injury. *Nat Rev Dis Primers*. 2019;5(1):e58. https://doi.org/10.1038/s41572-019-0105-0
9. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol*. 2017;23(47):8263–8276. https://doi.org/10.3748%2Fwjg.v23.i47.8263
10. Liss KH, Finck BN. PPARs and nonalcoholic fatty liver disease. *Biochimie*. 2017;136:65–74. https://doi.org/10.1016/j.biochi.2016.11.009
11. Mohamed F, Jean-Marc D, Stephane A, et al. Therapeutic approach out of IVF of ovulation disorders in Sub-Saharian African. *Open J Obstet Gynecol*. 2017;7(11):1116–1123. https://doi.org/10.4236/ojog.2017.711112
12. Khan T, Ali M, Khan A, et al. Anticancer plants: a review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*. 2019;10(1):e47. https://doi.org/10.3390/biom10010047

13. Nerdy N, Ritarwan K. Hepatoprotective activity and nephroprotective activity of peel extract from three varieties of the passion fruit (Passiflora sp.) in the albino rat. *Open Access Maced J Med Sci*. 2019;7(4):536–542. https://doi.org/10.3889%2Foamjms.2019.153
14. Hudaib MM, Tawaha KA, Mohammad MK, et al. Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. *Pharmacogn Mag*. 2011;7(28):320–324. https://doi.org/10.4103%2F0973-1296.90413
15. Jain A, Ranade R, Pritam P, Joshi N, Vavilala SL, Jain A. A comparative study of antioxidant activity, total phenolic and flavonoid contents in different parts of Helicteres isora L. *Am J Life Sci*. 2014;2(5):292–302.
16. Neupane P, Lamichhane J. Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. *Vegetos*. 2020;33:360–366. https://doi.org/10.1007/s42535-020-00116-7
17. Zhang A, Sun H, Yan G, Wang X. Recent developments and emerging trends of mass spectrometry for herbal ingredients analysis. *Trends Analyt Chem*. 2017;94:70–76. https://doi.org/10.1016/j.trac.2017.07.007
18. Montgomery DC. *Design and Analysis of Experiments*. Wiley; 2017.
19. Chaphalkar R, Apte KG, Talekar Y, Ojha SK, Nandave M. Antioxidants of Phyllanthus emblica L. Bark extract provide hepatoprotection against ethanol-induced hepatic damage: a comparison with silymarin. *Oxid Med Cell Longev*. 2017;2017:e3876040. https://doi.org/10.1155/2017/3876040
20. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines*. 2018;5(3):e93. https://doi.org/10.3390/medicines5030093
21. Sheoran S, Nidhi P, Kumar V, et al. Altitudinal variation in gallic acid content in fruits of Phyllanthus emblica L. and its correlation with antioxidant and antimicrobial activity. *Vegetos*. 2019;32:387–396. https://doi.org/10.1007/s42535-019-00048-x
22. Anto EJ, Syahputra RA, Silitonga HA, Situmorang PC, Nugaraha SE. Oral acute toxicity study extract ethanol of balakka fruit (Phyllanthus emblica). *Pharmacia*. 2022;69(1):187–194.
23. Naz M, Abbas N. Study of Hepatoprotective effect produced by the fruits of Phyllanthus emblicus and silymarin against cisplatin induced

hepatotoxicity. *Br J Pharm Res*. 2016;10(6):1–7. https://doi.org/10.9734/BJPR/2016/22904

24. Li L, Mo F, Hui EP, et al. The association of liver function and quality of life of patients with liver cancer. *BMC Gastroenterol*. 2019;2019:e66. https://doi.org/10.1186/s12876-019-0984-2
25. Kerdput V, Pradidarcheep W. *Effects Of Hua-Khao-Yen Extract (Dioscorea Membranacea Pierre) In Hepatocellular Carcinoma-Induced Rat* [dissertation]. Bangkok: Srinakharinwirot University; 2020.