Original Article

The Hepatoprotective and Anti-Nephrotoxic Potential of Methanolic Extract of a Polyherbal Preparation in CCl_4-Induced Liver Injury Model of Wistar Rats

Dose-Response:
An International Journal
July-September 2022:1–7

DOI: 10.1177/15593258221124728
journals.sagepub.com/home/dos
SAGE

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Abstract

The liver and kidneys are the vital organs of the body and perform important life-sustaining functions in the body. Synthetic drugs used in the treatment of liver and kidney diseases are sometimes inadequate and can lead to serious side effects. Medicinal herbs and plants were used to combat diseases for a long time and combination therapy is preferred over single plant therapy. In the current study, the *Asparagus racemosus, Mucuna pruriens, Anacyclus pyrethrum*, and *Tribulus terrestris* polyherbal preparation (PHP) was selected to evaluate its hepatoprotective, antioxidant, and anti-nephrotoxic potential. The methanolic extract of PHP was prepared following standard protocols. Fifty-six albino rats were divided into 7 groups (n = 8). The negative control (NC) having the healthy rats and the remaining 6 groups were induced liver toxicity by intraperitoneally injecting 0.5 mL/kg of 50% CCl_4 in olive oil. Group 2 was positive control and group 3 and 4 received silymarin standard drug at the dose of 100 and 200 mg/kg body weight. Groups 5, 6, and 7 (PHP-1, PHP-2, PHP-3) were the liver-damaged rats receiving the PHP at a dose of 50, 100, and 150 mg/kg body weight. Blood samples were collected at 21 of the trial, to evaluate oxidative stress, hepatoprotective and anti-nephrotoxic potential. Results of liver function tests revealed significant ($P < .05$) hepatoprotective activities of PHP after intoxication with CCl_4 of albino rats as compared to standard groups. Moreover, results of renal functions also showed that PHP has a significant ($P < .05$) restoring the capacity of blood urea, creatinine, and uric acid in intoxicated rats as compared with the control group. The PHP also reduced the oxidative stress in the treatment groups by increasing the total antioxidant capacity and reducing the total oxidative status. It can be concluded that selected medicinal plants have a potential role in the management of liver and kidney disorders. So, by running the clinical trial on a large scale and by isolating the phytochemical constituents responsible for hepatoprotective and nephroprotective activities, locally prepared drugs could be developed to manage liver and renal disorders.

Keywords

Asparagus racemosus, *Mucuna pruriens*, *Anacyclus pyrethrum*, *Tribulus terrestris*, polyherbal preparation, oxidative stress, hepatoprotective, anti-nephrotoxic

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Received 14 May 2022; received revised 18 August 2022; accepted 18 August 2022

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Introduction

The liver plays an important role in detoxifying poisons from the blood, digesting food, processing medication, and restoring nutrients and energy. Genetic factors obesity, infections, liquor utilization and long-term use of medicines, for example, anti-depression medications, analgesics, anabolic steroids, and oral contraceptives are a portion of the normal elements causing liver ailments. Untreated liver sicknesses may likewise bring about Liver Cirrhosis which is a major cause of death.[1,2] In the body, internal metabolism and toxicant exposure can be caused to produce reactive oxygen species in humans which lead to oxidative pressure.

The kidney is another vital organ of the body to play an important role in maintaining blood volume and electrolyte balance. Nephrotoxicity is one of the most overall kidney troubles that outcome from the introduction of an extrinsic or intrinsic toxicant. The medicinal plants extract has phenolic and triterpenoid compounds as well as vitamin C. The antioxidant potential of these extracts suppressed the CCl_4-induced oxidative stress by scavenging the reactive oxygen species and increasing the cellular antioxidant index. These extracts also reduce the CCl_4-induced inflammation by inhibiting the gene expression of NF-кБњ амг зМНСњ амг зм рнтатзмё тжд кдфдк не мзтрзв нцзгдћ[3] In past, it has been seen that medicinal herb treatment has developed as another option and better methodology for the treatment of kidney stones and urolithiasis, as the greater part of the traditional treatments accessible to date are not 100% powerful.[4]

Since long ago, herbal compounds are found to be used as traditional medicine for the treatment of several diseases. The manufacturer of traditional medicine had an edge as they do not have the requirement to prove claims about treating diseases are valid or not; in contrast, if they deal with a product which would be a drug, they need to provide several proofs. Some of those compounds from herbal products might be safe, while some are not. There are some assumptions for these reports with the possibility of the products being contaminated or being in contact with toxins, heavy metals, and drugs or possibly not containing the claimed/listed ingredients. There is also the possibility of some traditionally used herbal medicines interacting with drugs, resulting in serious side effects, or might be considered unsafe for numerous patients with specific conditions.[5] So, all the traditional and folk medicine with no research data must be scientifically approved for their efficacy and side effects. In the present study, we aim to evaluate the hepatoprotective and nephroprotective effects of a polyherbal preparation (PHP) comprising methanolic extracts of four plants, *A racemosus, M pruriens, A pyrethrum*, and *T terrestris*

Methods

Procurement of Plants

All four plants, *A racemosus, M pruriens, A pyrethrum*, *and T terrestris* were collected locally. The plant was identified by an expert Botanist and kept in the herbarium of the Department of Botany, Government College University Faisalabad, Pakistan.

Extract Preparation

After washing with distilled water, the plants were shade dried and ground into a fine powder and 50 g of each plant powder was mixed and soaked for 72 h in 250 mL of methanol with periodically stirring and mixing. The solution was subsequently sieved through Whatman® filter paper. The extract after filtration was evaporated and concentrated using a rotary evaporator (SCI100-Pro; SCILOGEX, USA) at 40°C and transferred into a Petri dish and kept in an incubator at 40°C until dried properly. The percentage yield was calculated as 15.7%. The extract was stored at 4°C till further analysis.

Qualitative Phytochemical Analysis

Phytochemical analysis of a methanolic extract of the polyherbal preparation was carried out qualitatively using standard methods as described by Mustafa et al.[6] to verify the presence or absence of potentially active phytochemicals.

Experimental Design

Following the approval of all animal protocols by the Animal Care and Ethical Committee, Government College University of Faisalabad, 56 albino (Wistar strain) rats of mixed gender, aged 8 ± 1 weeks, weighing 260 ± 10 g, were procured from the Department of Physiology Animal Experimental Station, Government College University, Faisalabad, Pakistan. The rats were housed in elevated wired cages with ad libitum access to food and drinking water, at standard conditions (temp = 26°C ± 2°C; light = 12 h light and dark cycle; ambient humidity = 40–60%). The rats were divided into 7 groups with an equal number of rats (n = 8). The negative control (NC) having the healthy rats and the remaining 6 groups were induced liver toxicity by intraperitoneally injecting 0.5 mL/kg of 50% CCl_4 in olive oil.[7,8] After the confirmation of liver damage, group 2 was named as a positive control with damaged liver receiving no drug. Groups 3 and 4 were the standard controls (SC-1 and SC-2) receiving silymarin standard drug at the dose of 100 and 200 mg/kg body weight, respectively.[7] Groups 5, 6 and 7 (PHP-1, PHP-2, PHP-3) were the liver-damaged rats receiving the PHP at a dose of 50, 100, and 150 mg/kg body weight, respectively. The dose of the PHP treatment was adjusted according to the previous literature.[9–12] After the completion of 21 days of treatment, all the rats were sacrificed by cervical decapitation for the collection of blood and serum was separated by centrifugation at 2000 r/min for 10 min and stored at −20°C till further analysis.

Total Antioxidant Capacity, (TAC, mmolTroloxequiv./L)

The method for evaluating total antioxidant capacity (TAC) in serum samples was previously defined by Nisar et al. (2017).[13]

In short, the presence of antioxidants in the sample bleaches the orthodianisidine color in the assay reagent. Increased antioxidant levels in the sample contribute to higher bleaching and reduced absorbance, suggesting an inverse standard curve. The Biolab® 310 semi-auto analyzer was used with biochromatic wavelength modification (660 and 870 nm) by calibrating vitamin C standards at 1, 3, 5, and 7 mmol/L concentrations. This assay had a minimum observable value of .18 mmol/L and linearity of up to 7 mmol vitamin C equivalent/L, with a coefficient of variance (CV) below 3% in the intraassay.

Total Oxidant Status (TOS; μmol H_2O_2 equiv./L)

The serum TOS was measured by using the technique previously adopted by Mustafa et al.[14] The standard curve was constructed from different hydrogen peroxide (H_2O_2) concentrations and the TOS was represented as μmol H_2O_2 equivalent/L. The assay's detection range was <3% and the CV of the Intra assay was held below 10% and linearity was up to 200 μMol H_2O_2 equivalent/L.[14]

Serum Liver Enzymes

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels were examined by commercially available kits from Sigma diagnostics Co. (Cat#: 112 024, Cat#: 112 003 respectively). Total protein and albumin were also measured by using colorimetric assay through commercially available kits by Sigma diagnostics (Cat#: 610, Cat#: 913, respectively).

Evaluation of Renal Function Tests

"Urease – GLDH": enzymatic UV test described by Thomas, (1998) was utilized to evaluate the urea level in the serum samples. A kinetic test without deproteinization as demonstrated by the Jaffé system was used to measure the serum creatinine level (Newman, 1999).[15] Serum uric acid level was examined using the methods for changed trinder peroxidase test using 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB; Fossati et al., 1980).[16]

Statistical Analysis

The SPSS software version 23 was used for statistical analysis. All datasets were statistically demonstrated as mean +standard error. One-way Analysis of Variance (one-way ANOVA) was observed to seek the significance of differences among various groups followed by Turkey's post hoc test. The level of significance was actively considered at $P < .05$.

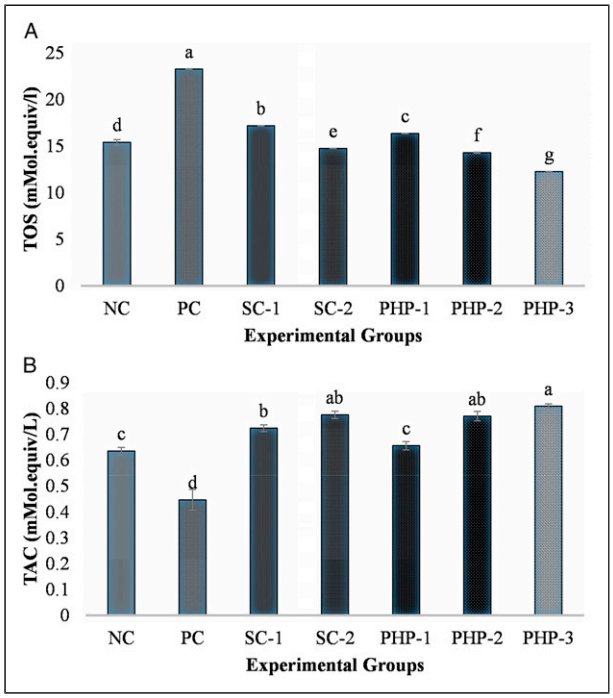

Figure 1. Effect of Silymarin (standard drug) and polyherbal preparation (PHP) methanolic extract on TAC (A) and TOS (B) in the serum of rats of various groups of the study. NC is Negative control, PC is Positive control, SC-1 and SC-2 means standard control groups receiving silymarin at the dose of and 100 and 200 mg/kg body weight, respectively, PHP-1, PHP-2, and PHP-3 shows the liver-damaged rats receiving the PHP at dose of 50, 100, and 150 mg/kg body weight, respectively. Different alphabets (a–c) on the bars show significant difference among the groups.

Results

Oxidative Stress Markers

The results of TAC and TOS are shown in Figure 1. The data are represented in mean ± standard error of a total of 8 samples for each experimental group. The mean TAC was decreased significantly ($P \leq .05$) in the PC group (.45 ± .04^b mM/L) as compared with other experiment groups (Figure 1). There was an increase in the TAC value in all the silymarin- and PHP-treated groups as compared with the PC and NC groups. The polyherbal preparation treatment significantly ($P \leq .05$) decreased the TOS in the PHP-1, PHP-2, and PHP-3 groups in a dose-dependent manner as compared with the PC and NC groups. The TOS levels were also decreased significantly ($P \leq .05$) in the silymarin-treated groups SC-1 and SC-2 in a dose-dependent manner.

Liver Enzymes and Bilirubin

The results of liver enzymes, ALT, AST, ALKP, and bilirubin are shown in Table 1. The data are represented in

Table 1. Effect of Silymarin (Standard Drug) and Polyherbal Preparation Methanolic Extract on Liver Enzymes and Total Bilirubin in the Serum of Rats of Various Groups of the Study.

	NC	PC	SC-1	SC-2	PHP-1	PHP-2	PHP-3
ALT (IU/L)	39.33 ± 2.08^{d}	110.33 ± 4.51^{a}	58.00 ± 2.0 ^{b}	55.67 ± 1.53^{bc}	55.00 ± 1.00^{bc}	53.67 ± 1.53^{c}	56.3 ± 1.5^{b}
AST (IU/L)	204.67 ± 7.08^{d}	432.67 ± 3.89 ^{a}	381.33 ± 4.60^{b}	225.00 ± 6.75^{b}	306.33 ± 3.89^{bc}	267.33 ± 6.38^{c}	282 ± 5.79^{c}
ALP (IU/L)	282.67 ± 13.01^{b}	451.00 ± 9.0 ^{a}	186.33 ± 10.26^{d}	179.67 ± 5.51^{d}	184.67 ± 3.21^{d}	148.67 ± 3.51^{e}	218 ± 6.56^{c}
T.Bil (IU/L)	0.47 ± 0.15^{d}	1.03 ± 0.15 ^{a}	0.80 ± 0.10^{b}	0.70 ± 0.02^{bc}	0.71 ± 0.08^{bc}	0.62 ± 0.03^{c}	0.63 ± 0.04^{c}

NC is Negative control, PC is Positive control, SC-1 and SC-2 means standard control groups receiving silymarin at the dose of and 100 and 200 mg/kg body weight, respectively, PHP-1, PHP-2, and PHP-3 shows the liver-damaged rats receiving the PHP at dose of 50, 100, and 150 mg/kg body weight, respectively. Different alphabets $^{(a–e)}$ on the values in a row show significant difference among the groups.

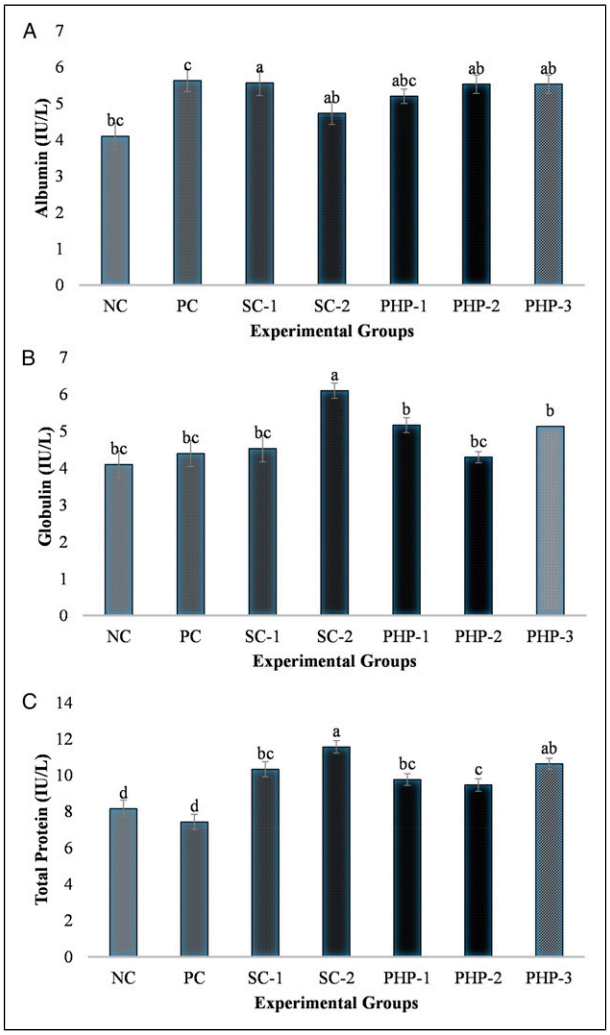

Figure 2. Effect of Silymarin (standard drug) and polyherbal preparation methanolic extract on albumin (A), globulin (B), and total proteins (C) in the serum of rats of various groups of the study. NC is negative control, PC is positive control, SC-1, and SC-2 means standard control groups receiving silymarin at the dose of and 100 and 200 mg/kg body weight respectively, PHP-1, PHP-2, and PHP-3 shows the liver-damaged rats receiving the PHP at dose of 50, 100, and 150 mg/kg body weight, respectively. Different alphabets (a–c) on the bars show significant difference among the groups.

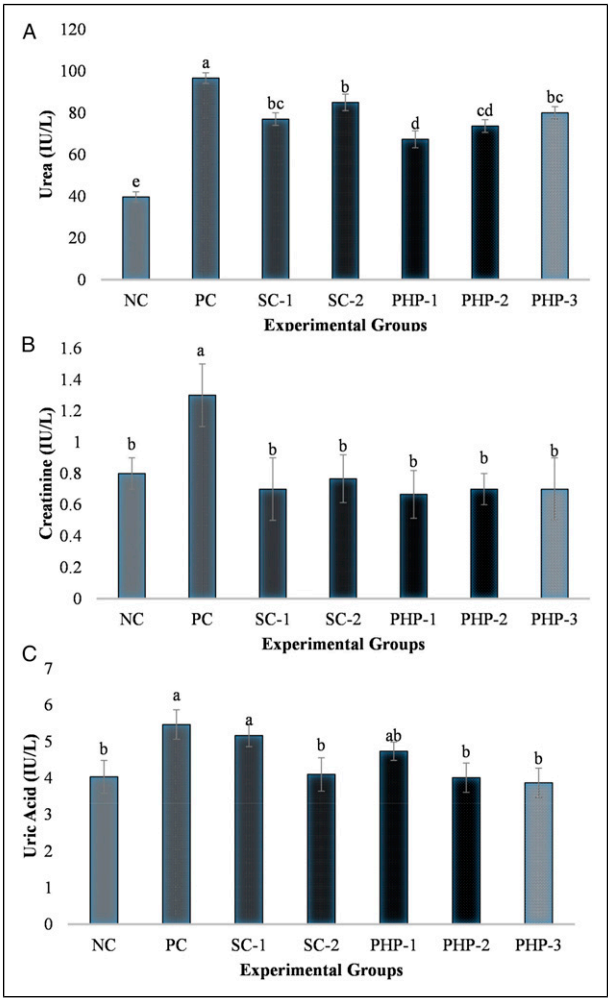

Figure 3. Effect of silymarin (standard drug) and polyherbal preparation methanolic extract on urea (A), creatinine (B), and Uric acid (C) in the serum of rats of various groups of the study. NC is negative control, PC is positive control, SC-1 and SC-2 means standard control groups receiving silymarin at the dose of and 100 and 200 mg/kg body weight respectively, PHP-1, PHP-2, and PHP-3 shows the liver-damaged rats receiving the PHP at dose of 50, 100, and 150 mg/kg body weight, respectively. Different alphabets (a–c) on the bars show significant difference among the groups.

mean ± standard error of a total of 8 samples for each experimental group. The mean ALT was increased significantly ($P \leq .05$) in the PC group as compared with all other experimental groups including the NC group, which shows liver damage due to CCL_4 toxicity. However, this rise was normalized by treatment with the silymarin and PHP in the SC-1, SC-2, PHP-1, PHP-2, and PHP-3 groups that show a significant ($P \leq .05$) decrease in the ALT levels as compared with the PC group. A similar trend was seen in the case of AST, ALKP, and bilirubin levels. The serum levels of AST, ALKP, and bilirubin were increased significantly ($P \leq .05$) in the PC group as compared with all other experimental groups. And by treating with the silymarin and PHP in the SC-1, SC-2, PHP-1, PHP-2, and PHP-3 groups there was a significant ($P \leq .05$) decrease in the AST and ALKP levels as compared with the PC group.

Serum Proteins

The results of serum proteins, albumin, globulin, and total proteins are shown in Figure 2. The data are represented in mean ± standard error of a total of 8 samples. Serum albumin level was significantly increased in the PC group but was not significant in the silymarin- and PHP-treated groups. The serum globulin levels were increased significantly in the SC-2 and PHP-3 groups as compared with the PC group. While the total protein level was increased in all the silymarin- and PHP-treated groups as compared with the PC group.

Renal Function Tests

The results of serum levels of urea, uric acid, and creatinine are shown in Figure 3. The data are represented in mean ± standard error of a total of 8 samples. The results show that the serum urea level was significantly increased in the PC group as compared to the NC group. However, this rise was normalized by treatment with the silymarin and PHP in the SC-1, SC-2, PHP-1, PHP-2, and PHP-3 groups showing a significant ($P \leq .05$) decrease in the serum urea levels as compared with the PC group. A similar trend was seen in the case of serum creatinine levels that were increased significantly ($P \leq .05$) in the PC group as compared with all other experimental groups. While the treatment with the silymarin and PHP in the SC-1, SC-2, PHP-1, PHP-2, and PHP-3 groups significantly ($P \leq .05$) decreased the serum urea level as compared with the PC group. Serum uric acid level was also increased significantly ($P \leq .05$) in the PC group as compared with the NC group. Silymarin showed no effect on the serum uric acid level at the low dose of 100 mg/kg in the SC-1 group but its higher dose of 200 mg/kg was effective significantly ($P \leq .05$) decreasing the serum uric acid level in the SC-2 group. Similarly, PHP treatment was effective in significantly ($P \leq .05$) decreasing the serum uric acid levels at the dose of 100 and 150 mg/kg in PHP-2 and PHP-3 groups, respectively.

Discussion

Medicinal plants have various bioactive compounds, including terpenoids, flavonoids, glycosides, and alkaloids, having antifungal, antibacterial and antioxidant activities. Selected portions of plants in the current study were screened for the presence of phytochemical constituents. Results showed that phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids were present in studied medicinal plants. Phenolic constituents are reported to be present in almost all types of plants. They comprise phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans, and flavonoids. Significant advancements in the research field concluded that phenolic compounds have been found to have therapeutic and nutritious value. Phenolic contents possess a significant role in reducing oxidative stress.[6,17] In the current study, polyherbal preparation increased the TAC level and decreased the TOS level in the toxic rats. In an in vitro study, Karuna et al.[18] described the antioxidant activity of roots of *A racemosus* that is part of the current polyherbal preparation. Similarly, different studies also described the antioxidant activities of the other plants of polyherbal preparation. In another study, Tumbas-Saponjac et al.,[19] demonstrated the *M pruriens* as a good antioxidant source. *A pyrethrum and T terrestris* plants also have demonstrated antioxidant effects.[20,21] In our study, polyherbal preparation of these plants also shows the in vivo antioxidant activity by decreasing the TOS and increasing the TAC which might be due to the presence of phenolic and flavonoid constituents in the plants included in the polyherbal preparation.[22]

In the current study, polyherbal preparation shows the hepatoprotective response by normalizing the ALT, AST and ALKP levels. ALT, AST, ALKP and bilirubin have great importance in the liver function test. Similar to our study, *A racemosus* decreased the ALT and AST levels in the t-BHP-treated rats.[23] In a study, *T terrestris* at the dose of 200 mg/kg protects the liver against cadmium toxicity.[24] Recently, Okorie et al.[25] demonstrated the *M pruriens* leaf extract effect on the liver. The result showed that ALP, AST, ALT, and albumin were found to be statistically significant among the treated groups when compared with the control group.[25] Various studies have reported the presence of phenolics, flavonoids, and anthraquinones in the *A racemosus, M pruriens, A pyrethrum*, and *T terrestris* used to prepare the PHP as a trweatment of liver injury in current study.[26-31] The presence of these phytochemicals with proven antioxidant potential are responsible for the reversal of liver injury in current study.[32]

Kidneys play a vital role in the excretion of toxins such as creatinine, uric acid, and urea. The nephron is the functional unit of the kidney and the renal function test is important to reveal the nephrotoxicity in the patients. In the current study, a protective response toward nephrotoxicity was revealed by performing the renal function test. Polyherbal preparation decreased the urea and creatinine levels in all the groups treated with PHP. However, PHP decreased the uric acid level

in a dose-dependent manner in PHP-2 and PHP-3 groups. *T terrestris* showed a protective kidney response in nephrotoxic rats.[33] *M pruriens* at the dose of 700 mg/kg b.wt show a protective response against arsenic-induced liver and kidney toxicity by reduced levels of kidney and liver enzymes.[34] *A racemosus* showed the kidney protective effect against alloxan induced diabetes rats.[35] Similar to above-described findings, polyherbal preparation showed the nephroprotective response in the current study due to the presence of phytoconstituents in plants of PHP which reversed the oxidative stress that caused the nephrotoxicity induced by CCl4.[30]

Conclusion

The current study concludes that the methanolic extract of the poly herbal preparation consists of the four plants named, *A racemosus, M pruriens, A pyrethrum*, and *T terrestris* contains antioxidant, hepatoprotective and nephroprotective activities. The active constituents of these plants can be separated to evaluate the above-mentioned activities of each constituent of each plant separately and a cheaper drug can be prepared from the most active constituents with maximum therapeutic efficacy and less side effects.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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References

1. Giannini EG, Farinati F, Ciccarese F, et al. Prognosis of untreated hepatocellular carcinoma. *Hepatology*. 2015;61(1):184-190.
2. Gillessen A, Schmidt HHJ. Silymarin as supportive treatment in liver diseases: A narrative review. *Adv Ther*. 2020;37(4):1279-1301.
3. Abu-Serie MM, Habashy NH, Maher AM. In vitro antinephrotoxic potential of *Ammi visnaga, Petroselinum crispum, Hordeum vulgare, and Cymbopogon schoenanthus* seed or leaf extracts by suppressing the necrotic mediators, oxidative stress and inflammation. *BMC Compl Alternat Med*. 2019;19(1):1-16.
4. Gupta G, Kuppachi S, Kalil RS, Buck CB, Lynch CF, Engels EA. Treatment for presumed BK polyomavirus nephropathy and risk of urinary tract cancers among kidney transplant recipients in the United States. *Am J Transplant*. 2018;18(1):245-252.
5. Liu MZ, Zhang YL, Zeng MZ, et al. Pharmacogenomics and herb-drug interactions: Merge of future and tradition. *Evid Base Compl Alternat Med*. 2015;2015:321091.
6. Mustafa I, Faisal MN, Hussain G, et al. Efficacy of *Euphorbia helioscopia* in context to a possible connection between antioxidant and antidiabetic activities: A comparative study of different extracts. *BMC Complement Med Therap*. 2021;21(1):1-12.
7. Shokrzadeh M, Jouybari HB, Hosseinpour M, Ziar A, Habibi E. Antioxidant and protective effect of hydroalcoholic extract of *Celtis australis* L. on CCL_4 induced liver toxicity. *Pharm Biomed Res*. 2018;4(3):26-31.
8. Zangeneh MM, Zangeneh A, Tahvilian R, Moradi R. Evaluation of the nephroprotective effect of *Glycyrrhiza glabra* L aqueous extract on CCl_4-induced nephrotoxicity in mice. *Comp Clin Pathol*. 2018;27(5):1119-1126. DOI: 10.1007/s00580-018-2707-4.
9. Abdel-Kader MS, Al-Qutaym A, Saeedan ASB, Hamad AM, Alkharfy KM. Nephroprotective and hepatoprotective effects of Tribulus terrestris L. growing in Saudi Arabia. *J Pharm Pharmacog Res*. 2016;4(4):144-152.
10. El-Senosiy YA, Ahmad SA, Farid AS, Wessam EM. Hepatoprotective effect of asparagus racemosus in paracetamol induced hepatotoxicity in rats. *BVMJ*. 2015;28(1):133-137.
11. Obogwu MB, Akindele AJ, Adeyemi OO. Hepatoprotective and in vivo antioxidant activities of the hydroethanolic leaf extract of Mucuna pruriens (Fabaceae) in antitubercular drugs and alcohol models. *Chin J Nat Med*. 2014;12(4):273-283.
12. Usmani A, Mujahid MD, Khushtar M, Siddiqui HH, Rahman MA. Hepatoprotective effect of Anacyclus pyrethrum linn. Against antitubercular drug-induced hepatotoxicity in SD rats. *J Compl Integr Med*. 2016;13(3):295-300.
13. Nisar J, Mustafa I, Anwar H, Sohail MU, Hussain G, Ullah MI, and Basit A. Shiitake culinary-medicinal mushroom, Lentinus edodes (Agaricomycetes): a species with antioxidant, immunomodulatory, and hepatoprotective activities in hypercholesterolemic rats. *International journal of medicinal mushrooms*. 2017;19(11).
14. Mustafa I, Anwar H, Irfan S, Muzaffar H, Ijaz MU. Attenuation of carbohydrate metabolism and lipid profile by methanolic extract of *Euphorbia helioscopia* and improvement of beta cell function in a type 2 diabetic rat model. *BMC Complement Med Therap*. 2022;22(1):1-12.
15. Sabbagh M, Rick W, Schneider S. A kinetic method for the direct determination of creatinine in serum with 3, 5-dinitrobenzoic acid without deproteinization. *Journal of Clinical Chemistry and Clinical biochemistry. Zeitschrift fur Klinische Chemie und Klinische Biochemie*. 1988;26(1):15-24.
16. Fossati P, Prencipe L, Berti G. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clinical chemistry*. 1980;26(2):227-231.
17. Presti G, Guarrasi V, Gulotta E, et al. Bioactive compounds from extra virgin olive oils: Correlation between phenolic content and oxidative stress cell protection. *Biophys Chem*. 2017;230:109-116.
18. Karuna DS, Dey P, Das S, Kundu A, Bhakta T. In vitro antioxidant activities of root extract of *Asparagus racemosus* linn. *J Trad Complem Med*. 2018;8(1):60-65.

19. Tumbas-Saponjac V, Akpoveso OOP, Oyeniran OI, Desančić J, Četojević-Simin D. Antioxidant activity and enhanced cytotoxicity of aqueous *Mucuna pruriens* L. leaf extract by doxorubicin on different human cancer cell lines. *Phcog Mag*. 2020; 16(68):224.
20. Elazzouzi H, Zekri N, Zair T, Alaoui El Belghiti M. Total phenolic and flavonoid contents of *Anacyclus pyrethrum* Link plant extracts and their Antioxidant activity. *Karbala Int J Mod Sci*. 2019;5(4):10.
21. Tian C, Chang Y, Zhang Z, et al. Extraction technology, component analysis, antioxidant, antibacterial, analgesic and anti-inflammatory activities of flavonoids fraction from *Tribulus terrestris* L. leaves. *Heliyon*. 2019;5(8):e02234.
22. Muhammad YB, Adam AA, Jamil DU, Lukman OA, Opke JM. Effect of aqueous extract of mucuna pruriens leaves on liver and kidney function markers in wister albino rats. *Am J Innov Res Appl Sci*. 2015;1(10):379-383.
23. Jayashree GV, Kumar KH, Krupashree K, Rachitha P, Khanum F. LC–ESI–MS/MS analysis of *Asparagus racemosus* Willd. roots and its protective effects against t-BHP induced oxidative stress in rats. *Ind Crop Prod*. 2015;78: 102-109.
24. Farhan AS. Alcohol extract of *Tribulus terrestris* ameliorates liver of mice exposed to cadmium acetate. *JUAPS*. 2019;13(2): 10-15.
25. Okorie N, Fasogbon SA, Ajileye AB, Adegeye FO. Effects of *Mucuna pruriens* leaf extract on the liver of wister rats. *J Med Lab Sci*. 2020;30(1):107-117.
26. Acharyaa SR, Acharyaa NS, Bhangalea JO, Shahb SK, Pandyac SS. Antioxidant and hepatoprotective action of Asparagus racemosus Willd. root extracts. *Indian J Exp Biol*. 2012;50: 795-801.
27. Altay D, Dogan Y, Orhan C, et al. Hepatoprotective effects of *Tribulus terrestris*, Ashwagandha and N-acetylcysteine on liver fibrosis in carbon tetrachloride-induced rats. *Acta Pol Pharm -Drug Res*. 2019;76(5):805-813.
28. Gunarathne R, Nadeeshani H, Lu A et al. Potential nutraceutical use of *Tribulus terrestris* L. in human health. *Food Rev Int*. 2022;38(1): 1-30.
29. Kamkaen N, Chittasupho C, Vorarat S, et al. *Mucuna pruriens* seed aqueous extract improved neuroprotective and acetylcholinesterase inhibitory effects compared with synthetic L-dopa. *Molecules*. 2022;27(10):3131.
30. Ogunmoyole T, Ola-Awe AM, Fatile OG. Ethanolic extract of *Mucuna pruriens* leaves ameliorates carbon tetrachloride and rifampicin-induced hepatotoxicity and nephrotoxicity in wistar albino rat. *BMC Complement Med Therap*. 2021;21(1):1-11.
31. Rahiman F, Kumar MR, Mani TT, et al. Hepatoprotective activity of *Asparagus racemosus* root on liver damage caused by paracetamol in rats. *Indian J Nov Drug Deliv*. 2011;3(2): 112-117.
32. Sylvester EG, Israel EU, Olajumoke AD. The effect of Gongronema latifolium leaf extract on blood biochemical assay in diabetic rats. *J Scient Res Reports*. 2015;6(7):514-522.
33. Yadav HN, Sharma US, Singh S, Gupta YK. Effect of *Tribulus terrestris* in mercuric chloride-induced renal accumulation of mercury and nephrotoxicity in rat. *JAPTR*. 2019;10(3):132.
34. Concessao P, Bairy LK, Raghavendra AP. Protective effect of *Mucuna pruriens* against arsenic-induced liver and kidney dysfunction and neurobehavioral alterations in rats. *Vet World*. 2020;13(8):1555-1566.
35. Wesam EM, Yakout A, Samy AA, Souad AA. Antidiabetic and kidney protective effect of *Asparagus racemosus* in alloxan induced diabetes in rats. *World J Pharm Sci*. 2018;7:102-114.