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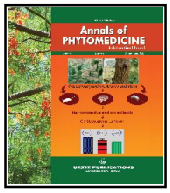

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Assessment of hepato and nephroprotective potential of polyherbal combinations against STZ-induced diabetic liver and kidney complications in wistar rats

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Abstract

Diabetes and medications with allopathic drugs in long term can lead to many complications, damage, and dysfunction of various organs, especially the kidneys, liver, heart, nerves and others. Thus, in diabetes management, a supportive drug is required that may protect the health and function of liver and kidney. In the present research, the objective was to evaluate novel antidiabetic polyherbal combinations for their hepatoprotective and nephroprotective potential. Thus, this may decrease the load of different drugs to control alignments associated with diabetes. Experimentally diabetes mellitus in albino rats was induced by using streptozotocin, followed by nicotinamide. Diabetic rats were evaluated against newly developed polyherbal combinations by studying biochemical parameters to evaluate their effect on liver and kidney. Hepatoprotective activity was assessed by liver function markers: alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), total protein, albumin, globulin, total, direct and indirect bilirubin levels (TBILI, DBILI and IDBILI). The nephroprotective activity was assessed by studying kidney function markers like blood urea nitrogen, urea, uric acid, and creatinine. By the treatment of four test polyherbal combinations of 250 mg/kg body weight, the altered liver and kidney function in streptozotocin-induced hyperglycemic albino rats significantly got ameliorated. The reversal of hepatorenal biochemical alterations might be due to the presence of active phytoconstituents. Thus, by the study, it was concluded that the polyherbal combination number 2 out of the 4; effectively treats the diabetes-induced complications associated with liver and kidney and protects from further complications.

1. Introduction

Diabetes in long term can lead to many complications, and thus can increase the overall risk of dying prematurely due to the severity of associated complications. Most prevalent complications include kidney failure, liver problem, hyperlipidemia, coronary heart disease, neuronal, peripheral vascular disease and others. Patients with diabetes also have a two to three times more risk of getting heart attacks and strokes (Martín-Timón *et al.*, 2014). Living well with diabetes requires early diagnosis and treatment. The longer you live with undiagnosed and untreated diabetes, the worse health outcomes are likely to be (WHO, 2021). A sequence of cost-effective steps that can improve patient health outcomes includes a combination of diet, physical activities and medication, followed by regular screening for possible damage to the eyes, kidneys, liver and feet (Tuso, 2014). It is very well known that diabetes is aggravated by and associated with metabolic complications; it is also proven that chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, especially of the kidneys, liver, heart, nerves, and blood vessels (ADA, 2011).

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Kidneys contain millions of small blood vessels in clusters that act as filtering units for blood. Diabetes when uncontrolled and over time can harm the kidneys by causing damage to these blood vessels, thus making these vessels narrow and clogged. This leads to kidney failure or chronic kidney disease, which may end up with a kidney transplant. As per a study, diabetic nephropathy is a serious kidney-related complication of diabetes (Lim, 2014), reported affecting about 25% of people with diabetes or uncontrolled diabetes sooner or later. Pathologically, it is diagnosed by performing a kidney function test (KFT). Another diabetes-related severe complication that affects about 50% or every second diabetic patient is associated with the liver which causes non-alcoholic fatty liver disease and other liver diseases associated are glycogen deposition, steatosis, nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis, biliary disease, cholelithiasis, and cholecystitis. Chronic or mild elevation or reduction in liver function is commonly found in type-2 diabetes, which can be analyzed by performing a liver function test (LFT) (Smith and Adam, 2011).

Apart from complications developed due to diabetes, synthetic drugs that are used as antidiabetic in the long term may develop severe complications associated with alteration in liver and kidney function (Ganesan *et al.*, 2021; Triplitt, 2006). Thus, while treating diabetes, an additional supportive drug may be required to protect and treat against developed complications. As diabetes is considered a lifelong disease, hence there is always a need for safe, efficacious drugs with minimum side, adverse effects and less contraindication.

Hence, it was planned to choose the drugs from natural origin (herbs), which have several advantages and relatively fewer side effects compared to synthetic drugs (Karimi *et al*., 2015; Kooti *et al*., 2016). Hence, the research was focused to study antidiabetic polyherbal combinations (Jyothilekshmi *et al*., 2020) that will not only protect the patients from serious complications associated with kidney and liver, but will also help reduce the load of different drugs that may be used to control different alignments associated.

Twenty-four polyherbal formulations were developed by using 50 % hydroalcoholic extracts, mixed in different proportions by changing ratio, *i.e*., 25:50:75:100, following geometrical dilution methods. Four most effective formulations out of the twenty-four were selected based on their potential for the management of hyperglycemia (Gupta and Kori, 2022) with nephroprotective (Wang *et al*., 2011; Hashmi *et al*., 2013; Kore *et al*., 2011; Natarajan *et al*., 2015), and hepatoprotective actions (Adeyemi *et al*., 2014; Suryawanshi *et al*., 2011; Singanan, 2007; Raj *et al*., 2009). Plants selected for study included leaves of *Annona squamosa* L. and leaves of *Aegle marmelos* (L.) Corr. stem bark of *Ficus religiosa* Roxb. and calyx of *Hibiscus sabdariffa* L. Polyherbal combinations evaluated against diabetic nephro and hepatotoxicity were named PHC 1 which contained 50% ethanolic extract in a ratio of *H. sabdariffa* (100), *A. marmelos* (25), *F. religiosa* (75) and *A. squamosa* (50), PHC 2 had a ratio of 100:50:75:25, PHC 3 had 75:25:100:50 and PHC 4 had a ratio of 75:50:100:25, respectively of four drugs, making 250 mg dose. In the study, diabetogen streptozotocin (STZ) was used to develop diabetes-associated nephro and hepatotoxicity (Yang *et al*., 2013; Qinna and Badwan, 2015; Venkatachalam *et al*., 2021). The prevalence and severity of injury produced by STZ in the pancreas, liver, kidney and GIT, progressively increased with time (Piyachaturawat *et al*., 1988; Piyachaturawat *et al*., 1990).

2. Materials and Methods

2.1 Identification of plant materials and extract preparations

Plants selected for the research study were collected and authenticated by Dr. S.N. Dwivedi, Department of Botany, Janata PG College, APS University, Rewa, M.P., India. Herbarium specimens of each were prepared and deposited with voucher specimen number JC/B/PAN 482, for future reference. All plant parts collected were cleaned, shade dried and powdered. Then, they were defatted with petroleum ether and finally were extracted using hydroalcoholic solvent (50% ethanol v/v) using Soxhlet. All four extracts were concentrated, dried and kept moisture-free using desiccators.

2.2 Equipments and chemicals

Glucometer (Accu-Check active; Roche Diagnostic India Pvt. Ltd), automated hematology analyzer (BC-5000; ASPEN Diagnostics), chemistry analyzer (BS-120) (Mindray Medical, India), spectrophotometer (Shimadzu UV-1800), glibenclamide tablets (Ozone International, Mumbai), streptozotocin (Sigma Chemical Co., Bangalore, India.), nicotinamide (Aster Pharmaceuticals India), Glucose-oxidase peroxidase kit (Merck, India) mild anesthesia, EDTA and all other chemicals of analytical grade.

2.3 Experimental animals

Hepatoprotective and nephro-protective study of novel polyherbal combinations was conducted by using 8-10 weeks old albino rats of wistar strain having 180-250 g weight. All were divided into 7 groups of 6 animals each (including male and female). All the female animals chosen were nulliparous and non-pregnant. Animals' were kept at 25 ± 2°C with a relative humidity of 30-60 % and were subjected to 12 h light/dark cycle. For acclimatization, animals were housed 7 days before experimentation started. Animals were fed with normal animals feed and water *ad libitum*. All the experimental steps performed were previously approved and were executed by guidelines of CPCSEA (Committee for Control and Supervision of Experiments on Animals), for the use of laboratory animals, at RKDF University, Bhopal, India (Registration number: 1693/PO/Re/S/13/CPCSEA).

2.4 Induction and confirmation of diabetes

Experimentally diabetes was induced in the overnight fasted rat by a single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (STZ) 60 mg/kg b.w. by using 0.1 M cold citrate buffer having 4.5 pH (Rerup, 1969; Rakieten *et al*., 1963). After 15 min, nicotinamide 120 mg/kg b.w., dissolved in normal saline was administered by the i.p. route. Permanent and severe hyperglycemia was confirmed when the rats, had fasting blood glucose concentrations of more than 300-350 mg/dl (Babu *et al*., 2008; Duraisami *et al*., 2021). To prevent experimental rats from hypoglycemic shock and associated mortality, 10% dextrose solution was given after 6 h of STZ administration for the next 24 h. Blood samples were collected from the tail of each rat and were measured for hyperglycemia using a glucometer. After the 7th day, animals that did not develop hyperglycemia were rejected and replaced.

2.5 Preparation of standard drug and polyherbal test suspension

The solution of the standard drug was prepared by dissolving 10 tablets of glibenclamide (GLB) 5 mg each in 100 ml distilled water, giving 0.5 mg of GLB/1 ml. Four different test suspensions of four selected polyherbal combinations (PHC) were prepared by dissolving 5 gm/100 ml 0.5% CMC, giving 50 mg/ml concentration. Standard and test suspensions were administered orally using the intragastric tube.

2.6 Experimental design

In the experiment setup standard drug and four PHC were given orally to the assigned groups once daily for 28 days.

Group I (Normal control)	Animals received normal saline
Group II (Diabetic control)	Diabetic animals received normal saline
Group III (Standard treated)	Diabetic rats treated glibenclamide 0.5 mg/kg b.w
Group IV (PHC 1 treated)	Diabetic rats treated with PHC 1 (250 mg/kg b.w.)
Group V (PHC 2 treated)	Diabetic rats treated with PHC 2 (250 mg/kg b.w.)
Group VI (PHC 3 treated)	Diabetic rats treated with PHC 3 (250 mg/kg b.w.)
Group VII (PHC 4 treated)	Diabetic rats treated with PHC 4 (250 mg/kg b.w.)

2.7 Assessment of liver and kidney functions markers

At beginning of the study, animals were assessed for fasting blood glucose (FBS) using a glucometer (Lanjhiyana *et al.*, 2011). Once the diabetes was confirmed, animals were treated as per an experimental regimen of 28 days, after the set duration animals were sacrificed by cervical dislocation under mild anesthesia and blood was collected from the arterial jugular with EDTA to prevent clotting. Plasma and serum were separated by centrifugation at 3000 rpm for 10 min at 30°C and were analyzed for liver and kidney function markers. Plasma and serum samples were stored in the refrigerator at 4-8°C before analysis.

2.7.1 Effect of PHC on liver function markers of diabetic rats

Liver function markers like ALP were carried out by the method described by Bassey *et al.* (1946) and modified by Wright *et al.* (1972). ALT activity was determined as described by IFCC, (1980) using diagnostic kits. AST activity was determined by following the same assay method as done for ALT, just by replacing the ALT with the AST reagent. Serum GGT activity was determined by the method of Szasz (1969). Estimation of total protein was done according to Henry (1964) and albumin by Doumasa *et al.* (1971), in the serum was estimated by the Biuret method by Gornall *et al.* (1949). Serum total and direct bilirubin levels (TBILI) were determined by the method of Jendrassik and Grof (1938), whereas IDBILI was calculated by subtracting DBILI from TBILI (Puri *et al.*, 2020)

2.7.2 Effect of PHC on kidney function markers of diabetic rats

Kidney function markers like; blood urea nitrogen (BUN), urea, uric acid, and creatinine in the serum were determined using diagnostic kits. Their presence in blood serum elevated than normal value is correlated with kidney disease and kidney damage. These were analyzed using Chemistry Analyzer. BUN is a normal metabolic waste product that is excreted by the kidneys. It was analyzed by the method described by Fawcett and Scott (1960). Serum urea is a byproduct of protein breakdown, its presence in the blood reflects kidney disease, resulting in its accumulation in the body, thus causing an increase in blood levels of urea. Urea in the serum was estimated by a diagnostic kit based on the method of Natelson *et al.* (1951). Uric acid in the serum was estimated by diagnostic kit based on the enzymatic method described by Fossati (1980). Estimation of serum creatinine was performed to monitor the function of the kidney. It is a normal metabolic waste product excreted by the kidneys. Its increase reflects possible kidney damage. Creatinine in the serum was estimated by diagnostic kit based on the method of Broad and Sirota (1948) using the Jaffes reaction.

2.8 Statistical analysis

All the results were expressed as mean ± SD for six rats in each experimental group. Statistical analysis was performed using Prism graph pad version 5.0. The data were evaluated using a one-way analysis of variance (ANOVA), followed by Dunnett's Test. *p*-values <0.05 were considered statistically significant, $p<0.01$ as very significant and $p<0.001$ as highly significant.

3. Results

Hyperglycemia was induced in the wistar albino rats by using STZ followed by nicotinamide (NA) and confirmed by the elevated fasting blood glucose of concentration between 300-350 mg/dl. During this procedure, those rats that did not develop diabetes as per requirement were rejected and replaced. It was noted that blood glucose elevated by 333.94 % was significantly reversed by PHC to a normal level after treatment of 28 days. PHC 1 reversed elevated blood glucose by 71.96%, PHC 2 reversed by 75.34 %, PHC 3 reversed by 70.51 and PHC 4 reversed by 73.47 %.

3.1 Significant effect of PHC on liver function markers of diabetic rats

It was noted that STZ-induced diabetes leads to increased liver function parameters including ALP, ALT, AST, GGT, total protein, albumin, globulin, total A/G ratio, total, direct and indirect bilirubin whereas AST/ALT ratio decreased. Treatment with PHC for 28 days significantly ($p<0.001$) ameliorated the altered liver functions marker to the normal, which have been represented in Tables 1, 2, and 3 and illustrated in Figures 1, 2, and 3. In terms of percentage, PHC 2 showed a highly significant reduction of 56.30 % in ALP, 43.52 % in ALT, 41.79 % in AST, and 44.58 % in GGT. Similarly, PHC 2 showed a significant and comparatively better reduction in total protein by 25.94 %, albumin by 31.41 %, globulin by 19.39 %, total bilirubin by 51.49 %, direct by 46.15 % and indirect by 46.08 %. Thus, the effect of PHC 2 on the overall improvement was found to be better compared to other PHC 1, 3, and 4.

Table 1: Effect of PHC on liver function marker ALP, ALT, AST, GGT and AST/ALT ratio

Groups	Liver function markers (IU/L)				
	ALP	ALT	AST	GGT	AST/ALT ratio
Group I (Normal control)	85.50 ± 2.59	29.50 ± 1.87	22.83 ± 1.17	19.50 ± 1.87	0.78 ± 0.07
Group II (Diabetic control)	174.67 ± 4.18	72.00 ± 3.03	44.67 ± 3.08	41.50 ± 3.02	0.62 ± 0.04
Group III (Standard treated)	103.17 ± 2.64**	48.17 ± 1.17**	36.00 ± 1.79**	28.50 ± 2.51**	0.75 ± 0.03
Group IV (PHC 1 treated)	93.33 ± 3.44**	46.33 ± 1.63**	31.83 ± 3.31**	27.00 ± 2.83**	0.69 ± 0.09
Group V (PHC 2 treated)	76.33 ± 1.97***	40.67 ± 2.94***	26.00 ± 2.10***	23.00 ± 2.19***	0.64 ± 0.07
Group VI (PHC 3 treated)	92.83 ± 4.12**	47.00 ± 1.55**	34.50 ± 1.87**	27.50 ± 2.26**	0.73 ± 0.04
Group VII (PHC 4 treated)	87.33 ± 3.27**	44.83 ± 2.32***	30.17 ± 3.76***	25.83 ± 2.93***	0.68 ± 0.10

Data are expressed as mean ± SD. (n=6); * ($p<0.05$) significant; ** ($p<0.001$) very significant; *** ($p<0.001$) highly significant when compared with normal control.

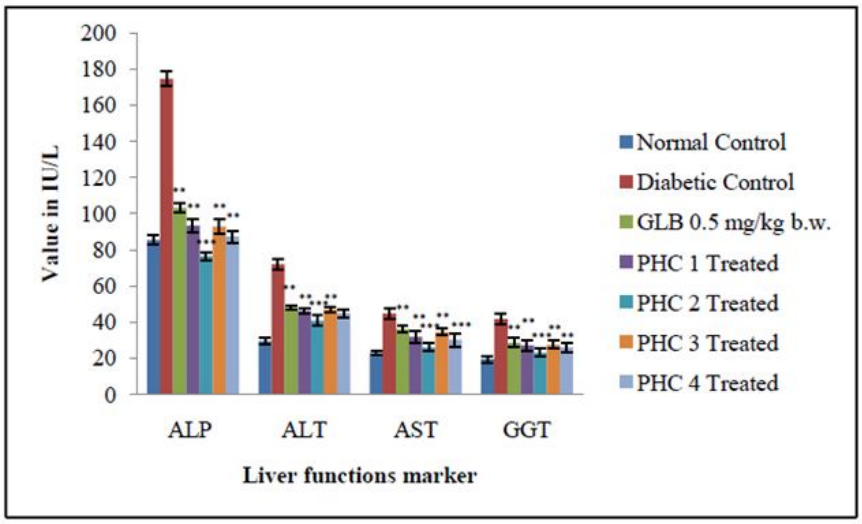


Figure 1: Effect of PHC on liver function marker ALP, ALT, AST and GGT.

Table 2: Effect of PHC on liver function markers (total protein, albumin, globulin and albumin/globulin ratio)

Groups	Liver function markers			
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Group I (Normal control)	6.16 ± 0.22	3.17 ± 0.08	2.99 ± 0.20	1.06 ± 0.08
Group II (Diabetic control)	9.62 ± 0.23	5.24 ± 0.23	4.38 ± 0.36	1.21 ± 0.14
Group III (Standard treated)	8.18 ± 0.11**	4.67 ± 0.04**	3.51 ± 0.11***	1.33 ± 0.05
Group IV (PHC 1 treated)	7.61 ± 0.05***	4.12 ± 0.07**	3.73 ± 0.12***	1.18 ± 0.04
Group V (PHC 2 treated)	7.12 ± 0.10***	3.60 ± 0.07***	3.52 ± 0.04***	1.02 ± 0.02
Group VI (PHC 3 treated)	8.10 ± 0.06**	4.20 ± 0.03**	3.91 ± 0.07***	1.08 ± 0.03
Group VII (PHC 4 treated)	7.40 ± 0.03***	3.70 ± 0.04***	3.71 ± 0.05**	1.00 ± 0.02

Data are expressed as mean ± SD. (n=6); * (p<0.05) significant; ** (p<0.001) very significant; *** (p<0.001) highly significant when compared with normal control.

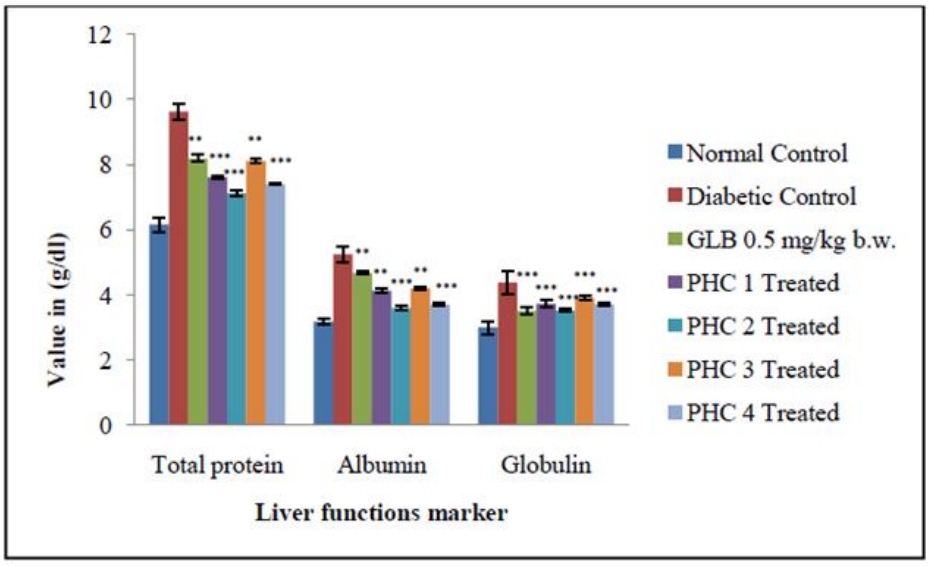


Figure 2: Effect of PHC on liver function markers (total protein, albumin, and globulin).

Table 3: Effect of PHC on liver function marker (total, direct and indirect bilirubin)

Group	Liver function markers in mg/dl		
	Total bilirubin	Direct bilirubin	Indirect bilirubin
Group I (Normal control)	0.43 ± 0.03	0.18 ± 0.03	0.25 ± 0.03
Group II (Diabetic control)	1.57 ± 0.10	0.37 ± 0.03	1.20 ± 0.08
Group III (Standard treated)	1.10 ± 0.05**	0.28 ± 0.02**	0.81 ± 0.07**
Group IV (PHC 1 treated)	0.92 ± 0.03***	0.29 ± 0.04**	0.63 ± 0.05**
Group V (PHC 2 treated)	0.76 ± 0.03***	0.20 ± 0.03***	0.56 ± 0.04***
Group VI (PHC 3 treated)	1.15 ± 0.04**	0.28 ± 0.03**	0.88 ± 0.05**
Group VII (PHC 4 treated)	0.82 ± 0.02***	0.26 ± 0.03**	0.56 ± 0.05***

Data are expressed as mean ± SD. (n=6); * (p<0.05) significant; ** (p<0.001) very significant; *** (p<0.001) highly significant when compared with normal control.

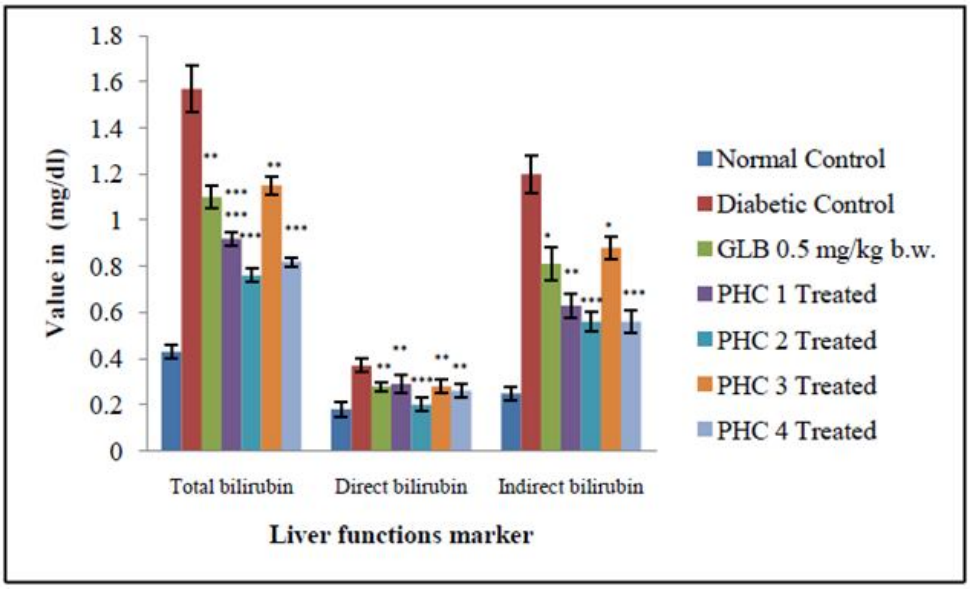


Figure 3: Effect of PHC on liver function marker (Total, direct and indirect bilirubin).

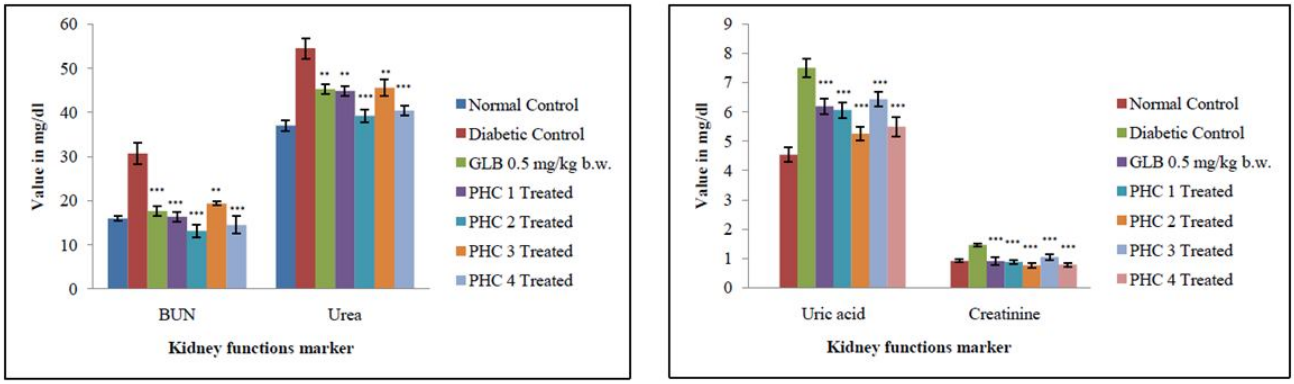
Table 4: Effect of PHC on kidney function marker of STZ-induced diabetic rats

Serum profile	BUN (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	BUN/ creatinine	Urea/ creatinine
Group I (Normal control)	15.95 ± 0.62	37.03 ± 1.17	4.54 ± 0.24	0.92 ± 0.05	17.46 ± 0.85	40.51 ± 1.38
Group II (Diabetic control)	30.67 ± 2.36	54.54 ± 2.36	7.50 ± 0.31	1.46 ± 0.06	21.11± 2.25	37.52 ± 2.70
Group III (Standard treated)	17.58 ± 1.11***	45.26 ± 1.03**	6.19 ± 1.26***	0.91 ± 0.14***	19.78 ± 3.60	50.92 ± 8.54
Group IV (PHC 1 treated)	16.33 ± 1.06***	44.88 ± 1.15**	6.06 ± 0.26***	0.88 ± 0.08***	18.59 ± 1.89	51.09 ± 3.87
Group V (PHC 2 treated)	13.13 ± 1.36***	39.24 ± 1.45***	5.25 ± 0.24***	0.76 ± 0.09***	17.45 ± 2.31	52.40 ± 6.92
Group VI (PHC 3 treated)	19.36 ± 0.48**	45.62 ± 1.98**	6.43 ± 0.24***	1.05 ± 0.10***	18.61 ± 2.31	43.86 ± 5.19
Group VII (PHC 4 treated)	14.49 ± 1.96***	40.38 ± 1.16***	5.49 ± 0.32***	0.79 ± 0.07***	18.57 ± 3.77	51.34 ± 4.52

Data are expressed as mean ± SD. (n=6); * (p<0.05) significant; ** (p<0.001) very significant; *** (p<0.001) highly significant when compared with normal control.

3.2 Effect of PHC on kidney function markers of diabetic rats

The effects of PHC on kidney function tests are depicted in Tables 4 and 5 and illustrated in Figures 4 (a) and (b). Percentage change in BUN level was calculated in comparison to STZ induced diabetic group. In STZ-induced diabetic rats, the BUN level which got raised by 92.28 %, was reversed by 46.77 %, 57.19 %, 36.88 %, and 52.77 % simultaneously. Serum urea levels which got raised by 47.28 % were decreased by 17.72 %, 28.05 %, 16.36 %, and 25.96 % simultaneously. The uric acid level which got raised by 65.19 %, decreased by 19.18 %, 30 %, 14.33 %, and 26.82 %. Creatinine levels which got raised by 59.20 % decreased by 39.36 %, 47.94 %, 27.92 % and 45.65 % by PHC 1, 2, 3 and 4 simultaneously. When BUN/creatinine ratio was studied, it was noted that, in STZ-induced diabetes, its level got raised to 21.11. However, on treatment with PHC 1, 2, 3, and 4 groups' a significant decrease (p<0.001) in ratio to 18.59, 17.45, 18.61, and 18.57 was reported. Urea/creatinine ratio in STZ-induced diabetes which got decreased to 37.52 was improved to 51.09, 52.40, 43.86, and 51.34 by PHC 1, 2, 3, and 4 simultaneously.



(a) **(b)**

Figure 4: Effect of PHC on kidney function marker of STZ-induced diabetic rats.

4. Discussion

In this study, it has been revealed that STZ-induced diabetes is also associated with liver damage or toxicity which can be assessed by the study of serum levels of ALP, ALT, AST, TP, and TB release in circulation (Hall and Cash 2012; Giannini *et al*., 2005). When the liver cell plasma membrane gets damaged, varieties of enzymes located in the cytosol got released into the bloodstream. Their estimation in the serum proved to be a useful quantitative marker of the extent and type of hepatocellular damage (Mondal *et al*., 2012). As result, it was noted that all four PHC significantly revert the altered liver function markers to the normal after treatment of 28 days, and thus showed hepatoprotective effects. The protection offered by the PHC might be due to the presence of flavonoids and alkaloids (Gupta *et al*., 2015; Sangale and Patil, 2017; Yoshikawa *et al*., 2002).

In the consecutive study, it was also noted that STZ having an inherent nephrotoxic potential, showed definite signs of nephrotoxicity and marked renal dysfunction. This was evidenced by the elevation of the blood urea nitrogen, serum urea, uric acid, and creatinine level, followed by an index of altered glomerular filtration rate (GRF) in diabetic nephropathy. However, four PHC treatments over 28 days resulted in the reversal of altered elevation to the normal in the kidney tissues and correction of altered GRF (Guo *et al*., 2021; Guoyi *et al*., 2021). It is reported that in diabetes protein glycation may lead to muscle atrophy and the increased output of purines, which is the main source of uric acid, as well as increased activity of xanthine oxidase, which may have been resulted due in metabolic disturbance in diabetes. Studies have shown that increased concentrations of urea and creatinine were due to excessive lipolysis in severe diabetes leading to ketosis and later on to acidosis. By acidification of urine and removal of metabolic wastes such as urea, uric acid, and creatinine, the kidney maintains the optimum chemical composition of body fluid. But, in the condition of renal function impairments or diseases, the concentration of these metabolites rises in the blood (Sharma *et al*., 2014). Elevated BUN/creatinine ratio may be a condition that causes a decrease in the flow of blood to the kidneys, such as in congestive heart failure. This elevated ratio thus increases the risk of coronary artery diseases in those with type 2 diabetes and also represents diabetic nephropathy (Uchino *et al*., 2012; Liu *et al*., 2022). Low urea/creatinine ratio or its impairment due to increased glucose level indicates the reduction in kidney function in diabetics, thus leading to diabetic kidney disease (Kene *et al*., 2021).

Thus by study, it was revealed that the altered biochemical conditions associated with hepato-renal functions were successfully reversed and normalized on treatment with PHC for 28 days on diabetic rats. The hepato-renal function restoring activity presented by PHC might be due to the reported phytochemical, phenyl compounds, coumarins, flavonoids, glycosides, saponins, monoterpenoids, diterpenoids, terpenoids, steroids, and alkaloids (Ross, 2003; Valan *et al*., 2010; Sundararajan *et al*., 2022; Mehan *et al*., 2019; Govindachari, 1983; Panda and Kar, 2007), that makes it an important source for the treatment from liver and kidney complications associated with diabetes. By the result, it was also reported that herbs used to develop PHC were nontoxic, and hence may be used as safe ingredients as antidiabetic with hepato and nephroprotective effects.

5. Conclusion

Usually, most drugs based on pharmaceutical substances cause contraindication effects on hepatic and renal functions. Hence, it was of great concern that any drug used in any treatment regime must be safe. And by a result, it was found that PHC may be considered as safe as liver and kidney function is concerned. This study revealed that out of 4 combinations, PHC 2 significantly ameliorated the altered liver and kidney functions associated with diabetes after 28 days of treatment. Polyherbal combinations might have restored the altered hepato-renal function due to the presence of active phytoconstituents. Therefore, the present study scientifically supports the use of PHC for the treatment of diabetes-induced liver and kidney function alterations, and thus helps protect from further complications.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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