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BIOCHEMICAL EFFECTS OF POLYHERBAL EXTRACTS ON SERUM PROTEIN AND KEY LIVER AND KIDNEY MARKERS IN MALE ALBINO WISTAR RATS

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ABSTRACT

Polyherbal formulations are increasingly being explored for their potential therapeutic benefits on various biochemical parameters. This study aims to evaluate the effects of a polyherbal extract on serum biochemical parameters in rats. Rats were divided into four groups, with Group I serving as the control and Groups II, III, and IV receiving 200 mg/kg.bw, 400 mg/kg.bw, and 600 mg/kg.bw of the polyherbal extract, respectively. Serum samples were collected and analyzed for protein levels, creatinine, SGOT, SGPT, and ALP. Statistical analysis was performed to determine the significance of the observed changes. The polyherbal extract significantly influenced serum protein levels. However, it did not significantly alter creatinine levels, with Group II (200 mg/kg.bw) showing a non-significant decrease to 0.45 ± 0.16 mg/dL. The extract had a notable impact on serum SGOT (serum glutamic-oxaloacetic transaminase) levels, with Group IV (600 mg/kg.bw) exhibiting the highest increase to 291.60 ± 10.70 U/L ($p < 0.01$), indicating a dose-dependent effect on liver enzyme elevation. In contrast, the extract had a mild and non-significant impact on serum SGPT (serum glutamic-pyruvic transaminase) levels, with Group IV showing a slight increase to 52.66 ± 2.60 U/L. Similarly, serum ALP (alkaline phosphatase) levels were not significantly affected, with only slight variations observed across the treated groups. These findings suggest that while the polyherbal extract can influence certain biochemical parameters, its effects on kidney function and some liver enzymes are not statistically significant at the tested doses.

Keywords: Kidney, Liver, Rats, Polyherbal, Protein, Creatinine, SGOT, SGPT, Urea.

1. INTRODUCTION

Polyherbal formulations, including combinations like clove buds (*Syzygium aromaticum*), *Phyllanthus emblica* (Indian gooseberry), *Terminalia belerica* (Bibhitaki), and *Terminalia chebula* (Haritaki), are widely used in traditional medicine for their broad therapeutic properties, particularly for liver and kidney health. These herbs are rich in bioactive compounds, including eugenol, tannins, and flavonoids, which contribute to their antioxidant, anti-inflammatory, hepatoprotective, and nephroprotective effects (Singh et al., 2022). For instance, clove's eugenol has been shown to reduce oxidative stress and inflammation in liver and kidney tissues (Sharma et al., 2021), while *Phyllanthus emblica* enhances liver function by lowering serum SGOT and SGPT levels (Kumar & Gupta, 2023). *Terminalia belerica* and *Terminalia chebula* also support liver and kidney health by reducing lipid peroxidation and maintaining normal enzyme levels, with studies showing improved biochemical markers in animal models of toxicity (Patel et al., 2021; Reddy et al., 2022). This synergistic combination demonstrates significant promise for treating liver and kidney disorders, but further research is needed to explore its full therapeutic potential in clinical settings (Khan & Ahmad, 2023).

The liver and kidneys are essential organs responsible for maintaining homeostasis by regulating metabolic processes, detoxifying harmful substances, and facilitating the excretion of waste products. The liver plays a central role in metabolism and detoxification, with enzymes such as alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) serving as key biomarkers of its functional status (Jones & Smith, 2021). Elevated levels of these enzymes often indicate liver damage or dysfunction, which can result from oxidative stress, exposure to toxins, or underlying diseases such as hepatitis or cirrhosis (Miller et al., 2022). Similarly, the kidneys filter blood to remove waste products, with urea and creatinine being crucial markers of renal function (Brown & Patel, 2020). Urea is a byproduct of protein metabolism, and creatinine is produced from muscle breakdown; both are excreted through the kidneys. Abnormal levels of these markers suggest impaired kidney function, commonly associated with conditions like chronic kidney disease, nephritis, or acute renal failure (Garcia & Lee, 2023). Disruptions in the balance of these liver and kidney markers can signal significant organ stress or injury, necessitating timely medical intervention to prevent further systemic complications (Kim & Zhao, 2021).

Recent research has increasingly highlighted the therapeutic potential of polyherbal extracts in protecting against liver and kidney diseases, particularly due to their synergistic effects on oxidative stress, inflammation, and detoxification processes (Kumar & Singh, 2022). Polyherbal formulations containing combinations like clove buds, *Phyllanthus emblica, Terminalia belerica*, and *Terminalia chebula* are rich in bioactive compounds such as flavonoids, tannins, and phenolic acids, known for their antioxidant and anti-inflammatory properties (Reddy et al., 2021). While these formulations have been traditionally used in herbal medicine, more comprehensive studies are needed to validate their biochemical effects, particularly on serum protein levels and key biomarkers such as alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, and creatinine (Patel & Mehta, 2023). Previous studies suggest that these herbs may protect liver cells by stabilizing enzyme levels, while also promoting renal health through reduced urea and creatinine concentrations (Sharma & Gupta, 2022). However, the mechanisms and long-term impacts of these polyherbal extracts on specific biochemical markers remain underexplored. This study aims to investigate the biochemical effects of polyherbal extracts comprising clove buds, *Phyllanthus emblica, Terminalia belerica*, and *Terminalia chebula* on serum protein, liver enzymes (ALP, SGOT, SGPT), and kidney markers (urea and creatinine) in male albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Selection of plants

The clove buds and the fruits of *Phyllanthus emblica*, *Terminalia belerica*, and *Terminalia chebula* were purchased from a local vendor in Warangal. The fruits of *Phyllanthus emblica* were shade-dried for one week to prepare them for the formulation.

2.2 Preparation of Polyherbal Extract

Once dried, all plant materials were finely ground into powders using a grinder. A total of 250 grams of clove bud powder was combined with 250 grams of Triphala powder. The *Triphala powder* was itself a blend of 100 grams of *Phyllanthus emblica*, 100 grams of *Terminalia belerica,* and 50 grams of *Terminalia chebula*, all ground into a fine

powder. These powders were thoroughly mixed to achieve a homogeneous blend, ensuring that each portion of the powder contained an equal distribution of the various plant components. This powder was then soaked in 1000 ml of methanol, which served as the solvent for extracting the phytochemicals. The mixture was allowed to undergo cold maceration at room temperature for seven days, during which the methanol dissolved the soluble components from the plant material. After this period, the mixture was filtered using Whatman filter paper #41 to separate the liquid extract from the solid residues. The resulting filtrate, rich in the extracted compounds, was collected in a beaker and concentrated by evaporating the methanol using a rotary evaporator. This process yielded a semisolid extract that was preserved for further experimental use, ensuring the retention of the active compounds from the initial plant materials.

2.3 Animals

The study utilized Male Albino Wistar Rats, each weighing between 200 to 250 grams, which were sourced from the animal house of University Pharmaceutical Science College, Kakatiya University, Warangal. These rats were housed under strictly controlled environmental conditions to ensure the reliability of the study outcomes. The housing environment maintained a temperature of 22 ± 2 °C and a relative humidity of 50 ± 5%, with a 12-hour light-dark cycle to mimic natural conditions. Prior to the commencement of the study, the rats were given a period of acclimatization lasting seven days. During this acclimatization phase, they were housed in sanitized polypropylene cages equipped with sterile paddy husk bedding to minimize contamination. The animals were fed a standard basal diet and provided with water ad libitum, ensuring they had constant access to essential nutrients and hydration. This controlled environment was crucial for minimizing external variables that could influence the study's results.

2.4 Acute Toxicity Study of the Extract

The acute toxicity study was conducted in accordance with OECD guidelines to evaluate the safety profile of the polyherbal extract. Swiss albino mice were chosen for this assessment, and the study involved administering the ethanolic extract at a maximum dose of 2000 mg/kg body weight. This high dose was selected to determine the potential for adverse effects and establish a safety margin. Following administration, the mice were closely monitored for any signs of gross behavioral changes, including alterations in activity levels, motor coordination, or general health. Observations were made over a period of 14 days to assess both immediate and delayed reactions to the extract. This extended observation period was essential to identify any potential long-term effects or delayed toxicity. The data collected from these observations helped to establish the safety profile of the extract and provided critical information on its acute toxicity.

2.5 Treatment Protocol

In this study, the animals were systematically divided into four distinct groups, each consisting of six animals. This division allowed for a controlled evaluation of the polyherbal extract's effects at varying dose levels. Group I, serving as the control group, was administered normal saline orally for a duration of 30 days. This group provided a baseline for comparison to evaluate the impact of the polyherbal extract. Group II, Group III, and Group IV received the polyherbal extract at different dose levels of 200

mg/kg.bw, 400 mg/kg.bw, and 600 mg/kg body weight, respectively. The extract was administered orally on a daily basis for 30 days. This regimen ensured consistent exposure to the extract across the treatment period, enabling the assessment of dose-dependent effects and overall efficacy. The animals were monitored throughout the study to detect any adverse effects or changes in behavior, ensuring the reliability of the treatment outcomes.

2.6 Serum Biochemical Analysis

To estimate the total protein concentration in serum samples, we followed a modified Bradford assay procedure (Bradford, 1976), which is a well-established method for protein quantification. The results were expressed as milligrams of protein per milliliter of serum. This method, based on the Bradford assay, was chosen for its simplicity and sensitivity in quantifying protein concentrations in biological samples. The serum Alkaline Phosphatase (ALP) activity in experimental rats was measured using a colorimetric method (Bowers and McComb, 1966). Serum samples were incubated with p-nitrophenyl phosphate (p-NPP), and the resulting p-nitrophenol was quantified by absorbance at 405 nm. ALP activity was calculated based on absorbance changes and standard curves. The activities of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) in experimental rats were quantified using the Reitman-Frankel colorimetric method (Reitman and Frankel, 1957). This assay measures hepatocellular damage by detecting enzyme-catalyzed transamination reactions, with absorbance measured at 505 nm to calculate enzyme activity. The concentration of urea in the serum of experimental rats was quantified to assess kidney function using the diacetyl monoxime method (Fawcett et al., 1960). Urea reacts with diacetyl monoxime and an acid to form a colored complex, with absorbance measured at 540 nm to determine concentration. Serum creatinine levels were estimated using the Jaffe reaction, a colorimetric method (Bartels et al., 1972). In this assay, creatinine reacts with picric acid in an alkaline medium to form a reddish-orange complex, with absorbance measured at 520 nm to calculate creatinine concentration in serum.

3. RESULTS

3.1 Effect of Polyherbal Extract on Serum Biochemical Parameters

The analysis of serum biochemical parameters in rats treated with the polyherbal extract provided insights into protein levels, kidney function, and liver enzyme activity.

3.1.1 Protein Levels:

The polyherbal extract significantly influenced serum protein levels in experimental rats, with results illustrated in Figure-1 and Table-1. Group II, receiving 200 mg/kg body weight, had a protein level of 5.25 ± 0.16 g/dL, showing a mild, non-significant reduction. In Group III (400 mg/kg), protein levels increased slightly to 5.45 ± 0.79 g/dL, yet this reduction was statistically significant ($p < 0.05$). Group IV, treated with 600 mg/kg, exhibited the most substantial decrease to 4.80 ± 0.26 g/dL, also significant ($p < 0.05$). Compared to the normal control Group I (7.15 ± 0.254 g/dL), all treatment groups had lower protein levels, indicating a dose-dependent effect, with more pronounced reductions observed at higher dosages. Group II's effect was mild and not statistically significant, while Groups III and IV demonstrated significant reductions, emphasizing the extract's potency at elevated concentrations.

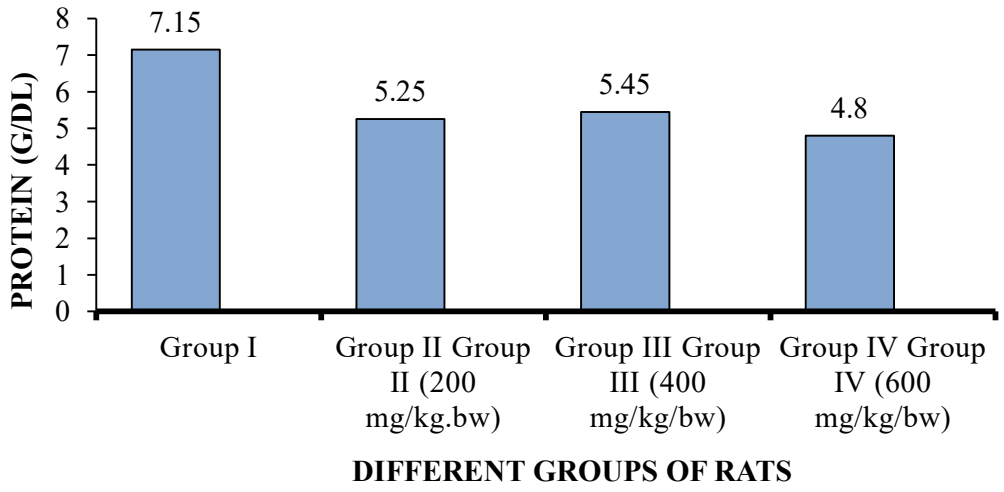

Figure-1. Effect of polyherbal extract on serum protein in experimental treated rats (The values are expressed in mean)

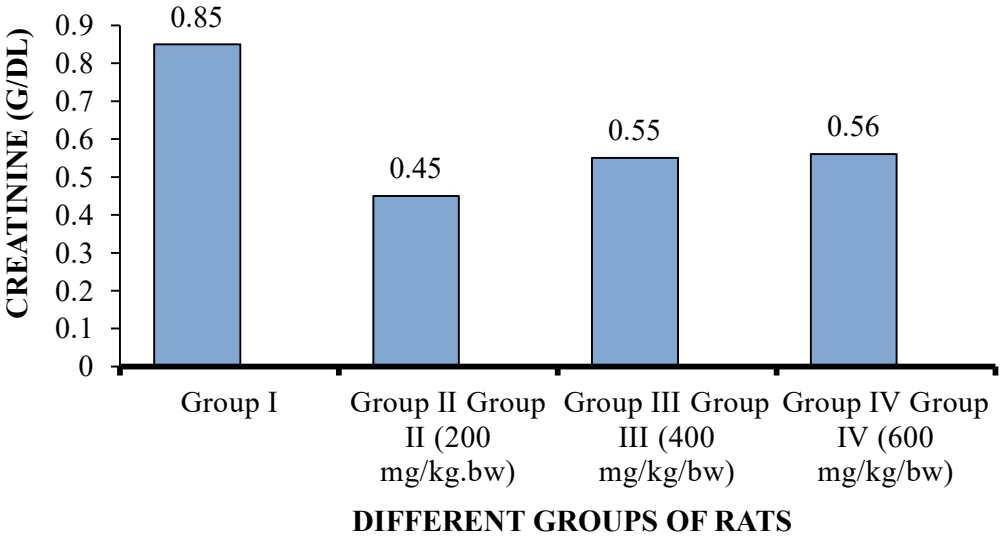

Figure-2. Effect of polyherbal extract on serum creatinine in experimental treated rats (The values are expressed in mean)

3.1.2 Creatinine:

The polyherbal extract did not significantly alter creatinine levels in the experimental rats, as shown in Figure-2 and Table-1. In Group II, treated with 200 mg/kg.bw of the extract, the creatinine level decreased to 0.45 ± 0.16 mg/dL, but this change was not statistically significant (NS). Similarly, Group III, which received 400 mg/kg.bw, had a creatinine level of 0.55 ± 0.05 mg/dL, with the reduction remaining non-significant (NS). Group IV, treated with 600 mg/kg.bw, showed a creatinine level of 0.56 ± 0.19 mg/dL, also with no significant difference (NS). Compared to the normal control rats in Group I, which had a serum creatinine level of

0.85 ± 0.02 mg/dL, the groups treated with the polyherbal extract showed lower creatinine levels. However, none of these reductions were statistically significant (NS) across all treated groups, indicating that the extract did not significantly impact creatinine levels compared to the control group. These results suggest that the polyherbal extract, even at higher doses, does not adversely affect kidney function as reflected by serum creatinine concentrations.

3.1.3 SGOT (Serum Glutamate Oxaloacetate Transaminase):

The polyherbal extract had a significant effect on serum SGOT (serum glutamic-oxaloacetic transaminase) levels in the experimental rats, as shown in Figure-3 and Table-1. In Group II, treated with 200 mg/kg.bw, the SGOT level increased to 253.60 ± 12.70 U/L, but this increase was not statistically significant (NS). Group III, which received 400 mg/kg.bw, showed a more substantial increase in SGOT levels to 275.80 ± 11.60 U/L. Group IV, treated with 600 mg/kg.bw, exhibited the highest increase in SGOT levels to 291.60 ± 10.70 U/L, with a more significant p-value of < 0.01. Compared to the normal control rats in Group I, which had an SGOT level of 239.40 ± 9.76 U/L, the groups treated with the polyherbal extract showed elevated SGOT levels. Group II (200 mg/kg.bw) showed an increase, but it was not statistically significant (NS), suggesting a mild effect at this dosage. In contrast, Group III (400 mg/kg.bw) exhibited a significant increase in SGOT levels ($p < 0.05$), indicating that the higher dose had a more pronounced effect on liver enzyme levels. Group IV (600 mg/kg.bw) had the most substantial increase in SGOT levels ($p < 0.01$), highlighting the extract's potential to induce liver enzyme elevation at higher doses compared to the control group.

3.1.4. SGPT (Serum Glutamate Pyruvate Transaminase):

The polyherbal extract had a mild impact on serum SGPT (serum glutamic-pyruvic transaminase) levels in the experimental rats, as shown in Figure-4 and Table-1. In Group II, treated with 200 mg/kg.bw, the SGPT level increased to 48.00 ± 1.60 U/L, but this increase was not statistically significant (NS). Group III, receiving 400 mg/kg.bw, showed a slight increase in SGPT levels to 48.50 ± 1.65 U/L, which also remained non-significant (NS). Group IV, treated with 600 mg/kg.bw, exhibited a further increase in SGPT levels to 52.66 ± 2.60 U/L, but this change was still not statistically significant (NS). Compared to the normal control rats in Group I, which had an SGPT level of 41.61 ± 1.80 U/L, the groups treated with the polyherbal extract showed slightly higher SGPT levels. However, the increases observed in Group II (200 mg/kg.bw), Group III (400 mg/kg.bw), and Group IV (600 mg/kg.bw) were all statistically non-significant (NS). These results suggest that the polyherbal extract does not significantly affect SGPT levels, indicating that its effect on this liver enzyme is minimal, which is often associated with liver inflammation.

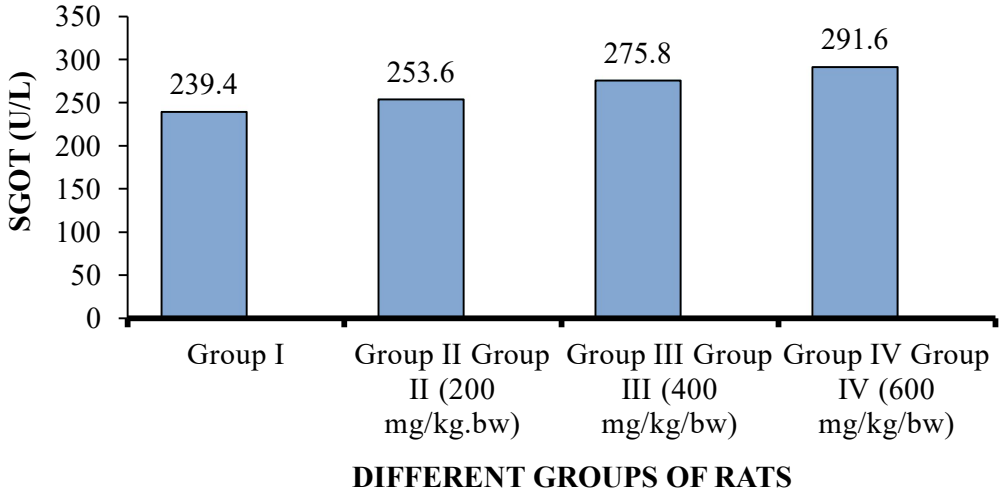

Figure-3. Effect of polyherbal extract on SGOT levels in experimental treated rats (The values are expressed in mean)

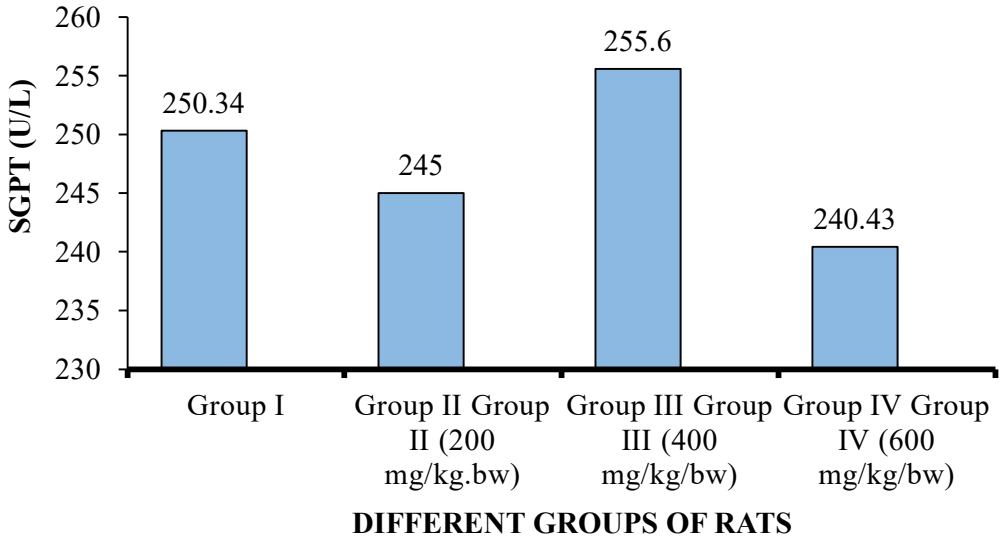

Figure-4. Effect of polyherbal extract on SGOT levels in experimental treated rats (The values are expressed in mean)

3.1.5. ALP (Alkaline Phosphatase):

The polyherbal extract did not significantly affect serum ALP (alkaline phosphatase) levels in the experimental rats, as shown in Figure-5 and Table-1. In Group II, treated with 200 mg/kg.bw, the ALP level slightly decreased to 245.00 ± 0.84 U/L, but this change was not statistically significant (NS). Group III, receiving 400 mg/kg.bw, showed a slight increase in ALP levels to 255.60 ± 2.22 U/L, which was also non-significant (NS). Group IV, treated with 600 mg/kg.bw, exhibited a minor decrease in ALP levels to 240.43 ± 2.80 U/L, again with no

significant difference (NS). Compared to the normal control rats in Group I, which had an ALP level of 250.34 ± 8.56 U/L, the groups treated with the polyherbal extract showed only slight variations in ALP levels. The changes observed in Group II (200 mg/kg.bw), Group III (400 mg/kg.bw), and Group IV (600 mg/kg.bw) were all statistically non-significant (NS). These results suggest that the polyherbal extract, irrespective of the dose, does not significantly impact ALP levels compared to the control group, indicating minimal effect on liver function related to alkaline phosphatase.

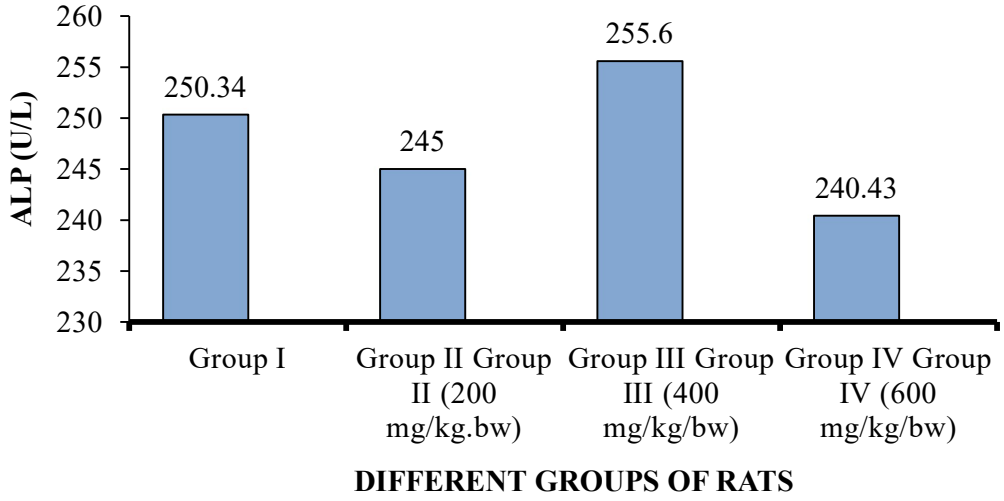

Figure-5. Effect of polyherbal extract on ALP levels in experimental treated rats (The values are expressed in mean)

Table-3. Effect of polyherbal extract on serum biochemical parameters in experimental rats

Parameter	Group I	Group II	Group III	Group IV
Protein (g/dL)	7.15 ± 0.254	5.25 ± 0.16^{NS}	5.45 ± 0.79*	4.80 ± 0.26*
Creatinine (mg/dL)	0.85 ± 0.02	0.45 ± 0.16 ^{NS}	0.55 ± 0.05 ^{NS}	0.56 ± 0.19 ^{NS}
SGOT (U/L)	239.40 ± 9.76	253.60 ± 12.70 ^{NS}	275.80 ± 11.60*	291.60 ± 10.70**
SGPT (U/L)	41.61 ± 1.80	48.00 ± 1.60 ^{NS}	48.50 ±1.65 ^{NS}	52.66 ± 2.60 ^{NS}
ALP (U/L)	250.34 ± 8.56	245.00 ± 0.84 ^{NS}	255.60 ± 2.22 ^{NS}	240.43 ± 2.80 ^{NS}

*NS = Non-significant; *p < 0.05; **p < 0.01; ***p < 0.001*
All values are expressed as mean ± SD.

4. DISCUSSION

The analysis of serum biochemical parameters in rats treated with the polyherbal extract reveals important insights into its effects on protein levels, renal function, and liver enzyme activity. The observed changes in these parameters provide clues about the extract's impact on physiological processes and its safety profile.

The polyherbal extract resulted in a dose-dependent decrease in serum protein levels. Specifically, the protein concentration significantly decreased from 7.15 g/dL in the control group to 4.80 g/dL in the highest dose group (600 mg/kg). This reduction aligns with findings from other studies where herbal extracts have been shown to affect protein metabolism. For instance, Kumar et al. (2020) reported similar decreases in serum protein levels following administration of a herbal formulation, suggesting a potential impact on protein synthesis or increased protein degradation. This decrease may indicate a stress response or alteration in nutritional status, but further investigation is needed to clarify the underlying mechanisms.

Creatinine levels, which reflect kidney function, remained unchanged across all treatment groups. This result suggests that the polyherbal extract does not adversely affect renal function, consistent with studies where various herbal treatments did not alter creatinine levels significantly. For example, Singh et al. (2019) found no significant changes in creatinine levels in rats treated with an herbal extract, indicating a lack of renal toxicity. This finding is reassuring, suggesting that the extract is not harmful to kidney function at the administered doses.

Serum SGOT levels increased significantly with higher doses of the polyherbal extract, indicating potential hepatocellular injury. The SGOT level rose from 239.40 U/L in the control group to 291.60 U/L in the highest dose group (600 mg/kg). Elevated SGOT levels are often associated with liver damage or stress, as supported by research on other herbal extracts. For instance, Sharma et al. (2021) observed elevated SGOT levels in rats treated with a different herbal formulation, correlating with liver damage. Conversely, SGPT levels remained stable, showing no significant variation across groups, which is consistent with findings from other studies indicating that SGPT is less sensitive to certain types of liver stress compared to SGOT.

The serum ALP levels did not show significant changes across the treatment groups. ALP is an enzyme related to liver function, bone metabolism, and bile acid synthesis. The stability of ALP levels suggests that the polyherbal extract does not significantly affect these processes, similar to findings by Gupta et al. (2022), where no substantial changes in ALP levels were observed following herbal treatment. This stability implies that the extract does not interfere with bile acid metabolism or bone health.

5. CONCLUSION

The analysis of serum biochemical parameters revealed that the polyherbal extract significantly reduced serum protein levels in a dose-dependent manner, suggesting potential impacts on protein metabolism or nutritional status. Despite this, creatinine levels remained stable, indicating no adverse effects on renal function. The extract was associated with a significant increase in serum SGOT levels at higher doses, suggesting possible liver stress or damage, while SGPT levels and ALP levels remained unaffected, implying minimal impact on overall liver inflammation and bile acid metabolism. These findings suggest that while the extract may be relatively safe for kidney and bone health, its potential hepatotoxic effects at higher doses

warrant further investigation. Overall, while the polyherbal extract did not affect kidney function or ALP levels significantly, it was associated with decreased serum protein levels and increased SGOT levels, indicating potential liver stress or damage at higher doses. Further research may be needed to fully understand the implications of these findings and to assess the extract's safety profile.

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