Exploring the Nontoxic Effect of Leaf Extract of Gynura Procumbent on Wister Albino Rat with Biochemical Investigation on Required Parameters

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ABSTRACT

Gynura procumbens (Lour.) Merr. (Family Asteraceae) is a medicinal plant used to treat a wide variety of health ailments such as blood pressure maintenance, kidney discomfort, Liver and Skin problems, rheumatism, diabetes mellitus, Heart disorders, cell damage repairing, cancer, constipation and hypertension. However, the use of plants by ethnic people for curing diseases without knowing their adverse effects may cause health complications in later stages.

Getting a positive result for many years from these populations has been scientifically studied by many researchers to know the therapeutic potentiality of G.procumbens for the treatment of various diseases making it a target for pharmacological studies aiming to validate and provide scientific evidence for the traditional claims of its efficacy. This Present study will also investigate the acute and sub-acute or sub chronic toxicity effects of the extract of *G.P.* leaves on different organs and the potentiality of leaves Extract of *G. procumbens* should be explored significantly.

Keywords: Gynura procumbens, medicinal plant, biological activity, therapeutic potentiality.

INTRODUCTION

There are thousands of herbal plants have already been recognized as blessing of Nature to mankind for the leading source of traditional medicines with a huge reservoir of various effective chemical substances with potential therapeutic properties. Regarding the medicinal properties of these plants, our modern scientists are very much interested and excited and globally admitted and accepted that the herbal elements are essential for World health prevention and protection without creating any side effects if justified with a scientific approach and compared to current synthetic drugs. For this reason, all types of pharmacologists or biologists are working for the search or invention of safe and effective natural remedies. Though all cultures throughout history have used these herbs and thus herbal medicine is the oldest form of health care known to mankind, it was noted that all medicinal plants are not safe to consume or use in medicine due to it's toxicity.

My subject medicinal plant named Gynura procumbens belongs to family Asteraceae is a fast-growing evergreen herb which is commonly found in tropical Asia countries such as China, Thailand, Indonesia, Malaysia, and Vietnam. Traditionally,and presently in India, Bangladesh, Myanmar etc. In Malay, *Gynura procumbens* is known as Sambung nyawa, meaning "prolongation of life," while in Chinese, it is referred to as Bai Bing Cao, meaning "100 ailments." This small plant typically grows to a height of around 1 to 3 meters, featuring a fleshy stem with a purplish tint. The leaves are ovate-elliptic or lanceolate, measuring 3.5–8 cm in length and 0.8–3.5 cm in width, while its flowering heads are narrow, yellow, and about 1–1.5 cm long. *G. procumbens* is widely used in various countries to address numerous health conditions, including kidney problems, rheumatism, diabetes, constipation, and hypertension. The plant is valued for its rich chemical composition, which contributes to its significant therapeutic potential [5,6,7]. The leaves contain important active chemical constituents such as flavonoids, saponins, tannins, terpenoids, and sterol glycosides[2]. It contains several constituents including kaempferol, quercetin, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-rutinoside, rutin, chlorogenic acid and 3,5-dicaffeoilquinate methyl ester, terpenoid, tannin, alkaloid, saponin, and astragalin [3].

MATERIAL & METHODOLOGY

Collection, Identification and authentication:

Fresh leaves of Gynura procumbens will be collected from RNTU botanical garden. Collected Plant was sent for Identification and authentication to Center for Microbiology & Bio-technology Research & Training (CMBT) Dr. Reena Upadhyay (Botanist, CMBT Research and Training Institute, and Institute, Bhopal Bhopal) Herbarium specimen with voucher no. CMBT/Res.22/1588.

After cleaning, the dried leaves of Gynura procumbens samples were ground into fine powder using mortar and pestle, and then a mechanical blender was used to ground them further to ready fine powder form. Then the sample was stored in an air-tight container for extract preparation in the lab.

For extraction, I preferred reflux technique with Soxhlet Extraction which is a process following Steam distillation. This process is useful for extraction bioactive compounds from plant materials.

The hydroalcoholic solvent is effective in dissolving both polar and nonpolar compounds, making this method suitable and logical for a wide range of plant extractions.

Animal used for Experiment – As per the OECD GUIDELINE NO 423, for this toxicity study Fifteen Wistar albino rats weighing 150-220g age 12-15 weeks were arranged and permitted after approval of the ethics committee and management of the Department of Pharmacy, VNS College, Bhopal. According to the Instruction of the Expert faculty of Pharmaceutical Institute, those animals were supervised and raised throughout the study properly.

Dose preparation according to Animal Groups –

The significance of different doses preparation and administration can ensure the accuracy of experimental results.

Animal Grouping

- **Group I (Control Group):** Three Wistar albino rats receive a normal diet without drug administration. This group serves as the baseline for comparison.
- **Group II (Low Dose Group):** Three Wistar albino rats receive the drug at a dose of 750 mg/kg body weight.
- **Group III (Moderate Dose Group):** Three Wistar albino rats receive the drug at a dose of 1500 mg/kg body weight.
- **Group IV (High Dose Group):** Three Wistar albino rats receive the drug at a dose of 2250 mg/kg body weight.
- **Group V (Maximum Dose Group):** Three Wistar albino rats receive the drug at a dose of 3000 mg/kg body weight.

Dose Preparation

I. Determine the Average Body Weight:

Each rat was Weighted before starting the experiment. The average weight of the rats in each group was determined to ensure precise dose calculation.

II. Calculate the Dose for Each Group:

- For Group 2, multiply the average body weight by 750 mg/kg to determine the total dose to be administered.
- For Group 3, multiply the average body weight by 1500 mg/kg.
- For Group 4, multiply the average body weight by 2250 mg/kg.
- For Group 5, multiply the average body weight by 3000 mg/kg.

This was maintained that each rat receives the drug in a consistent volume based on its body weight. The drug solution was administered orally at the calculated dose daily for 28 days. The control group received an

equivalent volume of the solvent without the drug. Accurate dosing is very important for the validity of experimental results, particularly in pharmacological and toxicity studies involving different drug concentrations in various groups.

Acute Toxicity Study – This investigation is to assess the acute or morderate toxicity study of a drug administered at 3000 mg/kg body weight in Wistar albino rats over a continuous 7-day period. Select healthy Wistar albino rats, weighing approximately 150-200 grams.

Divide the rats into a single experimental group consisting of three rats. Administer the drug orally using a gavage or another suitable method. Ensure that the drug is given once daily at the same time for 7 consecutive days. Monitor the rats closely during the administration period for any immediate adverse effects. Record any changes in behavior, physical condition, and weight of the rats throughout the 28-day trial.

Subacute Toxicity Studies – In three pathological sections like Biochemical, Hematological and Histological investigations were performed in a reputed Laboratory of Kolkata to determined the subjective required results for data analysis on several parameters as described below in topic "Pathological Studies" in detailed. Subacute studies involve repeated administration of certain doses (as per the dose calculation) throughout on 28 days continuously, to evaluate the cumulative effects of the plant extract on the health of experimental animals.

Sample Collection –

Blood Sample Collection from Albino Rats After Systemic Drug Administration

Here I performed the Cardiac puncture process for the large volume of blood sample as I had to do many biochemical tests with that sample.

- **Procedure for Cardiac puncture :**
  - Anesthetize the rat deeply to ensure it feels no pain.
  - Place the rat in dorsal recumbency.
  - Insert a needle into the heart, usually through the left ventricle, and aspirate the blood.
  - This procedure is typically followed by euthanasia.
- **Storage:** if required the plasma, serum, or whole blood samples should be stored at appropriate temperatures (e.g., -20°C) in the laboratory to preserve the integrity of the sample until analysis.

Pathological Studies

Biochemical Investigations

Biochemical Tests in Subacute Toxicity Studies of Drugs: A Detailed Overview

Subacute toxicity studies are essential in evaluating the safety of a drug over a prolonged period, typically ranging from a few weeks to three months. These studies help identify potential adverse effects and ensure that the drug does not cause significant harm to various organs and systems in the body. Biochemical tests play a crucial role in these studies, as they provide valuable insights into the physiological and metabolic changes that may occur due to drug administration. Below is a detailed note on why specific biochemical tests are conducted in subacute toxicity studies:

Used Analyzers for Pathological Investigation at Clinical Laboratory, Kolkata.

Biochemistry Department - Biochemistry Full Analyzer, **Beckman Coulter / Olympus AU480**. It's a closed system operative heavy instrument for accurate and quality controlled output with scientific presentable reports.

Data preparation and Statistical Analysis

The data were expressed as the mean ± standard error of the mean (SEM), and the results were analyzed using one-way ANOVA., Social Science Statistics Software to determine the significant outcome of this study, here which accepts the null hypothesis as true ($p > 0,05$).

RESULTS

Observation for Acute toxicity - The rats were observed in daily basis for any signs of toxicity, such as changes in behavior, physical appearance, feeding patterns, and overall health.. Record Any notable signs of distress, morbidity, or mortality were recorded properly. Body weights of every rats were measured and recorded before the start of the trial and daily during the study.

Findings:

- Throughout the 7-day trial, all three rats in the experimental group showed no signs of acute toxicity at the administered dose of 3000 mg/kg body weight.
- **Behavioral Observations:** The rats exhibited normal behavior, including regular feeding and grooming habits, without any signs of distress or discomfort.
- **Physical Observations:** There were no observable physical changes such as lethargy, abnormal posture, or changes in fur condition.
- **Body Weight Monitoring:** The body weight of the rats remained stable, with no significant weight loss or gain that could indicate toxicity.

These findings provide preliminary evidence of the drug's safety at the tested dose level, supporting further studies to explore its pharmacological properties and potential therapeutic applications. The results of this acute toxicity study suggest that the drug, when administered at 3000 mg/kg body weight over a 7-day period, does not induce acute toxic effects in Wistar albino rats.

Results of parameters for Sub acute toxicity studies -

Biochemical Investigations

Blood glucose

Table no. 1: Biochemical study of **Blood glucose** on Wister albino rat with different doses.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	98.66±3.05505
2	Group II	750 mg / kg BW	103.33±6.11
3	Group III	1500 mg / kg BW	110.00±11.13
4	Group IV	2250 mg / kg BW	116.66±13.61
5	Group V	3000 mg / kg BW	115.33±27.73

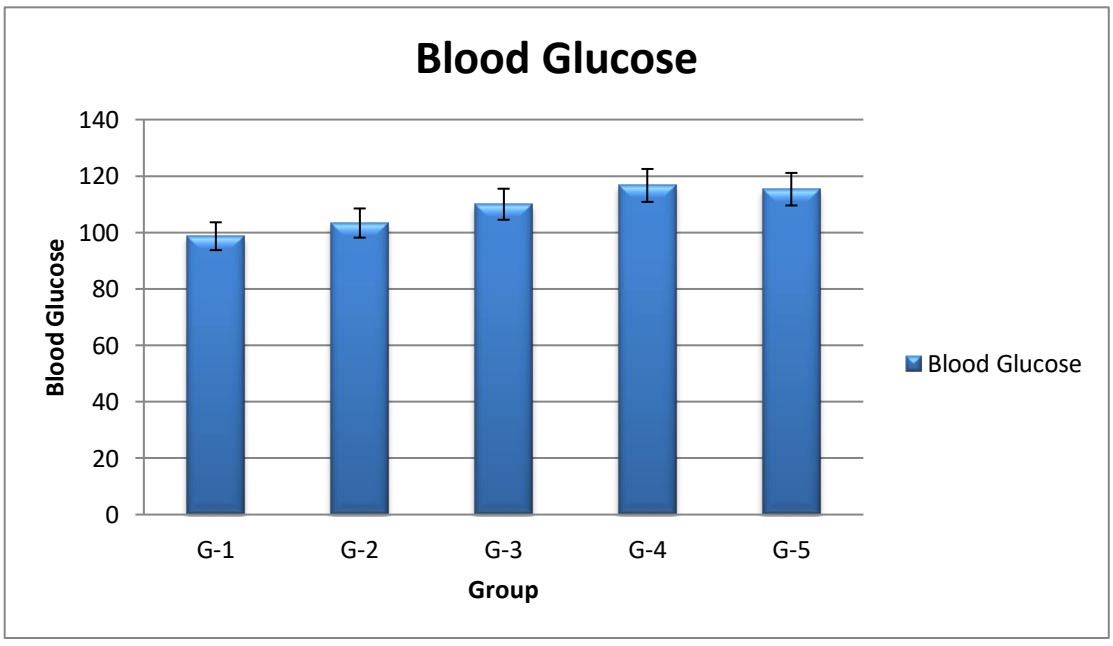

Fig 1: Graphical presentation of Blood glucose Study with Different Groups.

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Table no.2: Biochemical study of Alkaline Phosphate on Wister albino rat with different doses

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	114.66±16.19
2	Group II	750 mg / kg BW	147.66±12.70
3	Group III	1500 mg / kg BW	135.00±18.58
4	Group IV	2250 mg / kg BW	146.00±25.23
5	Group V	3000 mg / kg BW	142.66±34.21

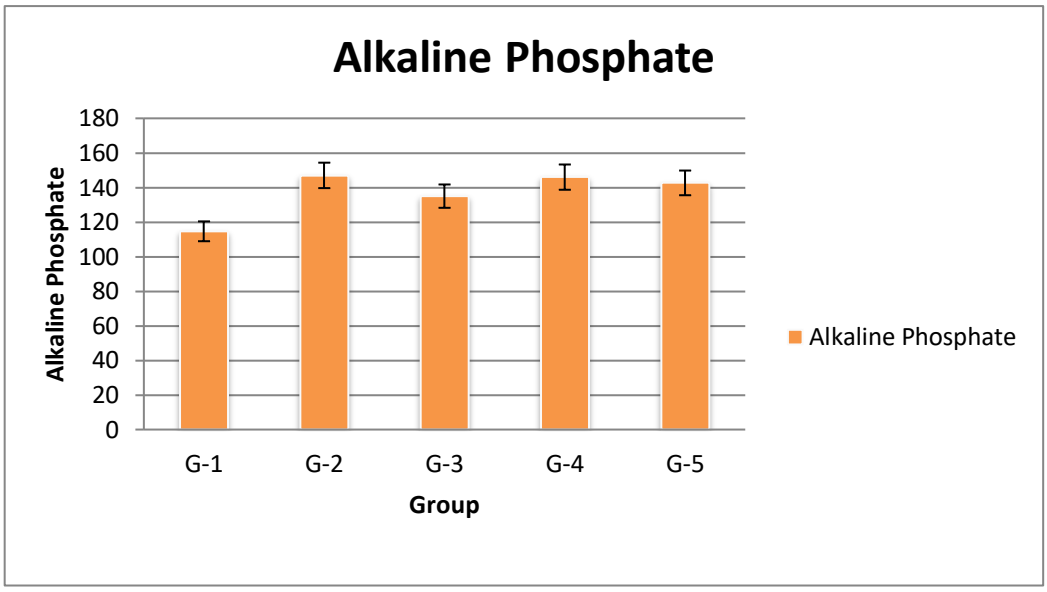

Fig 2: Graphical presentation of Alkaline phosphate Study with Different Groups.

Total Cholesterol

Table no. 3: Biochemical study of Total Cholesterol on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	84.33±6.18
2	Group II	750 mg / kg BW	77.66±4.19
3	Group III	1500 mg / kg BW	85.33±2.49
4	Group IV	2250 mg / kg BW	86.66±5.73
5	Group V	3000 mg / kg BW	73.66±5.43

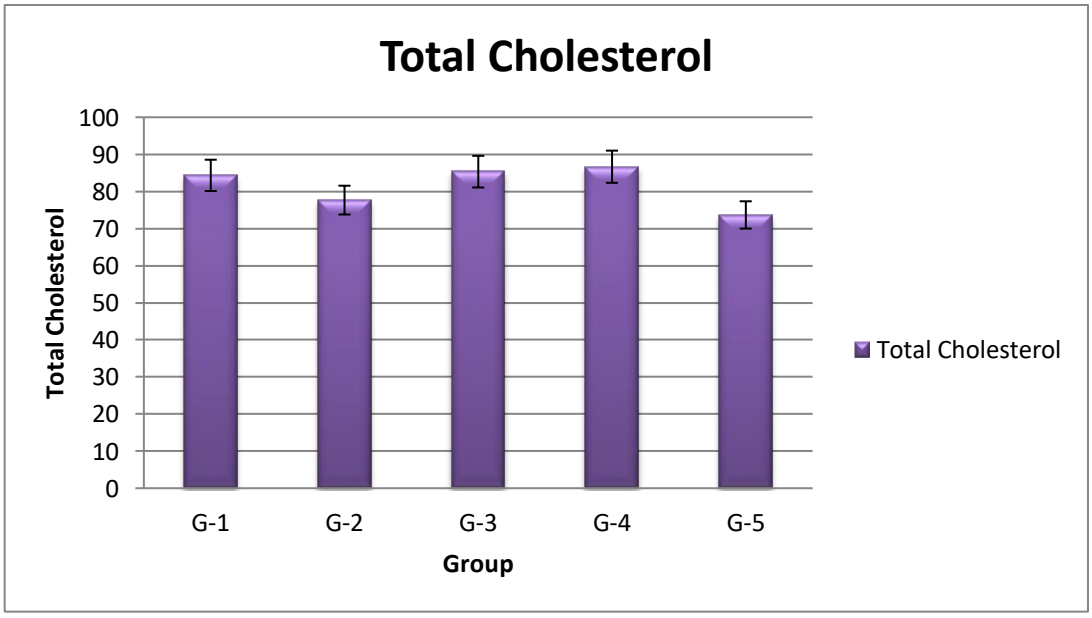

Fig 3: Graphical presentation of Total Cholesterol Study with Different Groups.

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Hdl Cholesterol

Table no. 4: Biochemical study of HDL Cholesterol on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	34.33±2.62
2	Group II	750 mg / kg BW	28.66±2.49
3	Group III	1500 mg / kg BW	34.00±0.82
4	Group IV	2250 mg / kg BW	36.83±4.09
5	Group V	3000 mg / kg BW	20.33±3.68

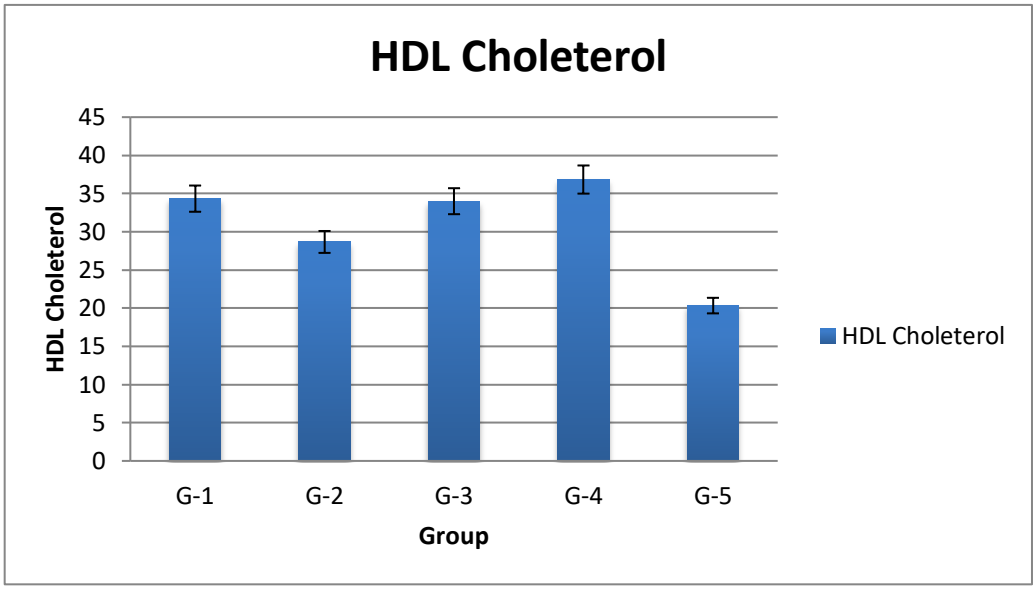

Fig. 4: Graphical presentation of HDL Cholesterol Study with Different Groups.

Ldl Cholesterol

Table no.5: Biochemical study of LDL Cholesterol on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	41.46±2.79
2	Group II	750 mg / kg BW	34.60±4.25
3	Group III	1500 mg / kg BW	42.00±2.51
4	Group IV	2250 mg / kg BW	35.90±2.49
5	Group V	3000 mg / kg BW	40.46±5.08

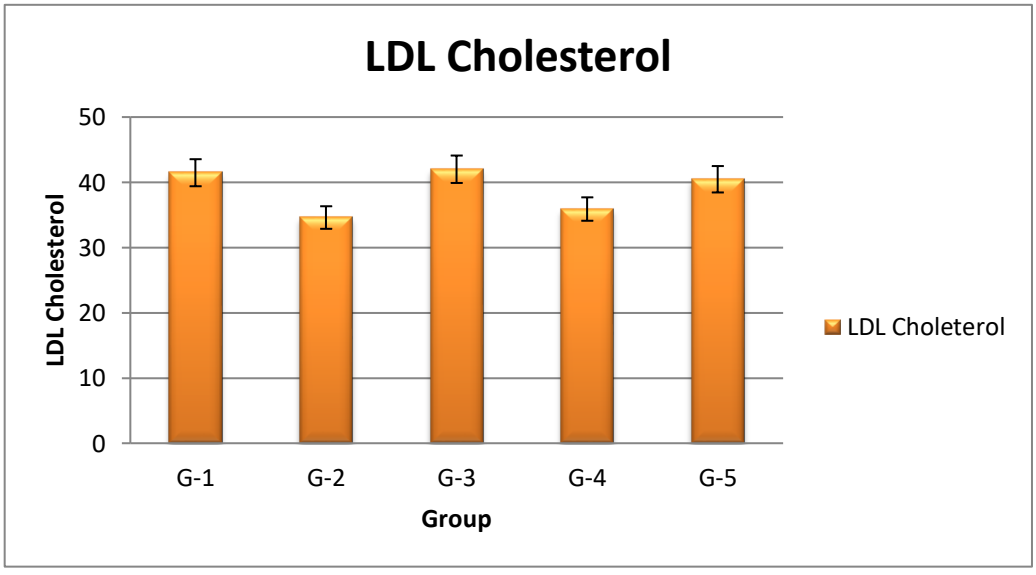

Fig. 5: Graphical presentation of LDL Cholesterol Study with Different Groups.

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Triglyceride

Table no. 6: Biochemical study of Triglyceride on Wister albino rat with different doses.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	43.33±5.24
2	Group II	750 mg / kg BW	72.00±5.72
3	Group III	1500 mg / kg BW	46.66±4.11
4	Group IV	2250 mg / kg BW	53.00±5.71
5	Group V	3000 mg / kg BW	61.00±6.16

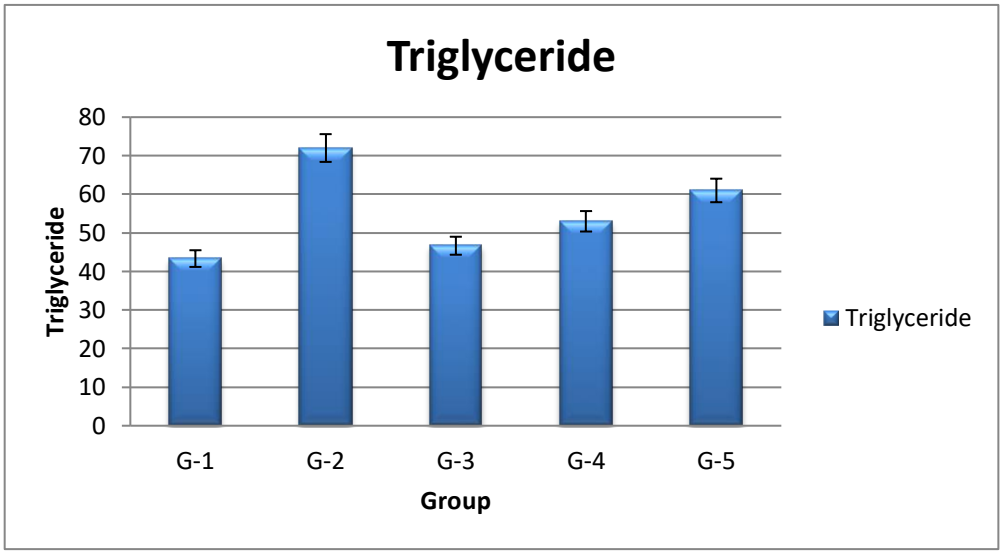

Fig. 6: Graphical presentation of Triglyceride Study with Different Groups.

Bilirubin Total

Table no.7: Biochemical study of Bilirubin on Wister albino rat with different doses.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	0.19±0.04
2	Group II	750 mg / kg BW	0.16±0.02
3	Group III	1500 mg / kg BW	0.18±0.02
4	Group IV	2250 mg / kg BW	0.11±0.04
5	Group V	3000 mg / kg BW	0.28±0.20

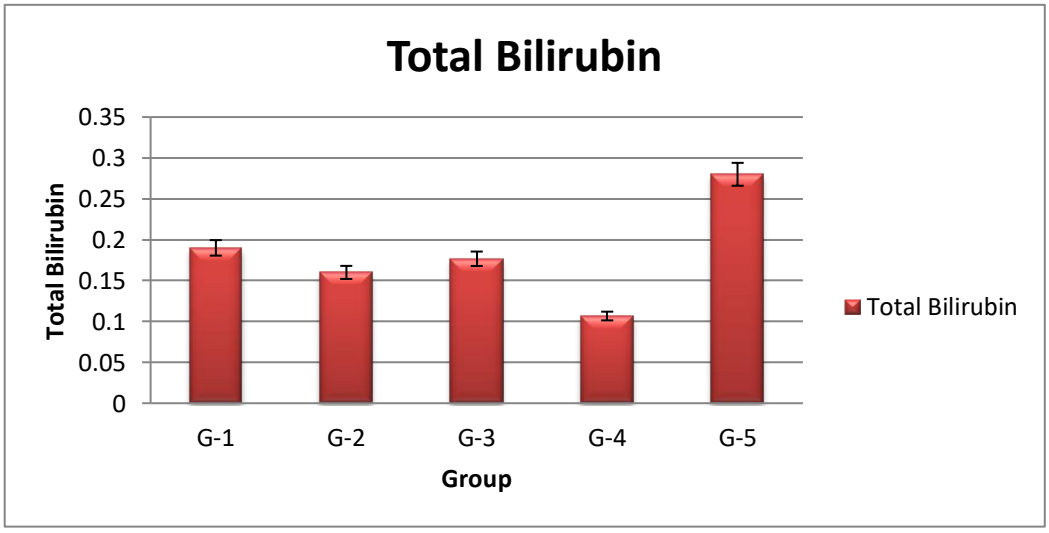

Fig. no 7: Graphical presentation of Total Bilirubin Study with Different Groups.

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Sgpt (Alt)

Table no.8: Biochemical study of SGPT (ALT) on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	188.00±16.57
2	Group II	750 mg / kg BW	185.66±28.59
3	Group III	1500 mg / kg BW	155.66±11.44
4	Group IV	2250 mg / kg BW	145.33±15.45
5	Group V	3000 mg / kg BW	194.00±17.04

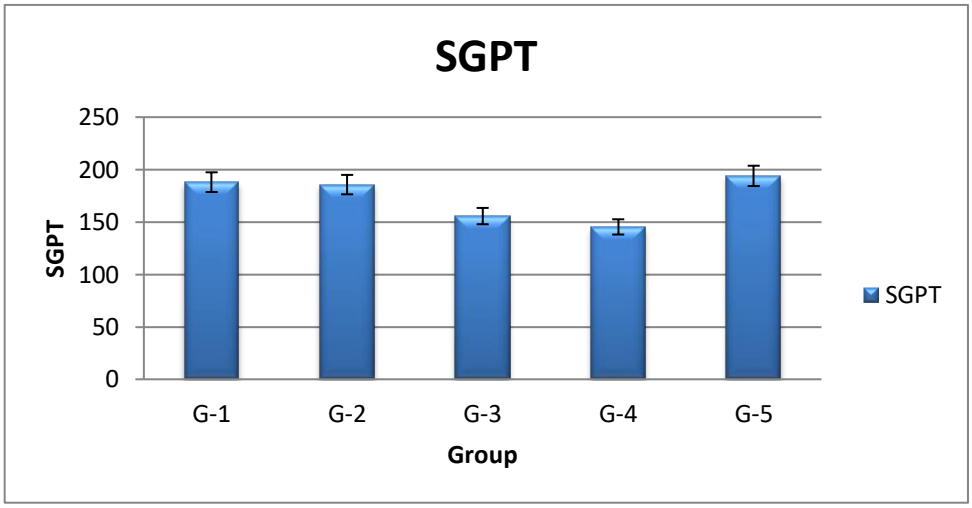

Fig. no. 8: Graphical presentation of SGPT (ALT) Study with Different Groups.

Sgot (Ast)

Table no. 9: Biochemical study of SGOT (AST) on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	191.00±21.92
2	Group II	750 mg / kg BW	154.00±29.88
3	Group III	1500 mg / kg BW	187.33±16.11
4	Group IV	2250 mg / kg BW	205.33±14.26
5	Group V	3000 mg / kg BW	188.33±26.04

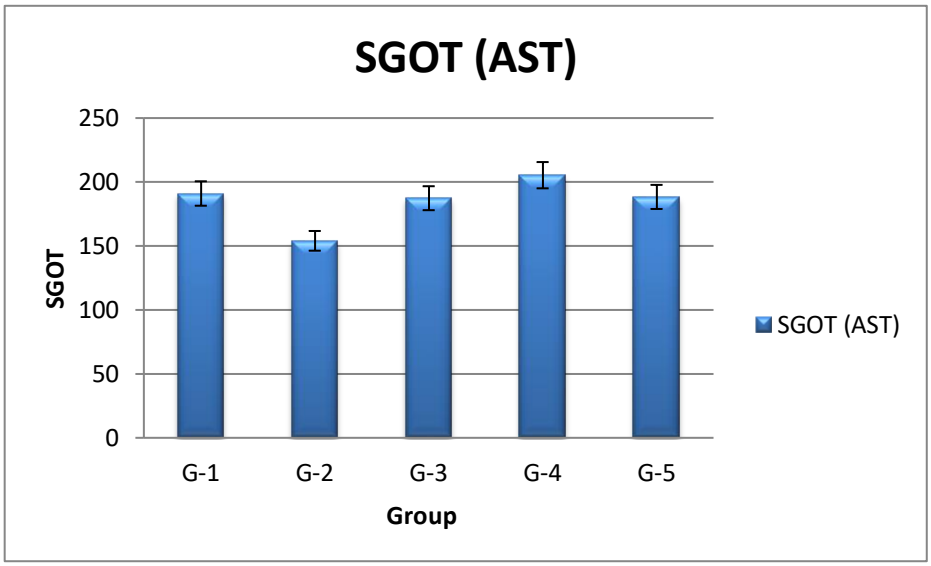

Fig. 9: Graphical presentation of SGOT (AST) Study with Different Groups

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Urea

Table no. 10 : Biochemical study of Urea on Wister albino rat with different doses.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	77.66±1327
2	Group II	750 mg / kg BW	48.66±3.30
3	Group III	1500 mg / kg BW	52.00±8.60
4	Group IV	2250 mg / kg BW	60.00±9.41
5	Group V	3000 mg / kg BW	43.33±3.77

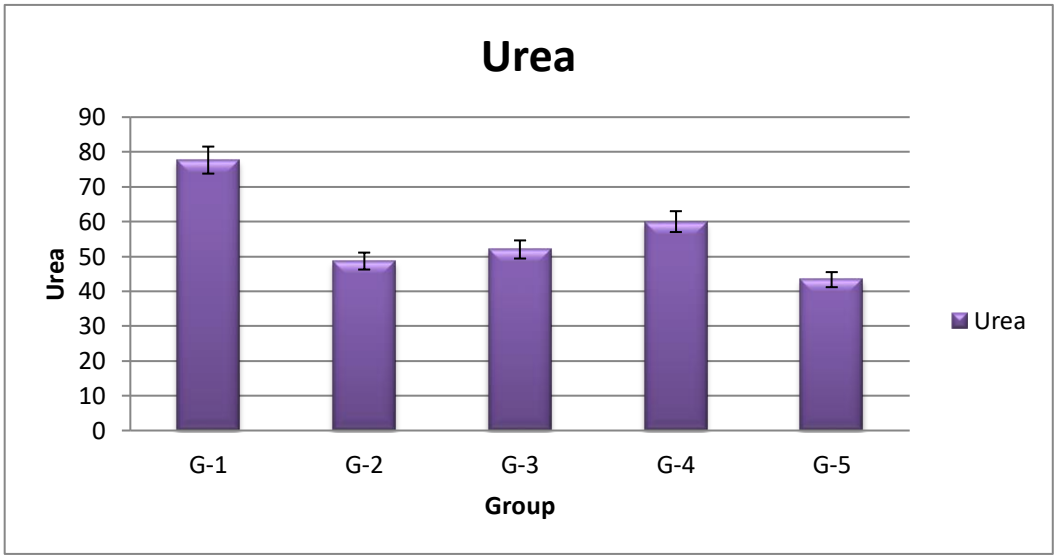

Fig. no. 10: Graphical presentation of Urea Study with Different Groups.

Creatinine

Table no. 11: Biochemical study of Creatine on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	0.61±0.04
2	Group II	750 mg / kg BW	0.48±0.01
3	Group III	1500 mg / kg BW	0.56±0.05
4	Group IV	2250 mg / kg BW	0.56±0,01
5	Group V	3000 mg / kg BW	0.57±0.05

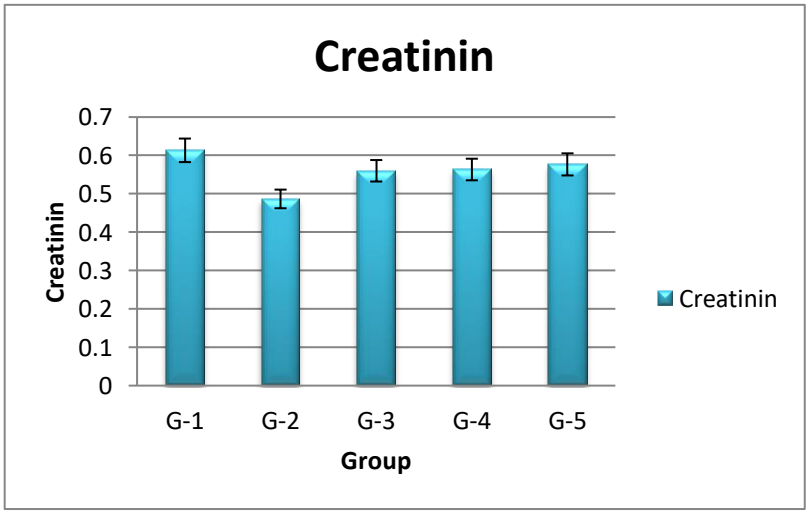

Fig.no. 11: Graphical presentation of Creatinine Study with Different Groups.

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Total Protein

Table no. 12: Biochemical study of Total protein on Wister albino rat with different doses.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	8.37±0.64
2	Group II	750 mg / kg BW	7.55±0.32
3	Group III	1500 mg / kg BW	8.59±0.36
4	Group IV	2250 mg / kg BW	8.11±0.50
5	Group V	3000 mg / kg BW	6.96±0.33

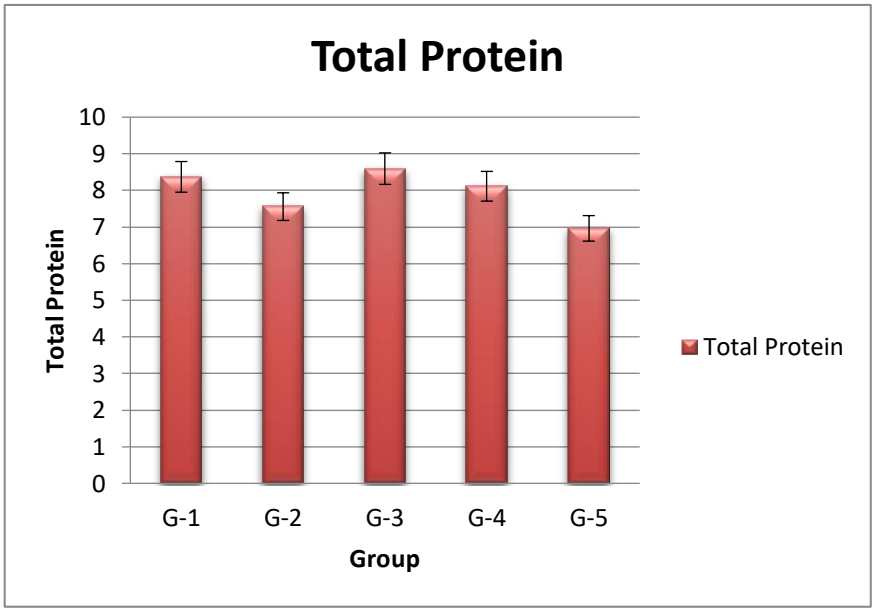

Fig. no. 12: Graphical presentation of Total Protein Study with Different Groups.

Uric Acid

Table no. 13: Biochemical study of Uric Acid on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	2.26±0.50
2	Group II	750 mg / kg BW	1.96±0.32
3	Group III	1500 mg / kg BW	1.95±0.16
4	Group IV	2250 mg / kg BW	2.15±0.41
5	Group V	3000 mg / kg BW	2.75±0.63

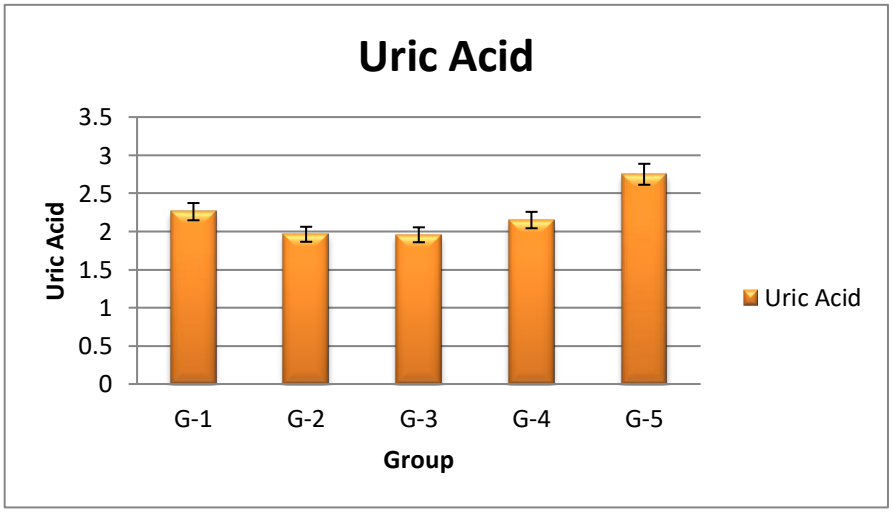

Fig. no 13: Graphical presentation of Uric Acid Study with Different Groups of Albino Rat.

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Bicarbonate

Table no. 14: Biochemical study of Bicarbonate on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	26.23±1.45
2	Group II	750 mg / kg BW	24.53±0.94
3	Group III	1500 mg / kg BW	25.43±0.66
4	Group IV	2250 mg / kg BW	25.56±0.24
5	Group V	3000 mg / kg BW	26.63±4.24

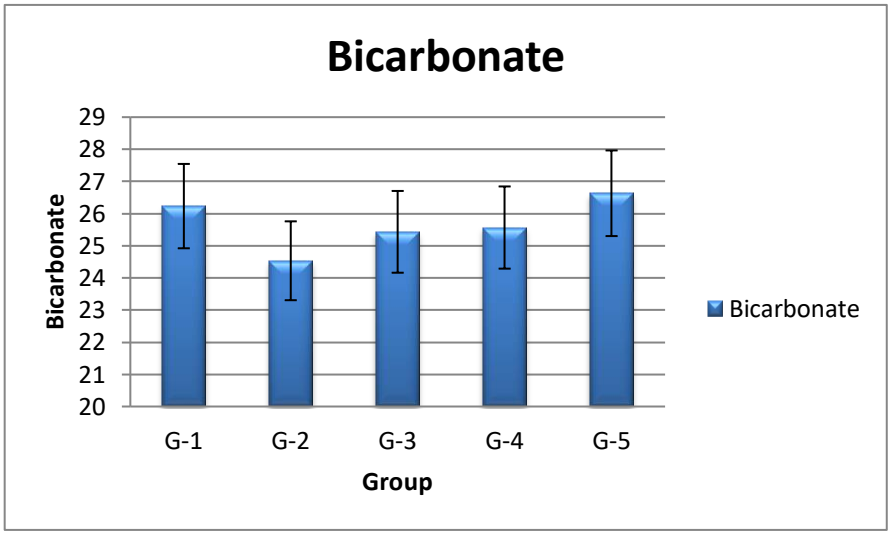

Fig. no 14: Graphical presentation of Bicarbonate Study with Different Groups.

Sodium

Table no. 15: Biochemical study of Sodium on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	151.66±2.86
2	Group II	750 mg / kg BW	143.00±1.63
3	Group III	1500 mg / kg BW	144.00±0.81
4	Group IV	2250 mg / kg BW	144.00±2.16
5	Group V	3000 mg / kg BW	148.00±11.77

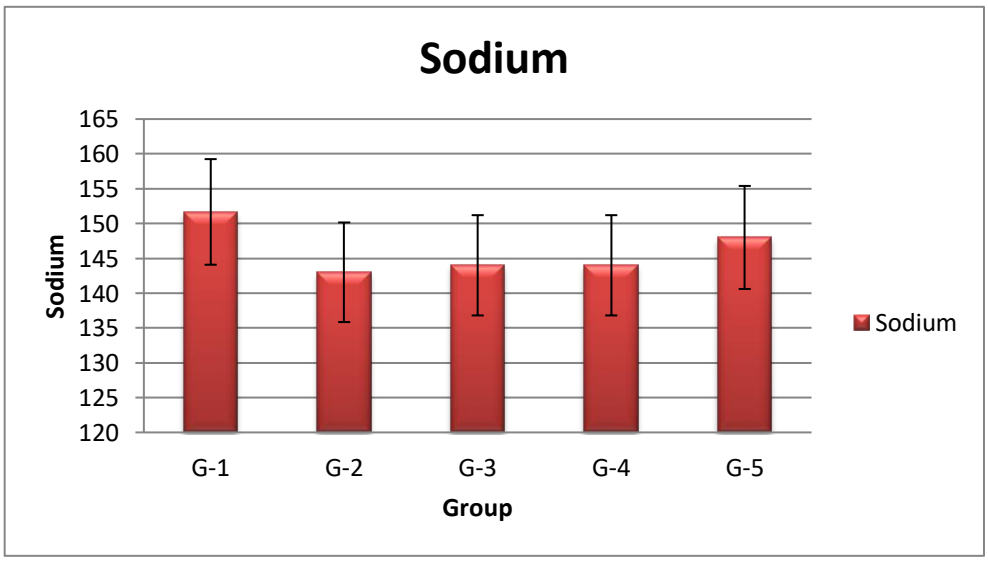

Fig. no. 15: Graphical presentation of Sodium Study with Different Groups

Potassium

Table no. 16: Biochemical study of Potassium on Wister albino rat.

SL NO	GROUPS	DIFFERENT DOSES (BW = Body Weight)	AVERAGE VALUES
1	Group I	Controlled	5.70±0.57
2	Group II	750 mg / kg BW	4.53±0.09
3	Group III	1500 mg / kg BW	5.20±0.57
4	Group IV	2250 mg / kg BW	5.56±0.23
5	Group V	3000 mg / kg BW	5.30±2.39

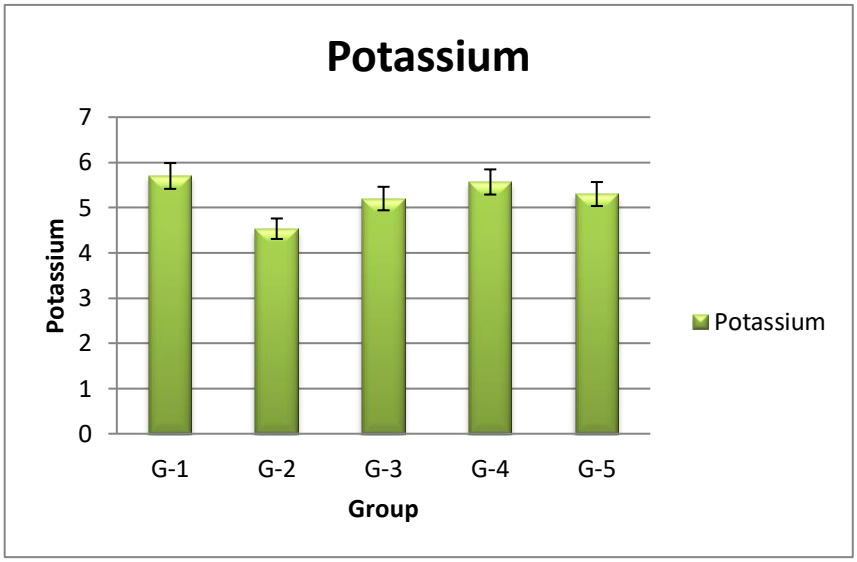

Fig. no. 16: Graphical presentation of Potassium Study with Different Groups.

DISCUSSION

The subacute toxicity study of *Gynura procumbens* extract on Wistar albino rats across five different groups, including a control group and four varying dosage groups (750 mg/kg BW, 1500 mg/kg BW, 2250 mg/kg BW, and 3000 mg/kg

BW), provided insightful data regarding the safety profile of the extract. As per the analysis with one way ANOVA, the overall result accepts the null hypothesis as true as the p value greater than 0.05 for every parameters of biochemical and hematological assay. Not only statistically it is found that the result is not significant, but also it showed that the result of every parameters of biochemical assay for different doses in subacute toxicity study are found with in normal range as data provided by National Library of Medicine and National Center for Biotechnology Information (NCBI). Despite the administration of high doses of *Gynura procumbens* extract over the study period, the findings revealed no significant alterations in the biochemical, hematological, or histopathological parameters.

The results of Acute & subacute toxicity study underscore the safety of *Gynura procumbens* extract when administered to Wistar albino rats over a prolonged period. The absence of any adverse effects on biochemical parameters, even at the highest tested dose of 3000 mg/kg BW, indicates that *Gynura procumbens* extract has a wide margin of safety. These findings support the potential therapeutic use of *Gynura procumbens* at various dosages without the risk of toxicity, thus providing a strong foundation for its continued use in traditional and alternative medicine. This finding also demands some advanced studies to use this plant in combined drugs for specific diseases for a upgradation of conventional as well as modern medicines.

CONCLUSION

In summery of this study this can be stated that the plant Gynura procumbens in all sense is a “God’s miracle gift to mankind” as it is a better availability, easy for plantation, non toxic as well as full of medicinal

properties can be highly usable in therapeutic application. Though it is not yet explored and promoted for lack of proper knowledge and advanced frequent researches, this plant can do miracle in medical science and can play a revolutionary role in health protection. Because having a huge medicinal benefits it also contains a significant nutritional values with required elements for health protection.

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