Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i6.6840

COMBINED EFFECT OF VITAMIN B (B_6, B_9, B_{12}) AND POTASSIUM SUPPLEMENTATION ON STROKE IN RAT MODEL

Noman Aslam[1], Muhammad Imran Hussain[2*], Khansa[3*], Imran Nawaz Bhatti[4], Qaswara Affaf[5], Hafiza Iqra Akram[6], Sahir Shahzad[1], Tasbiha Irfan [1], Moha Akram Khan[7]

[1]Department of Human Nutrition & Dietetics, Riphah International university QIE campus, Lahore, Pakistan
[2]Assistant Professor Department of Human Nutrition & Dietetics, Riphah International university QIE campus, Lahore, Pakistan
[3] Lecturer Al Razi Institute, Lahore
[4]Minhaj University, Lahore
[5]University of Veterinary & Animal Sciences, Lahore
[6]Government College University Faisalabad
[7] Department of Diet and Nutrition Sciences, Rashid Latif Khan University

***Corresponding author:** Muhammad Imran Hussain[1], Khansa[2]
[1]Assistant Professor Department of Human Nutrition & Dietetics, Riphah International university QIE campus, Lahore, Pakistan, Email: imran.hussain@riphah.edu.pk
[2]Lecturer Al Razi Institute, Lahore, Email: Khansamubeen123@gmail.com

ABSTRACT

Introduction: Stroke is second most common cause of death and the third most common cause of disability worldwide. The burden of stroke is rising quickly in low and middle-income nations, many of which are ill-equipped to handle the problem it poses. The objective of the current study was to determine the combined effect of Vitamin B (B_6, B_9, B_{12}) and Potassium supplementation on stroke in rat model. **Methods:** Vitamin B (B_6, B_9, B_{12}) and Potassium was bought in supplemented form. Rats were divided into five groups, including a control group Go). Complete Blood Count (CBC), Liver Function Test, Homocysteine Level and Blood Pressure were analyzed to check impact of B vitamins and potassium. The p-value <0.05 was considered significant. **Results:** Vitamin B (B_6, B_9, B_{12}) and Potassium supplementations significantly decrease the Homocysteine level by 55% (19.95 ± 0.08 g/dL) and (21.97 ± 0.06 g/dL) respectively. The systolic and diastolic blood pressure significantly de-escalated with the potassium supplementation diastolic (84.96 ± 0.06 mmHg) and systolic (122.97 ± 0.06 mmHg) in (G4 group). **Conclusion:** Our findings suggest that Vitamin B (B_6, B_9, B_{12}) and Potassium supplementation shod improving trend in the CBC, LFT, s Homocysteine levels and BP. Further research is warranted to explore the translational potential in human subjects.

Keywords: Vitamin B, Complete Blood Count (CBC), Homocysteine Level, Liver Function Tests (LFTs), Potassium

INTRODUCTION

Globally, stroke serves as the 2nd cause of morality worldwide and 3rd leading source of debility. Its burden is increasing rapidly in low to middle income and socio-economic countries which are unable to face its complications. In 2019, there were 95% cases of stroke including 101 million ubiquitous cases of stroke, 143 million DALYs due to stroke followed by 6.55 million mortalities resulted. Throughout the earlier three periods, worldwide stroke morbidity enlarged by 70%, its occurrence augmented by 85%, its death rate increased by 43%, and DALYs by 32%, with a more rise in stroke problem in low to middle income countries (LMICSs) in comparison to high income countries (HICs) [1].

Stroke prevalence is possibly will fluctuate to some extent between revisions because each study picks and staffs a trial of members to denote the target on people (e.g. state-run, area, or nation). A report represented the data that around 9 million Americans of 20+ age have stroke sign and symptoms according to NHANES report 2017–2020. 3.3% prevalence of stroke was estimated during this time frame. More males and females have stroke depending upon age is US. According to unpublished NHLBI tabulation data (BRFSS1 2021), in the United States, stroke prevalence rate was more than 3% in adults, with the highest prevalence rate in Mississippi recorded as 4.9% and the lowest in Vermont recorded as 1.9% [2].

Annually, ≈795000 people go through a new or recurrent stroke attack. Out of total, approximately 610000 stroke attacks occur for the first time and 185000 face recurrent attacks. Further, 87% counts for ischemic stroke, 10% are intracerebral haemorrhage and 3% are sub-arachnoid haemorrhage strokes [3].50 studies in 20 countries from a systemic review reveals that stroke incidences are diverging by age in HICs with less favorable trends at younger ages [4].

Stroke is a nervous disorder pigeon-holed by obstruction of plasma vessels. Accumulations in the brain or blood vessels disturb the blood movement, blocking arteries, initiating blood vessels to burst, leads to exploiting. Rupture of the arteries lead to the brain during stroke which results in the sudden death of brain cell sowing to a lack of oxygen. Stroke is also a leading cause of depression and dementia [5]. It is also described as sudden blood outburst caused due to impaired perfusion through the brain blood vessels. In ischemic stroke the blood flow and oxygen levels drop which develop stroke called IS, hemorrhagic stroke caused by flow of blood or holey plasma vessels. Ischemic blockings subsidize to around 85% of victims with patients having stroke, with the rest owed to bleeds in brain.

The ischemic obstruction develops thrombotic and embolic ambiance in the brain [6]. During thrombosis, the blood flow lowers due to thinning of vessels due to blood clotting. The plaques ultimately tighten up the vascular compartment and make clots, causing thrombotic stroke. The declined blood movement towards the brain area causes an embolism; the blood pressure reduces, producing severe anxiety which develops necrosis. Necrosis is by distraction of blood membranes, swelling of organelles and extra cellular content leakage into extracellular space and loss of neural function [7]. The key actions contributing to stroke levels include infections, level of free radical, oxidative stress and level of homocystein[8] .

The methylation pathway that breaks down homocysteine into methionine by producing methionine and folic acid (5-methyl tetrahydrofolate) and vitamin B12 (cobalaamin) as a vital factor. The conversion of homocysteine to cystathionine is catalyzed by cystathionine β-synthase and requires a cofactor such as vitamin B6. Hence, the total plasma level of homocysteine (tHcy) increases by blood meditations of the B vitamins folic acid (B9), cyanocobalamin (B12) and pyridoxine (B6) [9]. Tiredness associated with stroke could help from high-dose thiamine B1. Therefore, the writers started giving post stroke patients with oral or parenteral high dose of B1. There is a practical indication that riboflavin supplementation reduces oxidative damage and cerebral edema having stroke [10].

By 2020, there will be 2.2 million people living with a disability due to stroke. The increase in stroke cases in China depends on the cause of the stroke, uncontrolled diabetes, hypertension and hyperlipidemia. The national blood pressure survey launched in China in 2017 showed that 24.7%

of people had high blood pressure; The negative attitude of people with high blood pressure is 60.1%, and the control rate is 42.5%[11].

However, there is lack of knowledge on the combined influence of B6 (Pyridoxine), B9 (Folate), and B12 (Cobalamin) with combination of Potassium on the morbidity, and mortality on the stroke. Since B6, B9, and B12 are the cofactors of breakdown of the homocysteine and Moreover, Potassium will help in lowering the blood pressure their combine application may synergistically enhance their potentials against stroke. Therefore, this study will investigate on the rats with induced stroke to check the combined effect of B6, B9, B12 and potassium that is novelty of this study.

MATERIALS AND METHODS

The study design used for this research plan described is a Randomized Control Trial (RCT)an it included total 50 rat animals which would be divided into five groups, 10 each. Nonprobability Purposive Sampling was used. The study was conducted in the Pharmaceutical Department. Duration of this study was 6 months after the approval by the research board.The ethical approval was granted by the Research and Ethics Committee of University **Ref.NO.REC/RCR& AHS/23/0808 .**

For Data Collection Tools

The following tools were used for the assessment of rats during the study.

- Complete Blood Count (CBC)
- Body Weight
- Homocysteine Levels
- Liver Function Tests (LFTs)
- Blood Pressure (Tail-cuff Method)

For Selection of animal:

Male albino rats were taken from the animal house of the Riphah Institute of Pharmaceutical Sciences, International University, Lahore, Pakistan. 50 rats (4–5 weeks old) weighing 90-120 g were selected after a physical exam.

For Housing of rats:

The animals were housed in boxes with a numbered air system. Rats were initially acclimated to the laboratory area and received poultry meal (14 no.) for one week before starting the enriched diet. The rats housed in a well-aired room with a 12-hour light-dark cycle at a temperature of 25 ± 2 °C and a humidity of 55 ± 3%. Rats were given plenty of water in water bottles

Induction of Stroke in Rats:

The Animal Ethical Committee approved the tentative procedure as per the Guide for the Care and Use of Laboratory Animals prepared by the university. All rat was micro injected endothelin-1 into selected brain regions to induce stroke. 10 p moles of endothelin-1 were micro injected, after twenty-four hours loss of neurons was causing stroke. All rats were fed standard diet and water at the beginning of the trial. At least one week before the start of the treatments, the daily water intake of the animals was monitored to determine the water requirements per experimental animal. The test groups were divided in to four groups.

Treatment Plan

Groups	Treatment
$G_\circ$	Normal Diet
G_1	Normal Diet + Stoke Induced
G_2	Normal Diet + Vitamin B_6 (1200mg/kg) + Vitamin B_9 (200mg/kg) + Vitamin B_{12} (50mg/L) + Stroke Induced

G_3 Normal Diet + Potassium (10mg/kg BW) + Stroke Induced
G_4 Normal Diet + Vitamin B_6 (1200mg/kg BW) + Vitamin B_9 (200mg/kg BW) + Vitamin B_{12} (50mg/L per day) + Potassium (10mg/kg) +Stroke Induced

Statistical Analysis

Data was tabulated using percentage distribution then analyzed descriptively by mean, median, mode and graphically presented using bar chart. Results obtained from different assays were statistically analyzed using SPSS Statistics 26 software. Analysis of variance (ANOVA) under a completely randomized design was employed for B_6, B_9, B_{12} and Potassium while one-way ANOVA was used to check the level of significance, (α: 5%)

RESULTS

This study is conducted with the aim to compare pre-and post-CBC (Complete Blood Count), Liver Function Tests (LFTs), Homocysteine and Blood Pressure by giving Vitamin B_6, B_9 and B_{12} and Potassium supplementation on stroke induced rat model. 50 rats were divided into 5 groups.

DISCUSSION

The main aim of this study is to explore the potential of biochemical markers such as Complete Blood Count (CBC) and Liver Function Test (LFT) and Homocysteine Levels, in the prevention of stroke through supplementation with vitamins B6, B9, B12, and potassium. This investigation is based on the understanding of the significance of specific macronutrients in stroke prevention, which has been supported by multiple studies [12]. Our investigation confirms prior research implying that B vitamins, including folic acid, B6, and B12, may have a crucial function in stroke prevention. Our findings demonstrate a relationship between elevated homocysteine levels (tHcy) in the bloodstream and an increased likelihood of stroke. Furthermore, the experimental outcomes exhibit the potential of B vitamin supplementation, particularly B6, B9, and B12, in reducing homocysteine levels, thereby potentially mitigating arterial and thrombotic complications linked to stroke.

The hypothesis suggesting B vitamin therapy as a preventive measure against stroke emerged more than a decade and a half ago, initially stemming from early case studies and epidemiological investigations that reported a link between elevated plasma concentrations of tHcy and incident cardiovascular disease [13]. Subsequent laboratory results further highlighted the atherogenic and thrombogenic properties of elevated tHcy levels [14] . Randomized controlled trials (RCTs) subsequently validated this hypothesis by demonstrating that supplementation with B vitamins effectively lowers tHcy levels.Our study findings revealed that administering Vitamin B6 at a dose of 1 mg per Kg of body weight, Vitamin B9 at 2 mg per Kg of body weight, and Vitamin B12 at a concentration of 50 milligrams per liter per day led to a substantial decrease in homocysteine levels by approximately 40%. To achieve an effective reduction in homocysteine levels, it is recommended that a daily intake of at least 0.5 milligrams of folic acid be maintained.

Furthermore, while increasing folic acid intake up to 5 milligrams daily may not significantly influence homocysteine levels, Vitamin B12 supplementation within a dosage range of 0.02 milligrams to 1 milligram per day has been demonstrated to decrease homocysteine levels by approximately 40% [15]. Long-term blood pressure management post-stroke is crucial for preventing recurrent events, yet poor control persists, highlighting the need for novel approaches. Our study also investigates the potential of potassium supplementation alongside B vitamins, revealing a significantly lower systolic and diastolic blood pressure. This approach plays a vital role in addressing this challenge, offering alternative strategies with potentially improved efficacy and tolerability in stroke prevention [16].

Elevated AST levels often indicate liver damage but can also reflect muscle injury or be associated with conditions affecting other organs. In stroke, AST levels may provide insights into systemic inflammation, oxidative stress, or tissue damage beyond the brain. Our study observed higher AST levels in one group following supplementation post-stroke [17],[18]. This finding may suggest that

the administered supplementation, particularly potassium and B vitamins, could have influenced systemic factors beyond the brain, potentially affecting AST levels. Elevated AST levels often reflect tissue damage, and in the context of stroke, they may indicate a broader systemic response to injury [19],[20].

CONCLUSION

The study concluded that could be derived from the analysis of the study that investigated the combined impact of supplementation with Vitamin B6, B9, B12, and Potassium on the risk of stroke in a rat model.

The findings lend credence to the study hypothesis that there is a synergistic combination impact of supplementation with Vitamin B6, B9, B12, and Potassium on the stroke risk levels. Based on the data obtained from the experimental investigation, which most likely revealed a reduction in the incidents or severity of stroke with the combined supplementation of these nutrients, this conclusion is based on the findings.

As a result, the evidence rejects the null hypothesis, that identified there is synergistic combination impact of supplementation with vitamins B6, B9, B12, and Potassium on stroke. According to the findings, cumulative supplementation achieved the intended synergistic impact by avoiding or minimizing the effects of stroke in the rat model employed in the research.

It may be necessary to conduct more studies to investigate the processes responsible for the observed synergy and to confirm these findings using various experimental models or experiments involving humans. The current study offers encouraging data for the potential efficacy of combination supplementation with vitamins B6, B9, B12, and Potassium as a strategy for lowering the stroke risks or its effects.

Conflict of interest

Authors declare no conflict of interest.

References

[1] Feigin, V. L., Stark, B. A., Johnson, C. O., Roth, G. A., Bisignano, C., Abady, G. G., Abbasifard, M., Abbasi-Kangevari, M., Abd-Allah, F., Abedi, V., Abualhasan, A., Abu-Rmeileh, N. M., Abushouk, A. I., Adebayo, O. M., Agarwal, G., Agasthi, P., Ahinkorah, B. O., Ahmad, S., Ahmadi, S., & Ahmed Salih, Y. (2021). Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Neurology*, *20*(10), 795–820.

[2] Rolle-Lake, L., & Robbins, E. (2023). Behavioral Risk Factor Surveillance System. In *StatPearls*.

[3] GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019.

[4] Ovbiagele, B., Goldstein, L. B., Higashida, R. T., Howard, V. J., Johnston, S. C., Khavjou, O. A., Lackland, D. T., Lichtman, J. H., Mohl, S., Sacco, R. L., Saver, J. L., & Trogdon, J. G. (2013). Forecasting the Future of Stroke in the United States: A Policy

[5] Kuriakose, D., & Xiao, Z. (2020). Pathophysiology and Treatment of stroke: Present Status and Future Perspectives. *International Journal of Molecular Sciences*, *21*(20), 7609

[6] Musuka, T. D., Wilton, S. B., Traboulsi, M., & Hill, M. D. (2015). Diagnosis and management of acute ischemic stroke: speed is critical. *Canadian Medical Association Journal*, *187*(12), 887–893.

[7] Broughton, B. R. S., Reutens, D. C., & Sobey, C. G. (2009). Apoptotic Mechanisms After Cerebral Ischemia. *Stroke*, *40*(5).

[8] Woodruff, T. M., Thundyil, J., Tang, S.-C., Sobey, C. G., Taylor, S. M., & Arumugam, T. V. (2011). Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. *Molecular Neurodegeneration*, *6*(1), 11.

[9] Hankey, G. J. (2018). B vitamins for stroke prevention. *Stroke and Vascular Neurology*, *3*(2), 51–58.

[10] Costantini, A., Pala, M. I., Catalano, M. L., Notarangelo, C., & Careddu, P. (2014). High-Dose Thiamine Improves Fatigue After Stroke: A Report of Three Cases. *The Journal of Alternative and Complementary Medicine*, *20*(9), 683–685.

[11] Guo, J., Lv, J., Guo, Y., Bian, Z., Zheng, B., Wu, M., Yang, L., Chen, Y., Su, J., Zhang, J., Yao, J., Chen, J., Chen, Z., Yu, C., & Li, L. (2021). Association between blood pressure categories and cardiovascular disease mortality in China. *PLOS ONE*, *16*(7), e0255373.

[12] Zhao Y, Gu X, Sun X, Zhang Y, Gu J. The impact of potassium supplementation on stroke: A focus on stroke models and clinical trials. Nutrients. 2020;12(3):739.

[13] Qi, X., Sun, X., Xu, J., Wang, Z., Zhang, J., & Peng, Z. (2014). Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk in Chinese population. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine*, *35*(3), 1757–1762.

[14] França, C. F., & Vianna, L. M. (2010). Effectiveness of B vitamins on the control of hypertension and stroke events of SHRSP rats. *Journal of dietary supplements*, *7*(1), 71–77.

[15] Hunt, B. D., & Cappuccio, F. P. (2014). Potassium intake and stroke risk: a review of the evidence and practical considerations for achieving a minimum target. *Stroke*, *45*(5), 1519–1522.

[16] Li, Y., Huang, T., Zheng, Y., Muka, T., Troup, J., & Hu, F. B. (2016). Folic Acid Supplementation and the Risk of Cardiovascular Diseases: A Meta-Analysis of Randomized Controlled Trials. *Journal of the American Heart Association*, *5*(8), e003768.

[17] Park, J. H., Saposnik, G., Ovbiagele, B., Markovic, D., & Towfighi, A. (2016). Effect of B-vitamins on stroke risk among individuals with vascular disease who are not on antiplatelets: A meta-analysis. *International journal of stroke : official journal of the International Stroke Society*, *11*(2), 206–211

[18] Homocysteine Studies Collaboration (2002). Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*, *288*(16), 2015–2022.

[19] Spence, J. D., Yi, Q., & Hankey, G. J. (2017). B vitamins in stroke prevention: time to reconsider. *The Lancet. Neurology*, *16*(9), 750–760

[20] Collaboration H. L. T. (1998). Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. *BMJ (Clinical research ed.)*, *316*(7135), 894–898.

Table 1. Effect of Vitamin B (B_6, B_9, B_{12}) and Potassium Supplementation on Complete Blood Count Profile (CBC)

Serum Indices	Go	G1	G2	G3	G4
Hb	15.0±0.09	12.2±0.91	13.4±0.94	12.9±0.11	13.2±0.067
Total RBC	5.4±0.09	3.9±0.96	4.4±0.98	4.3±0.90	4.3±0.057
HCT	45.0±0.82	38.0±0.82	39.9±0.92	41.9±0.74	41.9±0.75
MCV	84.9±0.83	79.9±0.69	85.9±0.73	85.9±0.10	85.9±0.54
Platelets	275.0±0.95	199.9±0.77	224.9±0.56	199.9±0.56	219.9±0.58
WBC	7.46±0.83	4.02±0.87	5.49±0.69	5.01±0.79	5.68±0.10
Neutrophils	59.9±0.10	49.9±0.75	54.9±0.85	55.0±0.63	55.9±0.64
Lymphocytes	29.9±0.10	20.0±0.66	25.9±0.99	25.9±0.52	27.9±0.96
Eosinophils	3.49±0.72	3.35±0.10	2.97±0.78	2.96±0.11	2.00±0.92
Monocytes	5.98±0.97	2.01±0.57	5.00±0.97	4.98±0.74	2.99±0.63

Table 2: Effect of Vitamin B (B_6, B_9, B_{12}) and Potassium Supplementation on Liver Function Tests (LFTs),Homocysteine level & Blood Pressure

Serum Indices	Go	G1	G2	G3	G4
ALT	30.03±0.56	30.00±0.74	29.98±0.80	35.0±0.57	32.9±0.10
AST	19.9±0.97	199.9±0.11	120.0±0.64	123.0±0.69	118.9±0.74
GGT	35.0±0.96	550.0±0.68	149.9±0.60	224.9±0.50	145.0±0.59
Albumin	3.99±0.80	2.1±0.85	3.48±0.11	3.49±0.61	3.44±0.75
Total Protein	7.11±0.58	4.97±0.51	5.98±0.62	6.88±0.76	5.98±0.91
Homocysteine	9.96±0.62	60.0±0.66	19.9±0.83	52.9±0.61	21.9±0.64
Diastolic	78.9±0.93	99.9±0.57	98.9±0.67	84.9±0.61	87.9±0.70
Systolic	119.0±0.70	149.9±0.64	135.0±0.78	124.9±0.92	122.9±0.68

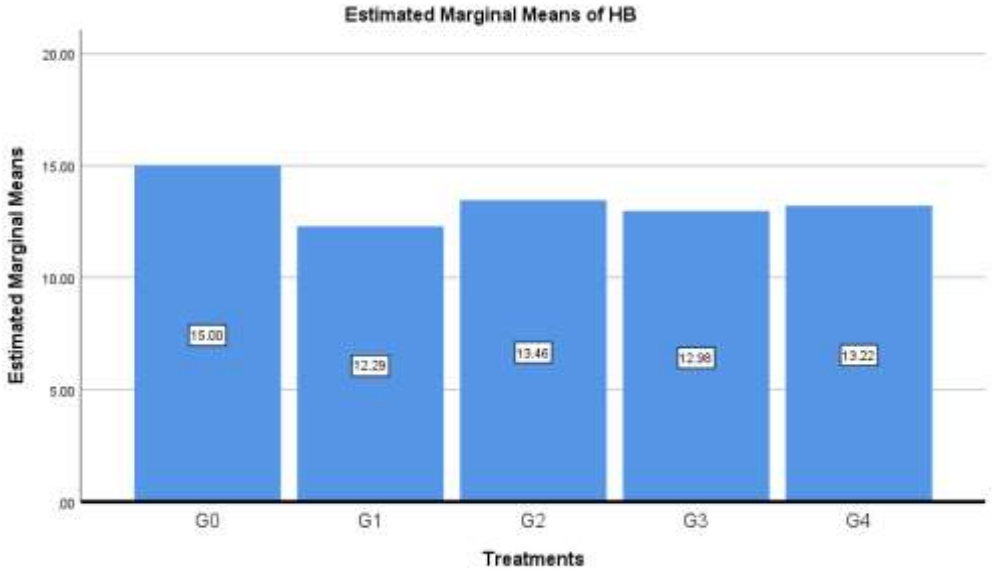

Figure 1: Mean Hemoglobin(Hb)

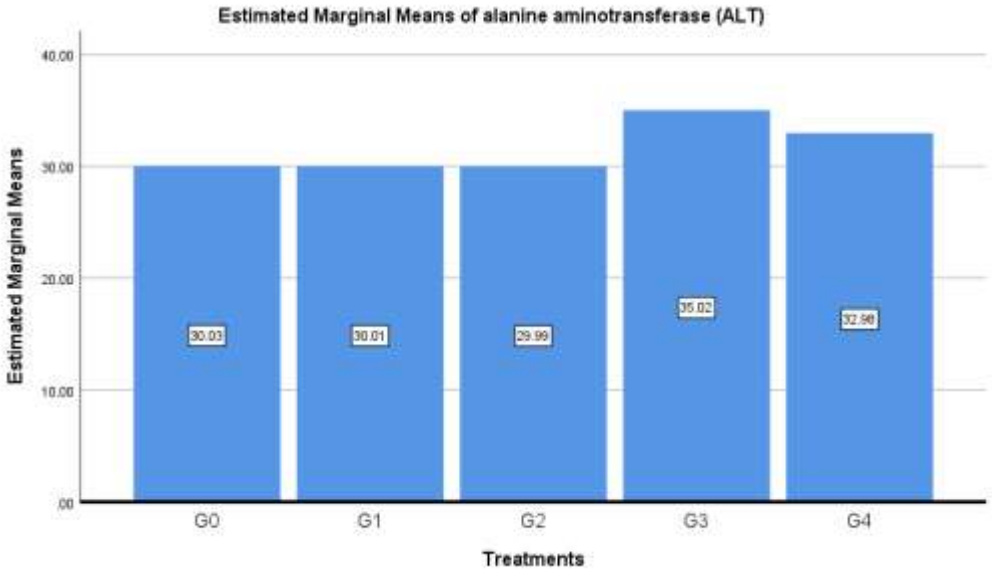

Figure 2: Mean Alanine Aminotransferase (ALT)

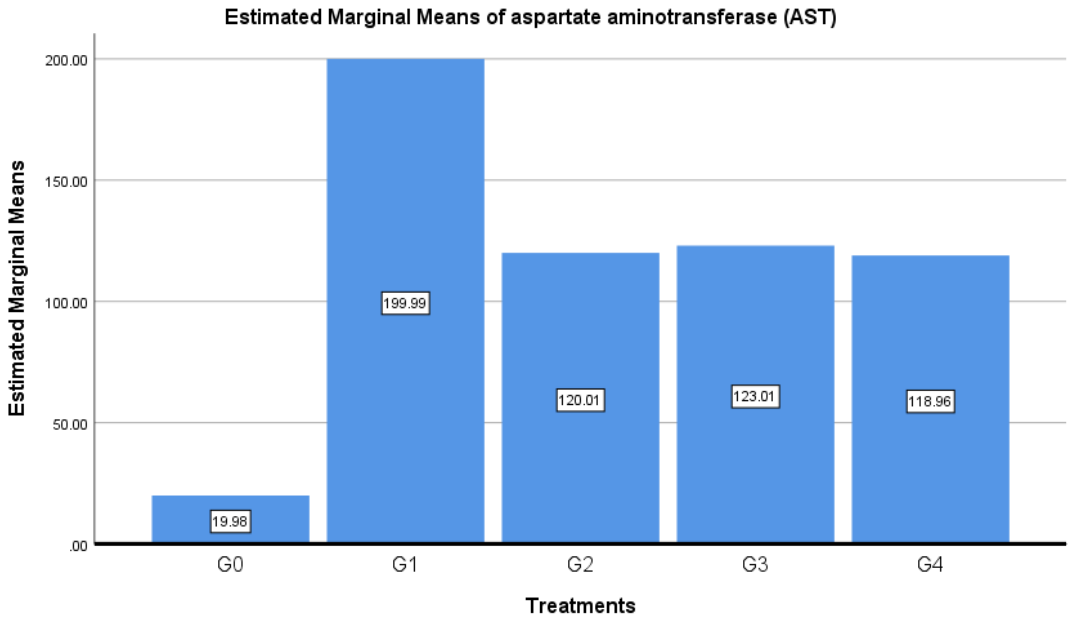

Figure 3: Mean of Aspartate Aminotransferase (AST)

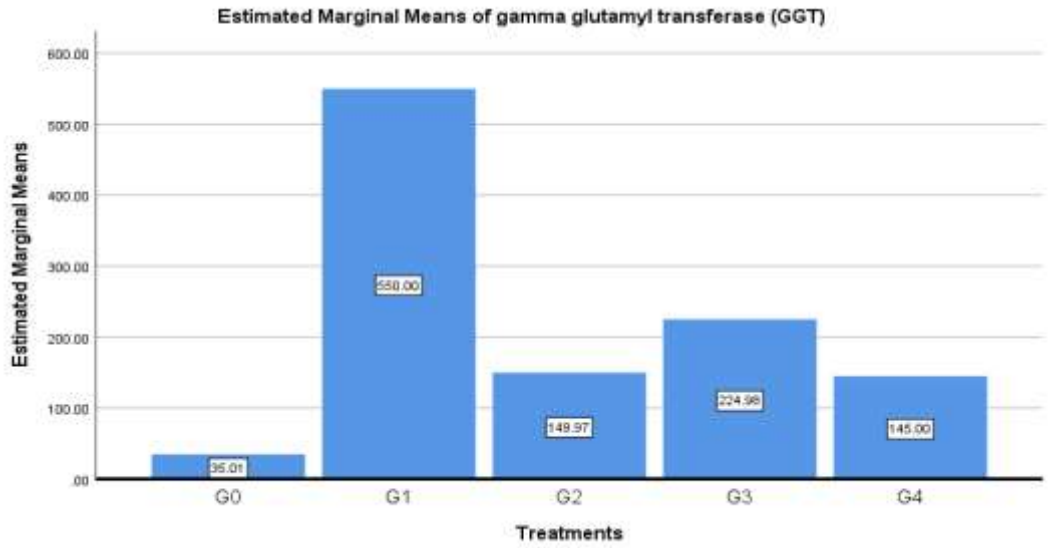

Figure 4: Mean of Gamma Glutamyl Transferase (GGT)

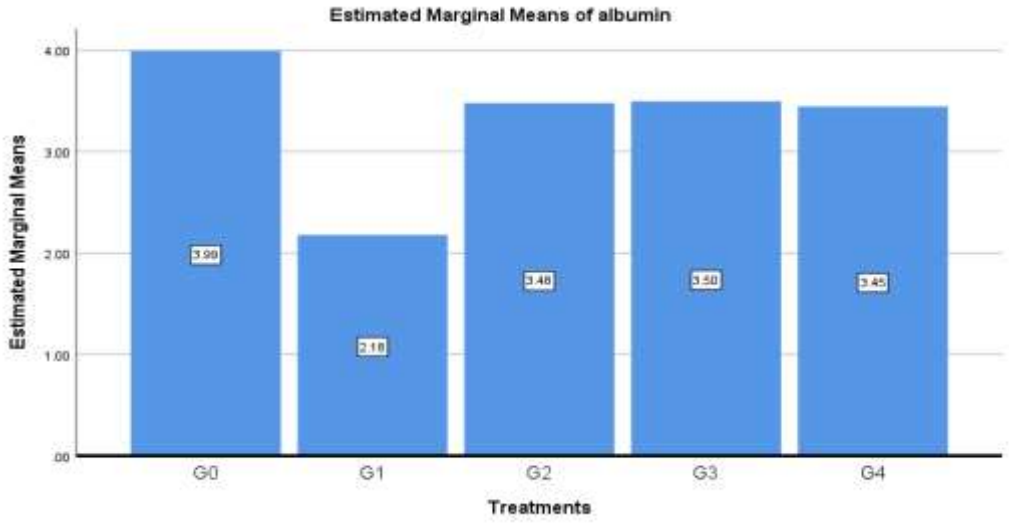

Figure 5: Mean of Albumin

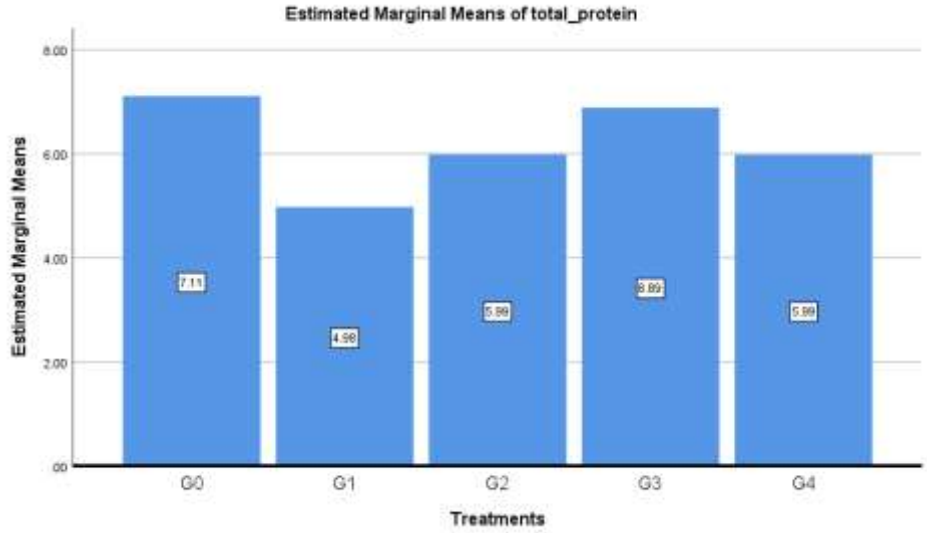

Figure 6:Mean of Total Protein

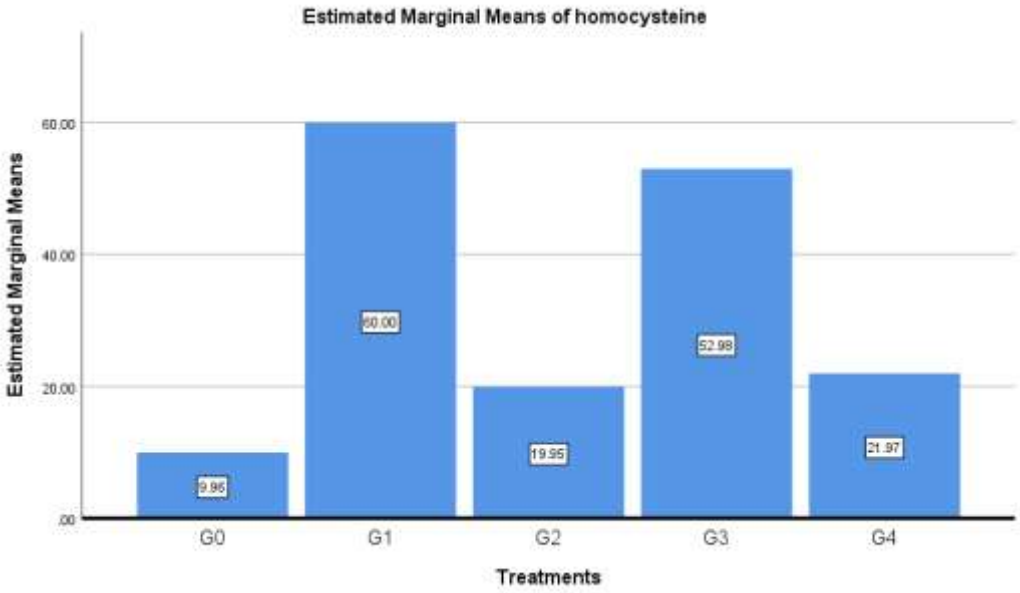

Figure 7: Mean of Homocysteine