Abstract

Arachis hypogea is believed to possess a broad spectrum of therapeutic properties. This plant's safety is crucial for its potential medicinal applications. Utilizing the methodologies outlined in the Lorke recommendations, the aim of the current study was to assess the acute and subacute toxicity characteristics of methanol leaf extract of *Arachis hypogea* (MLEAH) in an animal model. In the acute phase, MLEAH was orally administered to mature male mice at varying doses of 10, 100, and 1000mg/kg for phase I and 1600, 2900, and 5000mg/kg for phase II, respectively. General behavioral impacts, mortality rates, and mortality were monitored over a 7-day period. In the subacute phase, adult rats orally received MLEAH at doses of 1000 and 2000mg/kg daily for 28 days. Body weight and specific biochemical parameters were assessed at the conclusion of the treatment duration. The findings from the acute toxicity assessment indicated the safety of MLEAH even at the 5000mg/kg dose. The subacute toxicity evaluation of MLEAH revealed no notable alterations in body weight and tested biochemical parameters. In light of these outcomes, it can be inferred that MLEAH exhibits low toxicity levels. These results offer valuable insights into the toxicity profile of the medicinal plant, *Arachis hypogea*.

Keywords: *Arachis hypogea*, acute toxicity, subacute toxicity, MLEAH

Introduction

Plants have been integral to traditional medical systems globally, with over 80% of the world's population relying on plant-based traditional medicine for primary healthcare needs (Rukhshana, 2024; Parwiz *et al*., 2024). While herbal medications are perceived as safe with minimal side effects, it is crucial to acknowledge that approximately 1,50,000 plants contain toxic substances, highlighting the potential for adverse effects from medicinal plants (Mugale *et al*., 2024). The use of medicinal plants without assessing their toxicity can indeed pose health hazards, emphasizing the importance of studying the harmful effects induced by herbal remedies (Shamim *et al*., 2024; Rehan, 2023). Research has shown that some widely used plants exhibit significant toxicity, necessitating a comprehensive understanding of the safety and toxicity concerns associated with medicinal plant extracts for a complete safety assessment (Mugale *et al*., 2024).

Acute and subacute toxicity tests play a crucial role in assessing the potential harm and safety of chemicals by evaluating the effects of single or repeated doses on animals, aiding in hazard identification and risk management (Oladipo *et al*., 2024; Lei *et al*., 2024). Acute toxicity tests

involve administering a single dose to determine immediate toxic effects, while subacute toxicity tests assess long-term effects on specific tissues or organs through repeated dosing (Lei *et al*., 2024). These tests are essential for understanding the safety profiles of chemicals, as highlighted in studies evaluating the toxicity of medicinal plants and chemical compounds, emphasizing the importance of considering signs of toxicity alongside mortality in assessments (Oladipo *et al*., 2024; Alekseeva *et al*., 2024; Mukhlis and Idris 2023). By utilizing these tests, researchers can identify potential hazards associated with chemical exposure, guiding decisions related to production, handling, and use of chemicals for overall risk management and safety assurance.

The Fabaceae family, particularly the genus Arachis, is native to South America and comprises 83 described species, with Arachis hypogea being the most well-known and widely consumed species (Custodio *et al*., 2023). Peanuts, scientifically known as Arachis hypogea L., are oil-containing seeds cultivated extensively in Africa, Asia, and America, and are a staple food globally (Manoj *et al*., 2024; Yandra *et al*., 2023). In China, the leaves of *A. hypogea* are utilized for their medicinal properties in treating inflammation and sleep disorders (Singh *et al*., 2024). The diverse applications of peanuts extend beyond their nutritional value, encompassing environmental benefits, therapeutic potential, and even biotechnological uses, highlighting their significance in agriculture, medicine, and industry (de, Sousa *et al*., 2024).

The safety assessment of medicinal plants is crucial for their potential use in human health. Studies on various plant extracts like *Curcuma longa*, *Datura metel*, *Phoenix dactylifera* (Oladipo *et al*., 2024), *Ximenia americana*, and *Pappea capensis* (Gaichu, 2024), *Plectranthus neochilus* (Abu and Edah, 2024), and *Olax subscorpioidea* (Adekunle *et al*., 2023) have highlighted the importance of evaluating acute and subacute toxicity. While *Arachis hypogaea* leaves have been traditionally valued for their nutritional benefits, recent research emphasizes their therapeutic potential, necessitating toxicity evaluations (Singh *et al* 2024). These studies employ methods like LD_{50} determination, and biochemical analyses to assess the safety profiles of plant extracts and comprehensive toxicity assessments to ensure the safe use of medicinal plant extracts in humans.

Materials and methods

Materials

Plant material

The leaves of *Arachis hypogea L. (peanut)* were collected from a farmland in Umuaririogu, Okpuala Amakohia autonomous community in Ikeduru Local Government Area of Imo State. Nigeria. The leaves were identified by a taxonomist, Dr. M.C Nwoko. Department of Biological science, Federal University of Technology, Owerri. as those of *Arachis hypogea.* A voucher specimen of *Arachis hypogea* leaves was deposited in the herbarium.

Animals

Fifteen (15) male albino rats weighing 90-120g and eighteen (18) mice weighing 30-40g were used for the study. The rats were obtained from the Animal House of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were acclimatized to laboratory conditions for two weeks, under laboratory conditions and had free access to feed and water until the end of the experiments.

Methods

Acute toxicity and lethal dose test (LD_{50}) (Lorke, 1983)

It was carried out with modification according to the method of Lorke (1983). A total of 18 mice was used. They were divided into two phases: I and II. In stage one (phase 1), the animals were placed in three groups of three mice each, and were orally administered 10mg/kg, 100mg/kg and 1000 mg/kg body weight (b.wt) of the extract respectively. In the second phase, animals were given 1600, 2900 and 5000 mg/kg b.wt. orally. The mice were observed for signs of toxicity hourly in the first 12 hr and then daily for 7 days. They were observed for toxicity, signs of calmness and quietness, licking of forelimbs, passive movements, clustering together, prostration and death.

Subacute toxicity determination

Fifteen rats weighing 90 to 120g were assigned into three groups of 5 rats per group and the crude extract was administered to the animals daily for 4 weeks. The doses were calculated for humans and modified for rats using the method of Paget and Barnes (1964). The animals were treated as follows: Group 1 – normal control; Group 2- low dose (1000 mg/kg bw); Group 3-

high dose (2000 mg/kg bw). The administration of extract was done orally. The animals were sacrificed after 4 weeks and blood samples were collected for biochemical analysis.

Measurement of biochemical parameters

The sophisticated retro-orbital bleeding technique, as outlined by Sharma *et al*. (2014), was implemented to procure blood samples of sufficient quantity and quality. This method entails delicately retracting the eyelid and extending the upper eyelid to expose the eye, enabling precise blood retrieval using a Pasteur pipette to minimize vein irritation. Subsequent to the collection, the blood specimens are collected into a plain tube and subjected to centrifugation. The centrifuged samples are further handled to separate the serum designated for subsequent biochemical assessments.

the Randox kit was utilized for the biochemical analyses. Serum samples were collected and subjected to analysis for liver enzymes like aspartate aminotransferases (AST) and alanine aminotransferases (ALT), as well as lipid profile components such as LDL-cholesterol, HDL-cholesterol, TAG, and cholesterol. Furthermore, serum kidney parameters including urea and creatinine were assessed. The analysis was conducted using a spectrophotometer.

Results

The results for the acute toxicity (LD_{50}) of MLEAH is presented in Table 1. The data showed zero mortality up to 5000 mg/kg administered as single oral dose.
The results for the effect of MLEAH on lipid profile on rats are presented in table 2. The results showed no significant difference at ($p<0.05$) level of confidence in all the lipid parameter tested.

The study examined the effects of different doses (1000 mg/kg and 2000 mg/kg) on cholesterol (Chol), triglycerides (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels compared to a normal control (NC) group. Both treatment groups (1000 mg/kg and 2000 mg/kg) showed no statistically significant differences ($p>0.05$) from the control group in terms of Chol (107.5 ± 3.11 and 101.50 ± 2.64 vs. 113.00 ± 2.94), TAG (83.25 ± 3.86 and 82.25 ± 0.96 vs. 81.75 ± 1.50), HDL (51.25 ± 1.89 and 55.75 ± 2.21 vs. 42.00 ± 1.82), and LDL (41.75 ± 1.76 and 40.75 ± 0.95 vs. 50.25 ± 1.70), indicating that the treatments did not significantly alter lipid profiles compared to the control.

Each value represents the mean ± SD for 5 rats per group. NC, normal control; MLEAH1 and MLEAH2, methanol leaf extract of *Arachis hypogea* 1000 and 2000mg/kg respectively. The results showed no significant difference at ($p>0.05$).

Discussion

The utilization of the aerial components of Arachis hypogaea, also known as peanuts, has a longstanding history in Asia, notably in China, where it has been utilized for the management of insomnia. In this context, the leaves are employed as an oral supplement in sleep induction therapy as per traditional Chinese medical literature (Singh *et al*., 2024). Prior research has investigated the immediate toxicity of peanut leaf extract in vivo subjects, highlighting the necessity for further safety appraisals following the guidelines outlined by the OECD 407 to assess the toxicity associated with repeated doses (Singh *et al*., 2024). This particular protocol is widely used in studies focusing on the effectiveness and potential risks of isolated components and extracts derived from medicinal plants, underscoring the significance of comprehensive toxicity assessments in establishing the safety profiles of natural substances intended for therapeutic purposes (Yandra *et al*., 2023).

According to the classification of toxicity, substances that exhibit LD50 values ranging from 1 to 5 g/kg are generally categorized as having low toxicity, whereas those with values exceeding 5.0 g/kg are typically deemed to be non-toxic, as elucidated by Piyachaturawat *et al*. in 2002. The LD50 values determined for MLEAH in rats upon oral administration were identified to fall within the 1 to 5 g/kg range. These findings strongly indicate that the methanol extract of *Arachis hypogea* can be characterized as possessing a notably low level of toxicity when administered via oral routes, thereby underscoring its relative safety profile in this context.

During the subacute experiments, alterations in body mass were utilized as a parameter to assess the potential unfavorable impacts of the drugs administered. In both the experimental and control cohorts, there was an observed typical, gradual rise in average body weight (El-Sayed *et al*., 2015). The disparity in weight gain between the control cluster and the cohorts subjected to the MLEAH at a dosage of up to 2000 mg/kg did not display any statistically significant variance. This lack of distinction could potentially be attributed, at least partially, to the absence of any detrimental effects of the MLEAH on the subjects' appetite or food consumption. The results suggest that the MLEAH did not have a discernible impact on the subjects' dietary habits, which may have contributed to the similarity in weight gain across the different groups. It is plausible that the absence of negative effects on appetite or food intake

played a role in maintaining similar body weight changes between the control and treated groups in the study.

The investigation into the biochemical parameters of albino rats administered with the methanol leaf extract of Arachis hypogaea (MLEAH) revealed no significant alterations in the concentrations of ALT and AST, commonly used indicators of hepatic toxicity (Adeosun *et al*., 2023). This suggests that MLEAH did not induce notable harm to the liver. Additionally, the levels of creatinine, a key biomarker for renal function, remained consistent across the experimental groups, indicating that MLEAH did not have adverse effects on the renal system (Adeosun *et al*., 2023). These findings highlight the safety profile of MLEAH on both hepatic and renal functions in the albino rat model, supporting its potential for further exploration in medicinal applications.

Consumption of peanuts has been shown to have beneficial effects on risk factors associated with cardiovascular disease. Research conducted by various studies, including Wong *et al*. (2024) and Martínez-Ortega *et al*. (2023) has consistently shown that the regular intake of peanuts is associated with beneficial effects on blood cholesterol levels.

According to a study conducted by Ghadimi-Nouran *et al*. (2010), the consumption of peanuts resulted in substantial reductions in participants' TC/HDL and LDL/HDL ratios, as well as their plasma atherogenic index (PAI) values. Additionally, the consumption of peanuts led to an increase in HDL levels and overall antioxidant capacity. The impact of the extract on the lipid profile of the rats exhibited variable effects depending on the amount administered. Total cholesterol levels can indeed vary in different liver disorders. Research studies have shown significant differences in total cholesterol levels between acute and chronic liver diseases, with chronic liver disease patients exhibiting higher levels compared to acute liver disease patients (Mandal *et al*., 2013 and Bajaj *et al*., 2017). This study found that there were no statistically significant alterations in cholesterol and triacylglycerol levels among the treated groups compared to the control group. However, a notable increase in HDL-cholesterol was observed in the group treated with a dose of 2000mg/kg body weight of the MLEAH compared to the control group. These findings suggest that the MLEAH does not have an impact on liver metabolic function during the twenty-eight days treatment period in the rats.

It is of utmost importance to acknowledge that despite the thorough analysis provided in the current study, there are significant constraints that need to be acknowledged and dealt with accordingly. One such limitation pertains to the restricted number of dosage groups utilized

(1000 mg/kg and 2000 mg/kg). Although these dosages yielded valuable insights, it is imperative to incorporate higher dose levels in future investigations to ensure a comprehensive evaluation of the extract's safety, particularly with regard to hematological and hepatic parameters. Additionally, our study concentrated on the short-term subacute toxicity spanning 28 days, whereas longer durations would enable the assessment of prolonged effects and potential cumulative repercussions. A holistic assessment of toxicity should also encompass examination of other bodily systems and toxicological endpoints such as neurotoxicity, reprotoxicity, and genotoxicity. Addressing these limitations through further research will advance our comprehension of the safety profile of the *Arachis hypogea* extract, enabling more informed decision-making concerning its utilization.

Conclusion

The assessment of the acute and subacute toxicity of the methanol leaf extract of *Arachis hypogea* was conducted within the confines of this particular investigation. The findings of this analysis reveal that the extract exhibits minimal acute toxicity, as evidenced by the LD50 values determined for the oral administration route. Moreover, no adverse effects on various biochemical parameters were detected throughout the 28-day subacute toxicity assessment. To corroborate these results, further exploration involving higher dosages, alternative routes of administration, and prolonged exposure periods is imperative. Additionally, a thorough examination encompassing neurotoxic, reprotoxic, genotoxic, and bioactive constituent toxicities is warranted. It is essential to take into account the plausible risks related to molecular parameters prior to the utilization of *Arachis hypogea* extract.

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APPENDIX

Table 1 Results for the LD50 of MLEAH in healthy Wistar rats.

Group	Dosage (mg/kg)	Number of mortalities
Group 1	10	0
Group 2	100	0
Group 3	1000	0

Group	Dosage (mg/kg)	Number of mortalities
Group 4	1600	0
Group 5	2900	0
Group 6	5000	0

Treatment	Chol	TAG	HDL	LDL
NC	113.00 ± 2.94a	81.75 ± 1.50 a	42.00 ± 1.82 a	50.25 ± 1.70 a
1000mg/kg	107.5 ± 3.11 a	83.25 ± 3.86 a	51.25 ± 1.89 a	41.75 ± 1.76 a
2000mg/kg	101.50 ± 2.64 a	82.25 ± 0.96 a	55.75 ± 2.21 a	40.75 ± 0.95 a

Table 2: Lipid profile parameter of rats treated with methanol extract of *Arachis hypogea*.

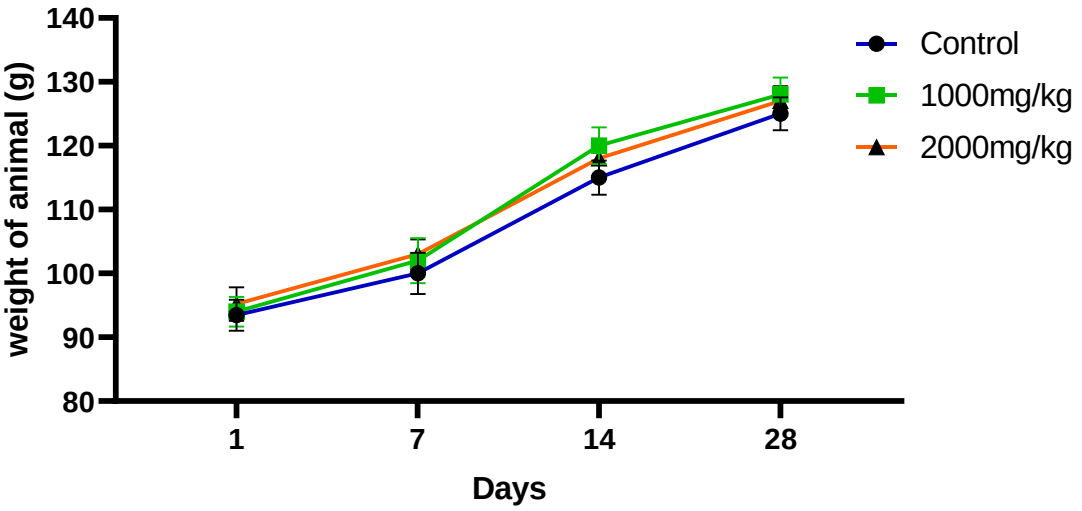

Figure 1. The effect of sub-acute oral treatment with the methanol extract of *Arachis hypogea* leaves on body weight in male rats. The MLEAH was given orally to rats in three groups at the following doses for a period of 28 days: Group I (control, 0 mg/kg), Group II (MLEAH dose, 1000 mg/kg), Group III (MLEAH dose, 2000 mg/kg). The data are expressed as mean ± S.E.M.

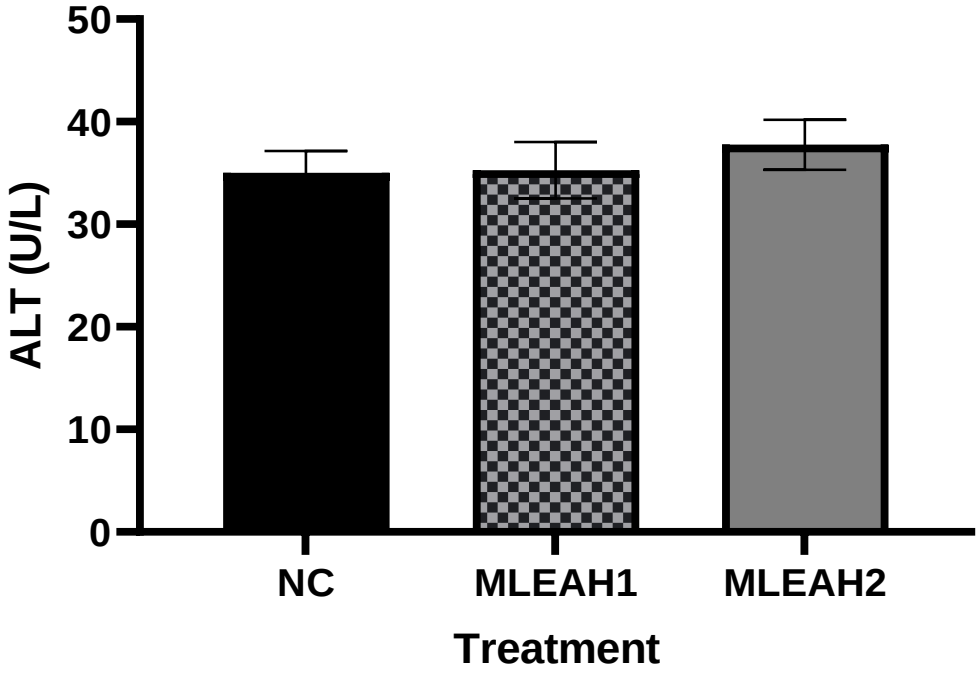

Figure 2. MLEAH on ALT level in Wistar rats. NC (control, 0 mg/kg), MLEAH1 (MLEAH dose, 1000 mg/kg), MLEAH2 (MLEAH dose, 2000 mg/kg). The data are expressed as mean ± S.E.M.

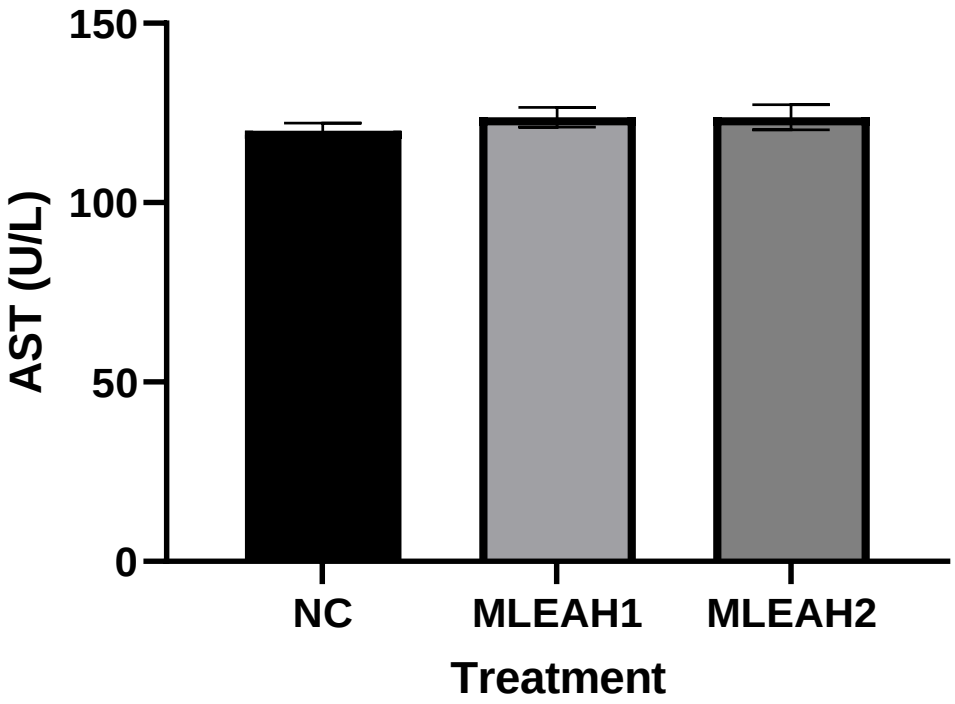

Figure 3. MLEAH on alkaline phosphatase level in Wistar rats. Each value is the mean ± SD for 5 rats per group. NC, normal control; MLEAH1 and MLEAH2, methanol leaf extract of *Arachis hypogea* 1000 and 2000mg/kg respectively. Above each column, different letters mean statistical significance at ($P<0.05$).

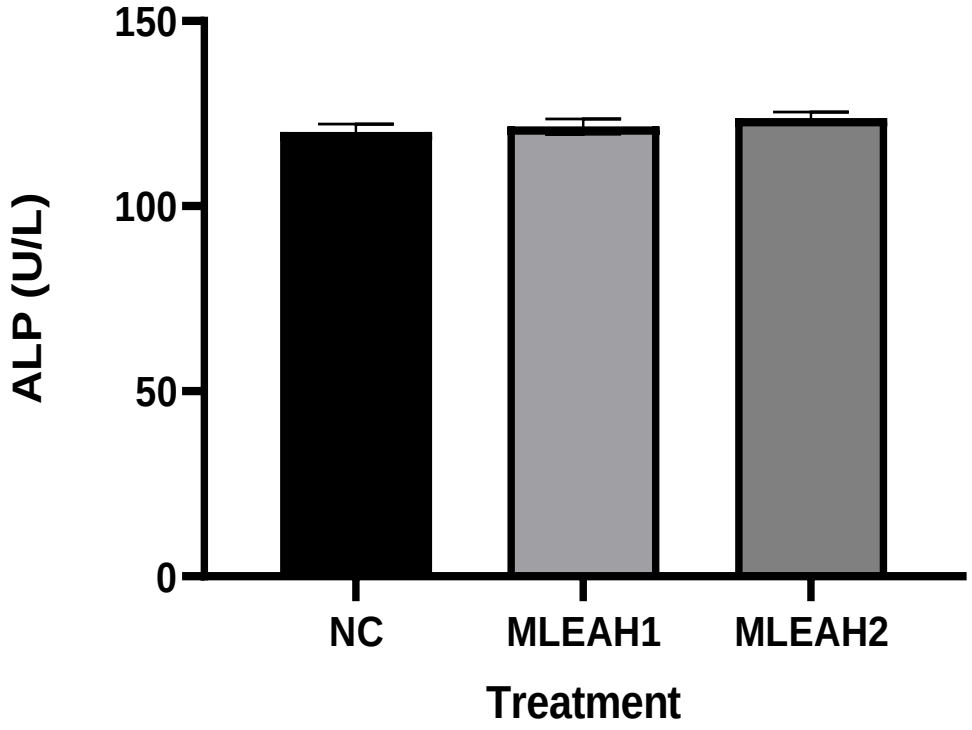

Figure 4. MLEAH on alkaline phosphatase level in wistar rats. Each value is the mean ± SD for 5 rats per group. NC, normal control; MLEAH1 and MLEAH2, methanol leaf extract of *Arachis hypogea* 1000 and 2000mg/kg respectively. The result showed no significant difference at ($p>0.05$).

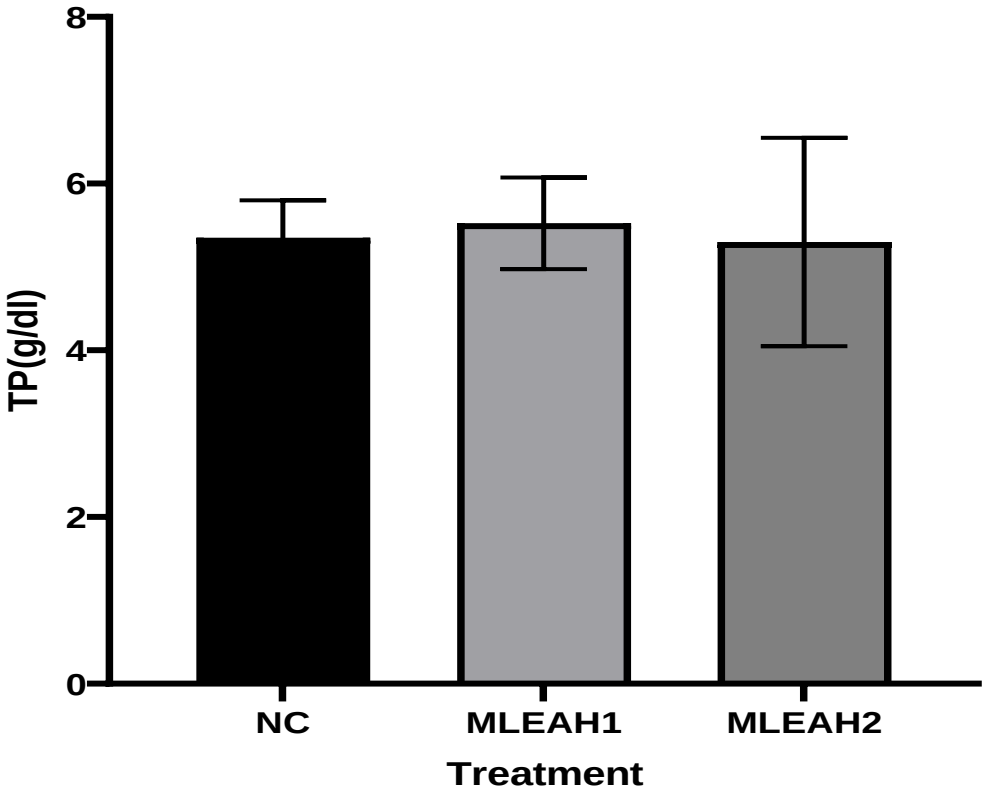

Figure 5. MLEAH on Total protein (mg/dl) level in Wistar rats.

Each value is the mean ± SD for 5 rats per group. NC, normal control; MLEAH1 and MLEAH2, methanol leaf extract of *Arachis hypogea* 1000 and 2000mg/kg respectively. The result showed no significant difference at ($p>0.05$).