



## COMPARATIVE EVALUATION OF THE NEUROPROTECTIVE POTENTIAL OF *PHYLLANTHUS AMARUS* AND *MENTHA PIPERITA* LEAF EXTRACTS FOR ALZHEIMER'S DISEASE

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**ABSTRACT:** *Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and neuronal damage, with oxidative stress and inflammation playing a major role in its pathogenesis. The search for natural bioactive compounds with therapeutic potential against Alzheimer's disease has led to the exploration of POTENTIAL from medicinal plants. This study aims to comparatively evaluate the phytochemical composition of Mentha piperita L. (Mentha spp.), Phyllanthus amarus, and their potential therapeutic effects on Alzheimer's disease through biochemical assessments of the brain, kidney, and liver in an experimental rat model. A total of 30 Wistar rats (n=3 per group) were divided into 10 experimental groups, including a normal control, Alzheimer's disease-induced groups, and treatment groups receiving different plant extracts. 27 Wistar rats were exposed to arsenite and divided into different treatment groups receiving different extracts from the different selected plants. The study assessed hematology, hepatic and renal biomarkers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), superoxide dismutase (SOD), DNA fragmentation, gamma-glutamyl transferase (GGT), and albumin (ALB). Statistical analysis was conducted using one-way ANOVA, with significant differences set at  $P < 0.05$  and  $P < 0.001$ . Results showed that the level of antioxidant assays of arsenite intoxication in the brain was reduced by the medicinal plants as compared to their level in the liver and kidney, which shows similar results. However, the level of DNA fragmentation was highly expressed in the arsenite-induced group as compared to the treatment group and control group. Also, the enzyme assay biomarker SOD shows that there is a high level of oxidative stress in the arsenite-induced group as compared to the treatment group. However, the level of oxidative stress in the Mentha piperita L. spp. treated group is lower in comparison to the other two bioactive plants. These findings suggest that Mentha piperita L., Phyllanthus amarus, and contain bioactive compounds with potential neuroprotective and antioxidative effects, which may contribute to therapeutic strategies for the maintenance of arsenate-induced Alzheimer's disease. Further studies could enhance the possible neurological treatment using the selected bioactive plant compounds.*

**Keywords:** Alzheimer's disease, Phytochemicals, Mentha Piperita L., Phyllanthus amarus, , Oxidative stress, Neuroprotection, Liver enzymes, Kidney biomarkers

### 1.0 INTRODUCTION

Alzheimer's Disease is a progressive brain disorder and the most common cause of dementia. It gradually damages brain cells, leading to worsening problems with memory, thinking, language, judgement, and eventually the ability to carry out everyday activities. In the brain, Alzheimer's is associated with abnormal changes including amyloid plaques (build-up of beta-amyloid protein) and tau tangles. Over time, neurons lose their connections and die, and parts of the brain shrink. The most disorder that primarily affects memory, cognition, and the ability to perform activities of daily living. AD is characterized by two hallmark features: tau tangles and amyloid-beta plaques, which build up in the brain and cause synaptic dysfunction, neuroinflammation, and neuronal death (Zhao et al., 2020). Currently, over 50 million people worldwide suffer from AD, and the number is predicted to triple by 2050 (World Health Organization, 2020). These pathological changes result in the gradual decline of cognitive functions, including memory loss, language difficulties, and spatial disorientation, eventually impairing the individual's ability to perform basic functions like eating or dressing (Kumar et al., 2019).

Current pharmacological treatments for AD, such as glutamate regulators (memantine) and cholinesterase (donepezin, rivastigmine), merely alleviate symptoms and do not change the course of the illness. These drugs are not the best for long-term care because they frequently cause adverse effects such as sleeplessness, lightheadedness, and gastrointestinal problems (Cummings et al., 2019). Additionally, the disease's increasing prevalence and the shortcomings of current therapies have prompted researchers to look into alternative therapeutic modalities, especially in traditional medicine, where medicinal plants have long been utilized for their neuroprotective qualities (Patel et al., 2018).

Of these, *Mentha piperita* (*Mentha* spp.) and *Phyllanthus amarus* (Stonebreaker) have attracted a lot of attention due to their possible medicinal uses. A tiny herbaceous plant called *Phyllanthus amarus* has long been used to treat kidney stones, liver problems, and jaundice (Baker et al., 2016). Its bioactive components, including flavonoids, alkaloids, and tannins, have been emphasized in recent research for their strong anti-inflammatory and antioxidant properties, which may offer neuroprotective benefits (Patel et al., 2018). Similarly, *Mentha piperita*, especially pepper *Mentha piperita* (*Mentha piperita*) and spear *Mentha piperita* (*Mentha spicata*), contain essential oils like menthol that have demonstrated anti-inflammatory, antioxidant, and cholinergic-enhancing properties, making them promising candidates for AD treatment (Zhao et al., 2017).

One of the major statements of the problem is that, despite the increasing evidence for the neuroprotective properties of *Phyllanthus amarus* and *Mentha piperita*, their therapeutic potential in the treatment of Alzheimer's disease has not been fully explored, especially when used in combination with other natural extracts. Most existing studies have focused on individual plant extracts or their isolated compounds, leaving a gap in understanding the synergistic effects of combining these extracts. Additionally, while the individual efficacy of each plant is promising, the combination of *Phyllanthus amarus* and *Mentha piperita* extracts could provide enhanced therapeutic benefits, as each plant's bioactive compounds target different pathways involved in AD pathology, such as oxidative stress and inflammation. Therefore, the main focus of this study is to investigate the effects of *Phyllanthus amarus* and *Mentha piperita*, both individually and in combination, on cognitive function, neuroinflammation, and oxidative stress in an experimental model of Alzheimer's disease.

The purpose of this study is to assess the medicinal benefits of extracts from *Phyllanthus amarus* and *Mentha piperita* in an animal model of Alzheimer's disease.

The Justification of the study is that, the study of therapeutic effects of *Phyllanthus amarus* and *Mentha piperita* extracts in an animal model of Alzheimer's disease holds significant promise due to their multifaceted mechanisms targeting oxidative stress, inflammation, and cognitive enhancement. Given the increasing prevalence of Alzheimer's disease globally and the urgent need for effective treatments beyond current pharmacological options, investigating these natural extracts could lead to novel therapeutic approaches that are both safe and effective

## 2.0 METHODOLOGY

### 2.1 Material

#### 2.1.1 Sample

The materials used were rat pellet,

#### 2.1.2 Apparatus

Apparatus used were water bath, spectrophotometer, analytical weighing balance, centrifuge, filter paper, mechanical shaker, pH meter, spatula, measuring cylinder, mortar and pestle, beakers, test-tube, test-tube rack, universal bottle, Whistar Albino rat, RIYTA pottle, stirrer, funnel and dissecting kits.

#### 2.1.3 Reagent

Methanol, Ethanol, Granulated, distilled water, Gallic acid, DDPH, Deoxyribose (Thymine), Randox ALP, Randox AST, Randox ALT, Sodium Phosphate Monobasic and Sodium Phosphate Dibasic, Ferric chloride, Hydrochloric Acid, Sodium Hydroxide, (Phosphate buffer of pH 7.4), Olive oil, Wagner's reagent, Glacial acetic acid, amyl alcohol, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Wagner's reagent, Ferric chloride solution, Salkowski reagent, ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), GGT (Gamma-Glutamyl Transferase), SOD (Superoxide Dismutase), BLB (Bilirubin), Total Protein (TP), Albumin (ALB), DPA (Deoxyribonucleic Acid Protein Assay). Gallic acid, Quercetin dehydrate, Creatinine were purchased from Olabest. (ALP, AST, ALT is Randox product), DDPH (Molychem product), Deoxyribonucleic Acid Protein Assay (DPA) (Accurex product), Aspirin (CDH product code 027039) TOTAL PROTEIN (spectrum product), Wagner's reagent (Burley-in-whar product), Gamma-Glutamyl Transferase ( $\gamma$ GT) (Accurex product).

#### **2.1.4 Preparation OF Phyllanthus Amarus and Mentha piperita SHEATHS**

Identification and preparation of plant Materials Dry leaf sheaths of *S. bicolor* plants were purchased from herb sellers at Oje Market Ede, Osun State Nigeria. The sample of the plant specimen was identified. The dry leaf sheaths of *Phyllanthus Amarus* and *Mentha piperita* plants was air dried for 3 weeks and subsequently dried (after which the lightly coloured part of the leaf sheath, were cut off leaving the dark red part of the leaf sheath) and ground into fine powder using electric dry mill. 100g was measured out from the powder and it was dissolved in 200ml of distilled water and it was shake with the aid of electromagnetic shaker for 30min at 1500rpm. Subsequently, appropriate concentrations of the extract were made by dilution with distilled water into 180-200 mg/kg body weight and administered to the rats.

### **2.2 METHODOLOGY**

#### **2.2.1 Phytochemical Analysis**

The following phytochemical analysis was carried out; Alkaloids, Steroids, Flavonoids, Saponins, and Tannins

#### **2.2.2 Animal Breeding**

Ethics rules were followed in compliance with National and institutional Guidelines for the protection of the animal welfare during experiments according to the Public Health Service (Garber *et al.*, 2011), and all experimental animals received human care in accordance with the criteria outlined in the guide for the care and use of laboratory animals prepared by the National Academy of Science and published by the National Institutes of Health (Garber *et al.*, 2011).

#### **2.2.3 Acclimatization**

The rats were breed in the animal house in Federal Polytechnic Ede. The rats were housed in different cage and were fed for two weeks with only rat feed and water only, to familiarize with them with their new surroundings, this was referred to as acclimatization. The caged were cleaned every day and the water was refilled to guarantee adequate hygiene. The aforesaid acclimation was done according to the previous study carried out by (Capdevila *et al.*, 2005).

#### **2.2.4 Experimental Design**

Eighteen (18) adult male and female rats weighing between 180-200 was obtained from the animal house at Redeemer's University, Osun State, Nigeria, were randomly separated into 7 groups of 4 animals per group. The rats were kept in a rubber plastic cage under controlled light cycle (12 light/12 dark); they were fed with commercial rat meal and supplied with water. The rats were randomly allocated into 8 groups as follows:

**Table 3.1 Experimental animal design group**

| GROUP | NUMBER OF ANIMAL | TREATMENT                      |
|-------|------------------|--------------------------------|
| A     | 3                | (CONTROL)                      |
| B     | 3                | (ARSENITE)                     |
| C     | 3                | (STANDARD) DONELPEZIL          |
| D     | 3                | (MENTHA PIPERITA ONLY)         |
| E     | 3                | (PHYLLANTUS AMARUS)            |
| F     | 3                | (Mentha piperita & PHYLLANTUS) |

## 2.2.5 Biochemical Analysis

### 2.2.5.1 Determination of Tissue Enzymes Activities (ALP)

Alkaline phosphatase (ALP) assay. Using Randox Kits, the technique outlined by Bassey et al. was modified by Wright et al. 5001 units of the reagent and 10ul of the sample were combined in a cuvette. The absorbance was first measured at 405 nm and then again over a period of three minutes.

#### ➤ Alanine transaminase (ALT) Assay for Tissue Enzyme Activity

Determination (ALT). the Randox Kits approach as outlined by the IFCC. In a test tube, 5001 ALT reagent and 301 sample were combined in a cuvette. After one minute, the initial absorbance was measured at 340 nm. At the same time, the timer was set, and after one, two, and three minutes, additional absorbance values were obtained.

#### ➤ Tissue Enzyme Activity Determination (ALP)

Alkaline phosphatase (ALP) assay. Using Randox Kits, the technique outlined by Bassey et al. was modified by Wright et al. 500p1 of the reagent and 10u! of the sample were combined in a cuvette. The absorbance was first measured at 405 nm and then again over a period of three minutes.

#### ➤ Aspartate Transaminase (AST) Determination

The AST reagent was employed in place of the ALT reagent in the identical assay procedure as previously reported for ALT. After a minute, the initial absorbance was measured at 340 nm. At the same time, the timer was set, and after one, two, and three minutes, additional absorbance values were obtained.

### 2.2.5.2 Superoxide Dismutase (SOD) measurement

300ul of freshly made adrenaline was added to 200ul of tissue homogenate and 100ul of carbonate buffer (pH 10.2). Using a spectrometer, the change in absorbance was measured for three minutes at 420 nm.

### 2.2.5.3 Determination of Bilirubin

Reagents 1 and 2 were combined with 200 ul of the serum, 50 ul of Reagent 2, and 200 ul of 0.9% NaCl. The mixture was incubated at 20–25 degrees Celsius for 10 minutes. At 540 nm, absorbance was measured.

### 2.2.5.4 Dipeptidyl Aminopeptidase (DPA) determination

To make the DPA Reagent, 1.5 ml of diphenylamine, 1.5 ml of acetic acid, and 0.5 ml of sulfuric acid were combined. For 30 minutes, 1 milliliter of the DNA sample and 2 milliliters of the DPA Reagent were incubated at 37 degrees Celsius. A spectrometer was used to detect the absorbance at 600 nm.

## 2.2.6 Hematological Analysis

Blood samples was analysed using an automated hematology analyzer for the following parameters:

Red Blood Cell Count (RBC); White Blood Cell Count (WBC); Hemoglobin Concentration (Hb); Packed Cell Volume (PCV); Platelet Count.

#### ➤ Total Protein Test

Three (3) tests—standard, blank, and sample (serum)—were conducted in order to obtain the total protein test.

For the Standard, 1 ml of the Reagent (R1) was combined with 20 ul of the standard solution.

For the Blank, 1 ml of Reagent R1 was combined with 20 ul of distilled water.

20 ul of serum and 1 ml of Reagent R1 were combined for the sample.

For ten minutes, the three mixtures were incubated at 37°C. A spectrometer was used to measure absorbance at 540 nm after 30 minutes.

#### ➤ **DPPH Radical Scavenging Activity Determination**

The scavenging activity was carried out using the methods that Brand-Williams et al. described. In an ethanol solution, the samples were exposed to the stable DPPH radical. 0.5 mL of the sample, 3 mL of 100% ethanol, and 0.3 mL of DPPH radical solution 0.5 mm in ethanol were added to create the reaction mixture. DPPH is diminished when it interacts with an antioxidant that has the ability to donate hydrogen. After 100 minutes of reaction, the colour changes (from deep violet to light yellow) were measured with a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA) at 517 nm [Absorbance (Abs)]. The sample (0.5 mL) and ethanol mixture (3.3 mL) act as a blank. Ethanol (3.5 mL) and DPPH radical solution (0.3 mL) were combined to create the control solution. According to Mensore et al., the scavenging activity percentage (AA%) was calculated.

#### ➤ **Animal Sacrifice and Sample Collection**

Methylated spirit, formaldehyde, chloroform, cotton wool, a desiccator, a pin, a clean flat board, and dissecting kits were all provided. In order to prevent contamination, the instruments were cleaned.

The rats were put to sleep with chloroform after being beheaded. To make the board available for dissection, the two hands and feet were pinned to it. The rats were dissected while they were still alive. The stomach was split apart using the dissecting kits, exposing all of the internal organs. To stop the blood from clotting, the heart's blood was first drawn using a syringe and placed in an EDTA bottle. The organ (kidney or liver).

### 3.0 **RESULT AND DISCUSSION**

#### 3.1 **Result**

##### 3.1.1 **Phytochemical Analysis Outcome for *Amarus phyllanthus*.**

To investigate the various components found in the concentrated ethanonic, methanoic, and aqueous extracts of *Phyllanthus amarus*, qualitative analytical assays were performed on the extracts. The outcome of the chemical experiments.

##### ➤ **Alkaloid Test Result**

After adding 3 ml of the sample filtrate to 3 ml of 1% HCl and steaming for 30 minutes, the mixture was allowed to cool before being centrifuged for 10 minutes at 3000 p.m. The presence of alkaloids was verified by mixing 1 milliliter of the supernatant with 1 milliliter of Mayer's reagent (creamy precipitate).

##### ➤ **Tannins Test Result**

2 ml of a 0.1% FeCl<sub>3</sub> solution was combined with 2 ml of the sample filtrate. Brownish green tint indicates the presence of condensed tannin, while blue-black pigment indicates hydrosoluble tannin.

##### ➤ **Saponin Test Result**

Five milliliters of distilled water were combined with the sample filtrate (0.5 milliliters), and the mixture was shaken violently. Persistence Frothing is a sign of success.

##### ➤ **Flavonoid Test Result**

Two milliliters of the sample filtrate were combined with two milliliters of diluted ammonia solution, and then one milliliter of concentrated H<sub>2</sub>SO<sub>4</sub> was added. The presence of flavonoids was shown by yellow coloring that vanished upon standing.

##### ➤ **Polysaccharide/starch Test Result**

Six (6) drops of iodine solution were combined with two milliliters of the sample filtrate. The presence of starch or polysaccharide is indicated by the blue-black coloring.

##### ➤ **Glucose/sugar Test Result**

After mixing two milliliters of the sample filtrate with two milliliters of Fehling's solution, the mixture was steam-cooked for half an hour. After boiling, the presence of reducing sugar was indicated by the crimson tint.

##### ➤ **Terpenoid Test Result**

Six drops of Brady's reagent were added to two milliliters of the sample filtrate. Yellowish orange coloring is a sign of a successful outcome.

##### ➤ **Steroids Test Result**

After mixing 0.5 ml of the sample filtrate with 0.5 ml of acetic acid anhydride, the mixture was cooled in an ice bath. Then, using a pipette, 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 ml of chloroform were carefully added until a reddish-brown ring formed at the separation level between the two liquids, indicating the presence of steroids.

### **3.1.2 Phytochemical Analysis Outcome for Piperita Mentha.**

#### **➤ Tannins and Phenol**

Two milliliters of a 2% FeCl<sub>3</sub> solution were combined with two drops of crude extract. Phenols and tannins are indicated by a black coloring.

#### **➤ Glycosides**

After treating 2 milliliters of the extract with 2 milliliters of glacial acetic acid, a few drops of ferric chloride solution and 1 milliliter of concentrated sulfuric acid were added. The presence of glycosides is confirmed by the formation of a brown ring at the contact.

The extract was combined with two to three pieces of magnesium ribbon, and four drops of concentrated hydrochloric acid were added as part of the flavonoid test. Flavonoids are present in the extract when it is red in hue.

#### **➤ Alkaloids**

2 milliliters of 2% HCl were combined with crude extract. After being boiled over a boiling water bath, the mixture was cooled, filtered, and separated into two tubes. A small drop of Mayer's reagent was added to the first tube's solution. Alkaloids are present when precipitation appears yellow or turbidity is present.

**Table 3.1: Phytochemical Screening for *Phyllanthus amarus***

| Test       | Methanoic |          | Ethanoic |          | Aqueous  |          | n-Hexane |          | Ethyl-acetate |          |
|------------|-----------|----------|----------|----------|----------|----------|----------|----------|---------------|----------|
|            | Positive  | Negative | Positive | Negative | Positive | Negative | Positive | Negative | Positive      | Negative |
| Tannis     | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | Nill          | -        |
| Flavonoid  | ++        | Nill     | ++       | Nill     | ++       | Nill     | +        | Nill     | -             | Nill     |
| Alkaloid   | +++       | Nill     | +        | Nill     | ++       | Nill     | +        | Nill     | +             | Nill     |
| Glycoside  | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | +             | Nill     |
| Phenol     | +         | Nill     | +        | Nill     | +        | Nill     | -        | Nill     | -             | Nill     |
| Terpenoids | ++        | Nill     | +        | Nill     | +        | Nill     | -        | Nill     | -             | nill     |
| Steroids   | Nill      | -        | +        | Nill     | Nill     | -        | -        | Nill     | -             | Nill     |
| Saponin    | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | +             | Nill     |

According to *Phyllanthus amarus* phytochemical screening, methanolic, ethanolic, and aqueous extracts contain tannins, flavonoids, alkaloids, glycosides, phenols, and terpenoids, whereas n-hexane and ethyl acetate extracts only contain trace amounts of these substances. Steroids and saponins are totally missing in all solvents, but alkaloids are moderately abundant (++) in aqueous extracts and substantially concentrated (+++) in methanolic extracts. According to the findings, n-hexane and ethyl acetate exhibit low extraction effectiveness, but methanolic and aqueous extracts are the most successful at removing bioactive substances.

**Table 3.2: Phytochemical Screening for *Mentha piperita*, (*Mentha* Spp)**

| Test       | Methanoic |          | Ethanoic |          | Aqueous  |          | n-Hexane |          | Acetic   |          |
|------------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|            | Positive  | Negative | Positive | Negative | Positive | Negative | Positive | Negative | Positive | Negative |
| Tannis     | +         | Nill     | +        | Nill     | Nill     | -        | +        | Nill     | Nill     | -        |
| Flavonoid  | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | -        | Nill     |
| Alkaloid   | +         | Nill     | +        | Nill     | Nill     | -        | +        | Nill     | +        | Nill     |
| Glycoside  | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | +        | Nill     |
| Phenol     | +         | Nill     | +        | Nill     | +        | Nill     | -        | Nill     | -        | Nill     |
| Terpenoids | +         | Nill     | +        | Nill     | +        | Nill     | -        | Nill     | -        | nill     |
| Steroids   | Nill      | -        | Nill     | -        | +        | Nill     | -        | Nill     | -        | Nill     |
| Saponin    | +         | Nill     | +        | Nill     | +        | Nill     | +        | Nill     | +        | Nill     |

**Keys:**+ = **Presence of compound**- = **Absence of Compound**++ = **Moderately Present compound**+++ = **Abundantly present of compound**

Tannins, flavonoids, glycosides, phenols, and terpenoids are found in methanolic, ethanolic, and aqueous extracts of *Mentha piperita* (*Mentha* spp.) leaves, although their presence is minimal in n-hexane and acetic extracts. All other solvents lack alkaloids, while n-hexane extracts include them. All extracts are totally devoid of steroids and saponins. According to the findings, n-hexane and acetic extracts have lower extraction efficiency than methanolic, ethanolic, and aqueous extracts, which are the most effective at removing bioactive substances.

**Table 3.3: Haematology Result**

| Parameter                               | Mentha piperita ONLY    | PHYLLANTHUS ONLY         | STANDARD                 | CONTROL                 | ARSENITE                |
|-----------------------------------------|-------------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| (RBC, $\times 10^6/\mu\text{L}$ )       | 7.466667 $\pm$ 0.450925 | 7.733333 $\pm$ 0.2081666 | 6.833333 $\pm$ 0.0707107 | 6.933333 $\pm$ 0.115470 | 2.033333 $\pm$ 0.152758 |
| (Hb, g/dL)                              | 13.16667 $\pm$ 0.251661 | 13.56667 $\pm$ 0.351188  | 14.2 $\pm$ 0.282843      | 12.53333 $\pm$ 0.251661 | 4.6 $\pm$ 0.360555      |
| (WBC, $\times 10^3/\mu\text{L}$ )       | 6.583333 $\pm$ 0.189297 | 7.333333 $\pm$ 0.351188  | 6.9 $\pm$ 0.141421       | 26.73333 $\pm$ 34.61305 | 3.166667 $\pm$ 0.152753 |
| Neutrophils (%)                         | 62.66667 $\pm$ 2.516611 | 62.33333 $\pm$ 2.516611  | 62.33333 $\pm$ 2.12132   | 62 $\pm$ 3              | 5.266667 $\pm$ 0.152753 |
| Lymphocytes (%)                         | 32 $\pm$ 2              | 25.33333 $\pm$ 2.516611  | 34 $\pm$ 1.414214        | 32.66667 $\pm$ 2.081666 | 1.1 $\pm$ 0.1           |
| Monocytes (%)                           | 5.066667 $\pm$ 0.11547  | 6.233333 $\pm$ 0.251661  | 5.266667 $\pm$ 0.141421  | 5.133333 $\pm$ 0.321455 | 2.7 $\pm$ 0.2           |
| Eosinophils (%)                         | 3.026667 $\pm$ 0.064291 | 3.05 $\pm$ 0.132288      | 3.233333 $\pm$ 0.212132  | 4.5 $\pm$ 0.5           | 3.066667 $\pm$ 0.057735 |
| Basophils (%)                           | 1.966667 $\pm$ 0.251661 | 1.15 $\pm$ 0.304138      | 2.15 $\pm$ 0.388909      | 1.266667 $\pm$ 0.251661 | 1.333333 $\pm$ 0.057735 |
| Platelets ( $\times 10^3/\mu\text{L}$ ) | 301.6667 $\pm$ 2.886751 | 321.66667 $\pm$ 2.886751 | 312 $\pm$ 2.428427       | 305 $\pm$ 5             | 122.6667 $\pm$ 2.516611 |
| (HCT, %)                                | 40 $\pm$ 2              | 44.33333 $\pm$ 2.516611  | 42.33333 $\pm$ 1.414214  | 42.66667 $\pm$ 2.516611 | 2.2 $\pm$ 0.2           |

The hematological statistics show how blood parameters are changed by various treatments. While the Arsenite group exhibits a significant reduction, indicating severe blood health degradation, the Phyllanthus Only and Mentha piperita Only groups had the greatest RBC count, indicating they support red blood cell formation. The standard group has the greatest hemoglobin levels, while the arsenite group has the lowest, indicating likely anemia. The control group has the greatest WBC count, which may be the result of an immunological response, while the arsenite group has a significantly lower WBC count, which suggests immune suppression. The Phyllanthus alone exhibits the highest neutrophil counts, indicating enhanced immune protection, while the Arsenite group shows a marked decrease. Monocytes and lymphocytes exhibit a similar pattern, with the arsenite group exhibiting a significant decline. The Arsenite group has the lowest values for eosinophils and basophils, while the Mentha piperita Only group has the highest. While the Arsenite group has the lowest platelet count, indicating hematotoxicity, the Mentha piperita/Phyllanthus combo group has the greatest platelet count, indicating preventive properties against clotting issues. With the exception of the Arsenite group, where they are considerably lower, hematocrit levels are constant across the majority of categories.

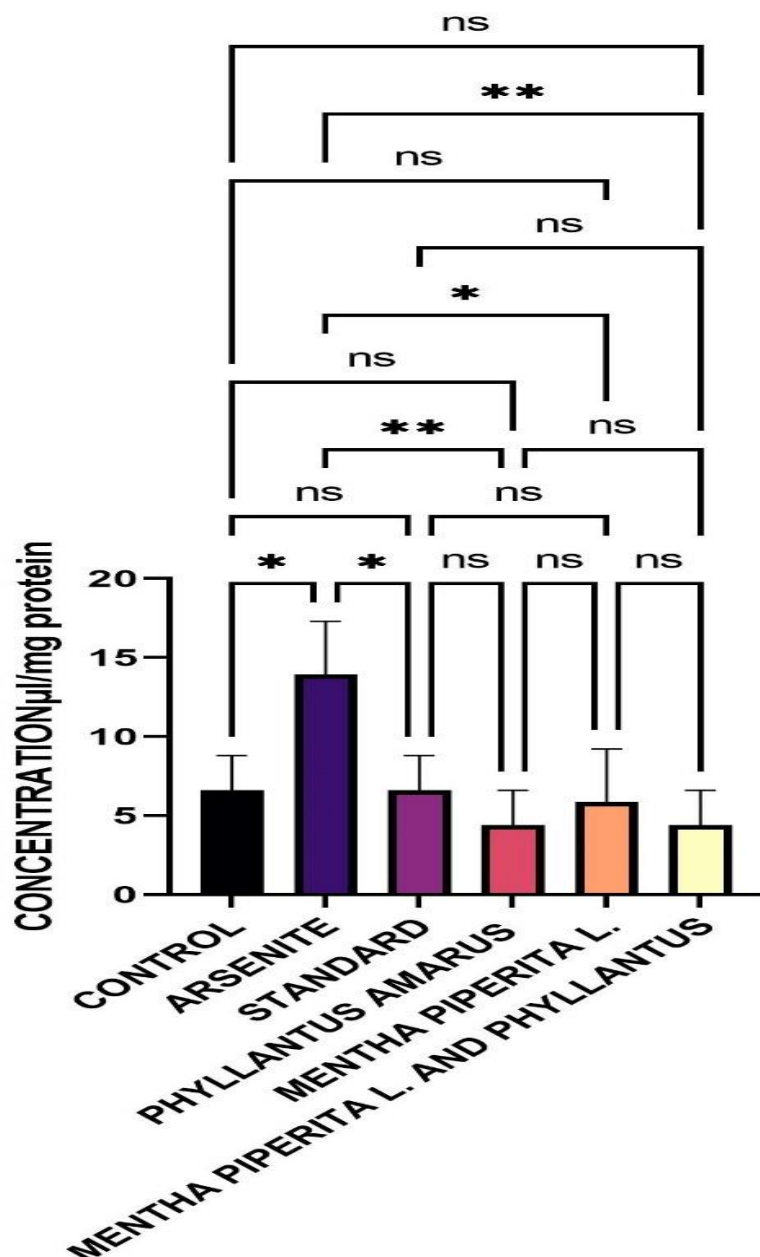


**Fig 3.1: AST BRAIN**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group showed a significant increase in AST levels ( $p < 0.01$ ), indicating probable brain toxicity. In contrast, therapy with *Mentha piperita* ( $p < 0.05$ ), *Phyllanthus amarus* ( $p < 0.01$ ), and their combinations considerably decreases AST levels compared to the arsenite group. The combined therapy of *Mentha piperita* + *Phyllanthus amarus* displays the most effective reduction ( $p < 0.01$ ), suggesting a synergistic neuroprotective effect against arsenic-induced brain damage.

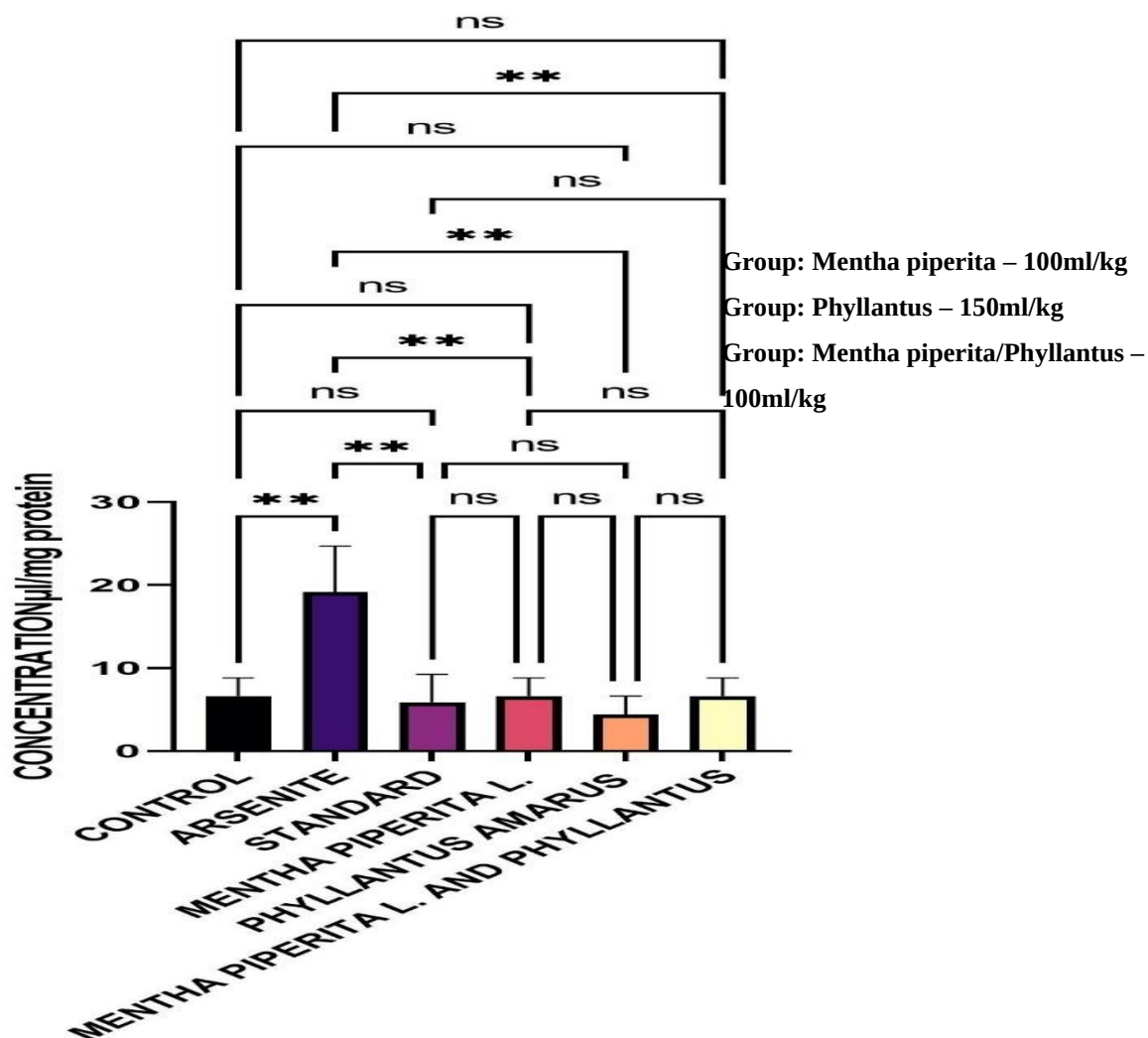


**Fig 3.2: AST KIDNEY**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in AST levels ( $p < 0.001$ ) compared to the control, indicating probable kidney injury. Treatment with *Mentha piperita* ( $p < 0.001$ ) and *Phyllanthus amarus* ( $p < 0.001$ ) dramatically decreases AST levels in comparison to the arsenite group. Furthermore, combination therapies, including *Mentha piperita* ( $p < 0.001$ ), *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.001$ ), and *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.001$ ), exhibit the most effective reduction, suggesting a strong synergistic nephroprotective effect against arsenic-induced kidney toxicity.

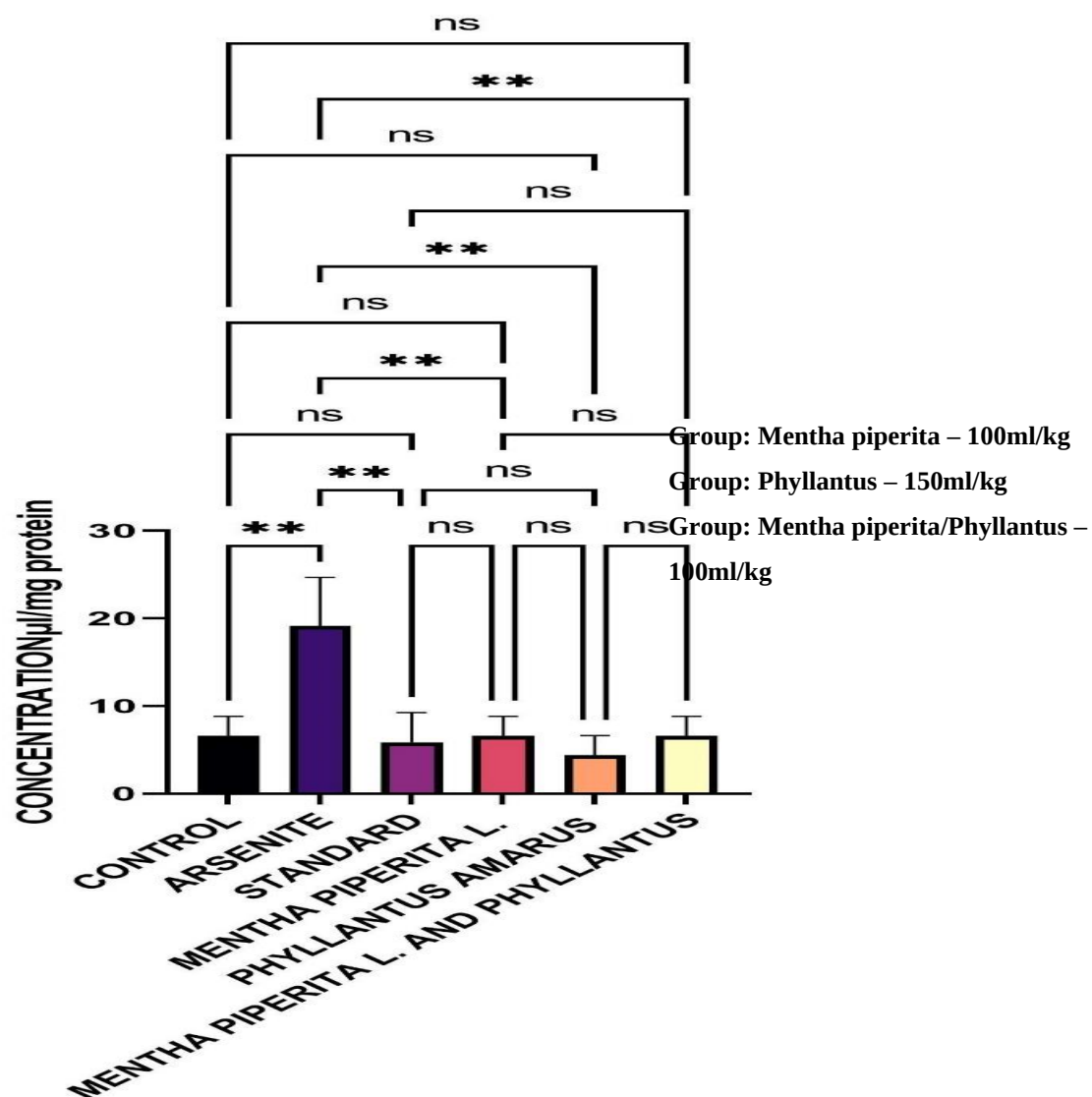
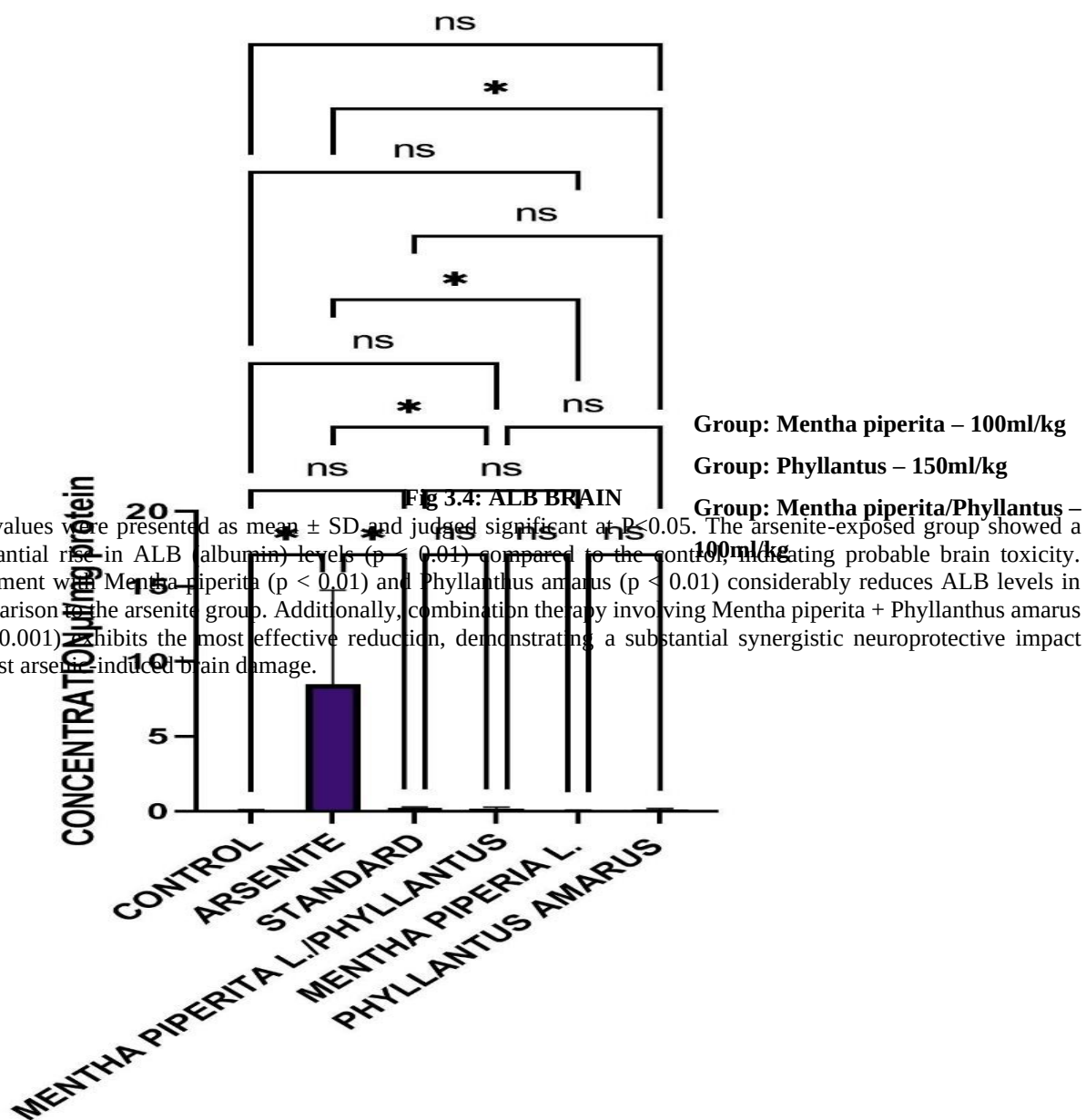


**Fig 3.3: AST LIVER**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in AST levels ( $p < 0.001$ ) compared to the control, indicating probable liver injury. Treatment with *Mentha piperita* ( $p < 0.01$ ) and *Phyllanthus amarus* ( $p < 0.01$ ) considerably decreases AST levels in comparison to the arsenite group. Additionally, combination therapies involving *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.001$ ) exhibit the most effective reduction, demonstrating a high synergistic hepatoprotective impact against arsenic-induced liver injury.



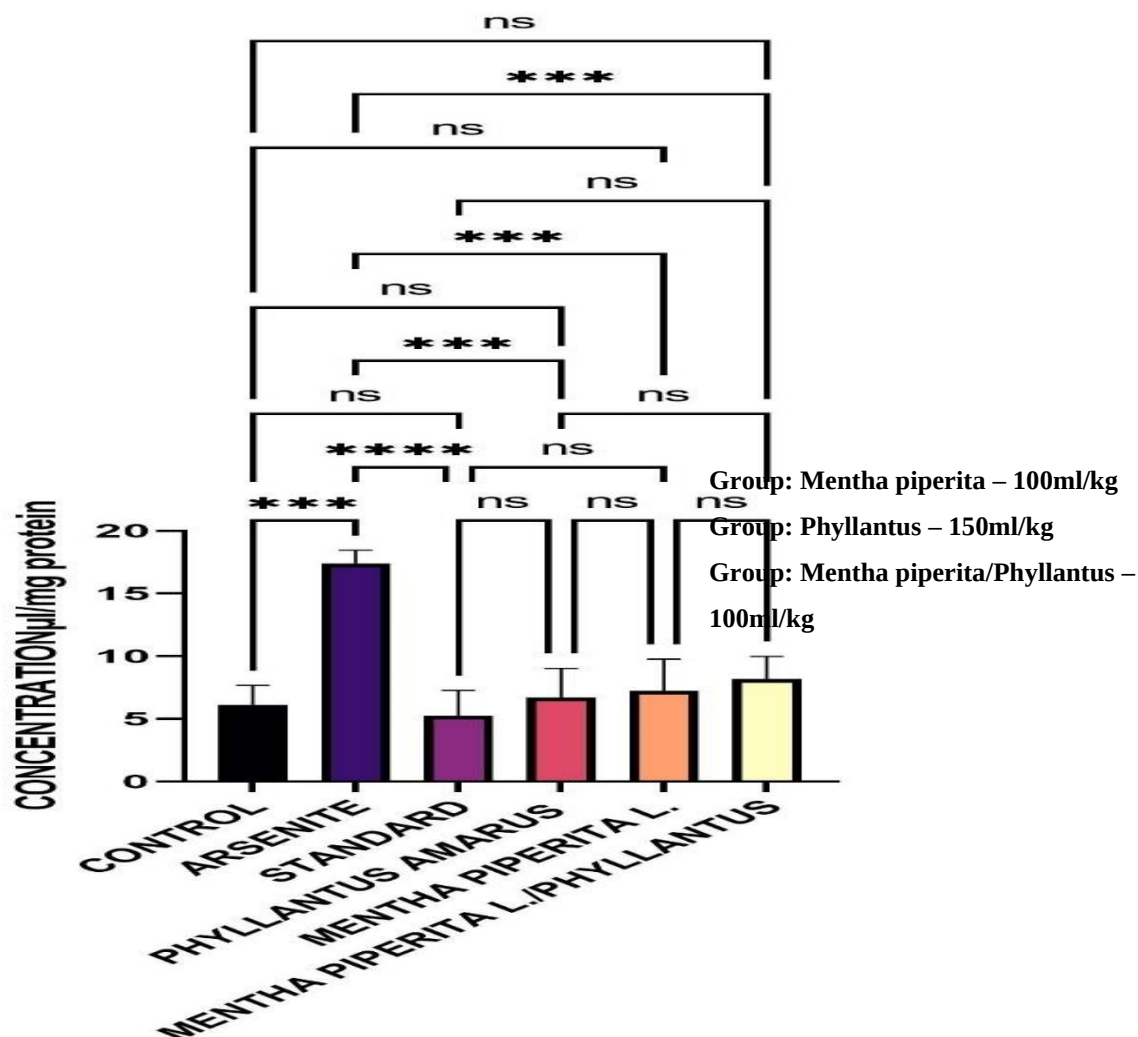


**Fig 3.5: ALB KIDNEY**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in ALB (albumin) levels ( $p < 0.0001$ ) compared to the control, indicating probable kidney injury. Treatment with *Mentha piperita* ( $p < 0.0001$ ) and *Phyllanthus amarus* ( $p < 0.0001$ ) considerably reduces ALB levels compared to the arsenite group. Additionally, combination therapy involving *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.0001$ ) exhibits the most effective reduction, demonstrating a high synergistic nephroprotective impact against arsenic-induced kidney injury.

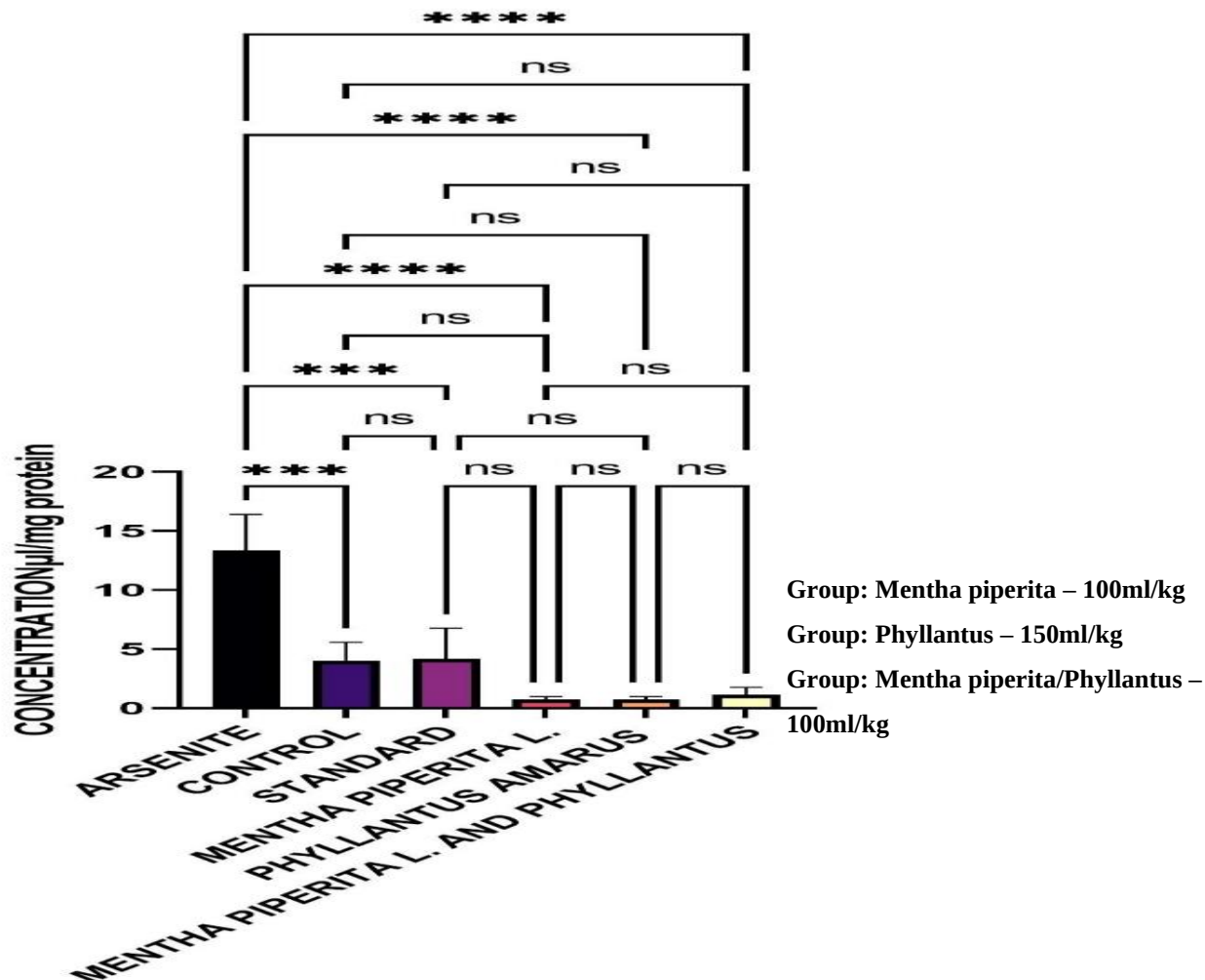


**Fig 3.5: BIL BRAIN**

These values were reported as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in bilirubin (BIL) levels ( $p < 0.0001$ ) compared to the control, indicating probable brain toxicity. Treatment with *Mentha piperita* ( $p < 0.0001$ ) and *Phyllanthus amarus* ( $p < 0.0001$ ) considerably reduces BIL levels in comparison to the arsenite group. Additionally, combination therapy involving *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.05$ ) exhibits the most effective reduction, demonstrating a substantial synergistic neuroprotective impact against arsenic-induced brain damage.

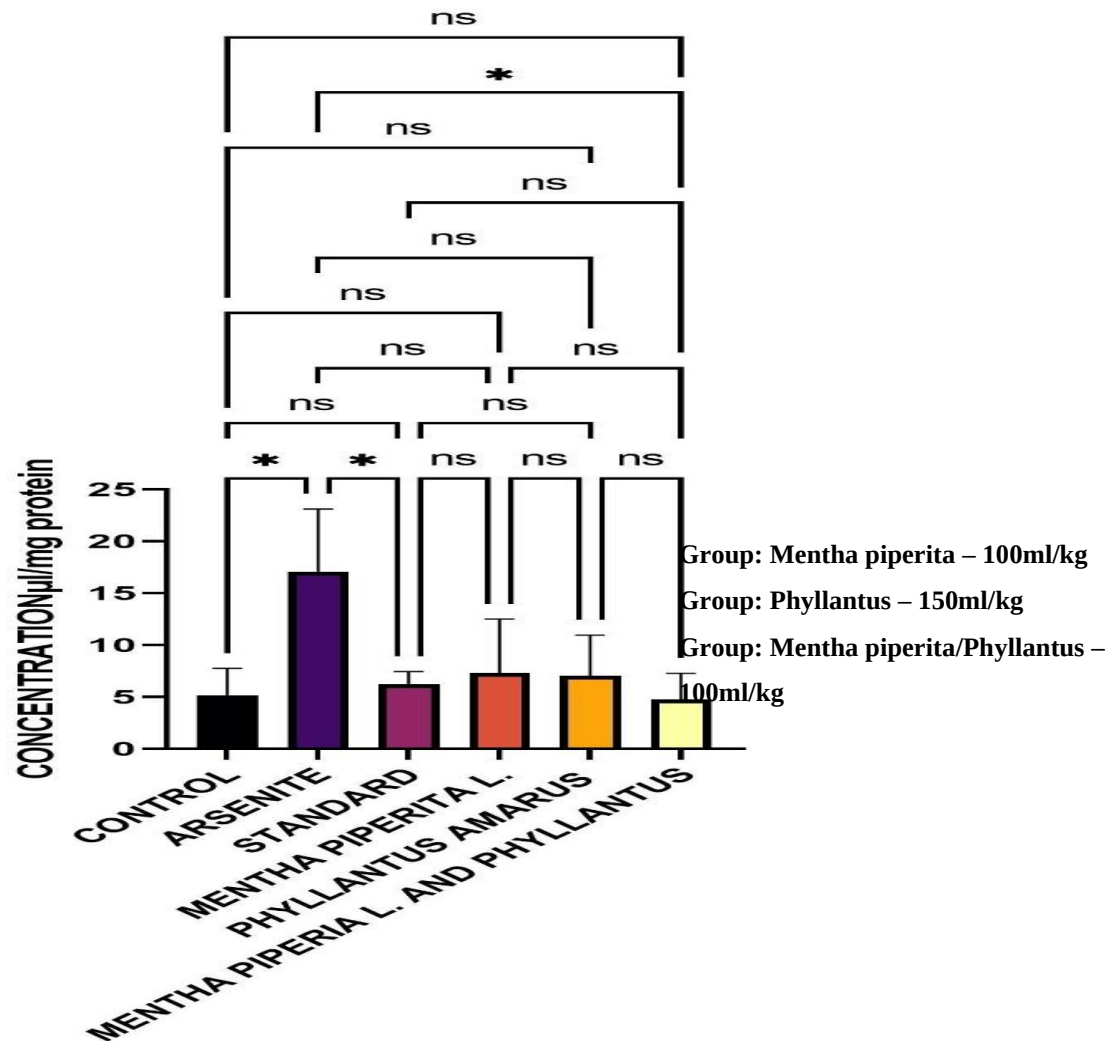


**Fig 3.6: BIL LIVER**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in bilirubin (BIL) levels ( $p < 0.05$ ) compared to the control, indicating probable liver injury. Treatment with *Mentha piperita* ( $p < 0.01$ ) and *Phyllanthus amarus* ( $p < 0.05$ ) considerably reduces BIL levels compared to the arsenite group. However, several combination therapies, notably *Mentha piperita* + *Phyllanthus amarus*, show no significant (ns) reduction, indicating that their effects on bilirubin levels in the liver may not be as pronounced as in other tissues. Nonetheless, *Mentha piperita* + *Phyllanthus amarus* displays a substantial reduction ( $p < 0.05$ ), suggesting a moderate hepatoprotective efficacy against arsenic-induced liver injury.



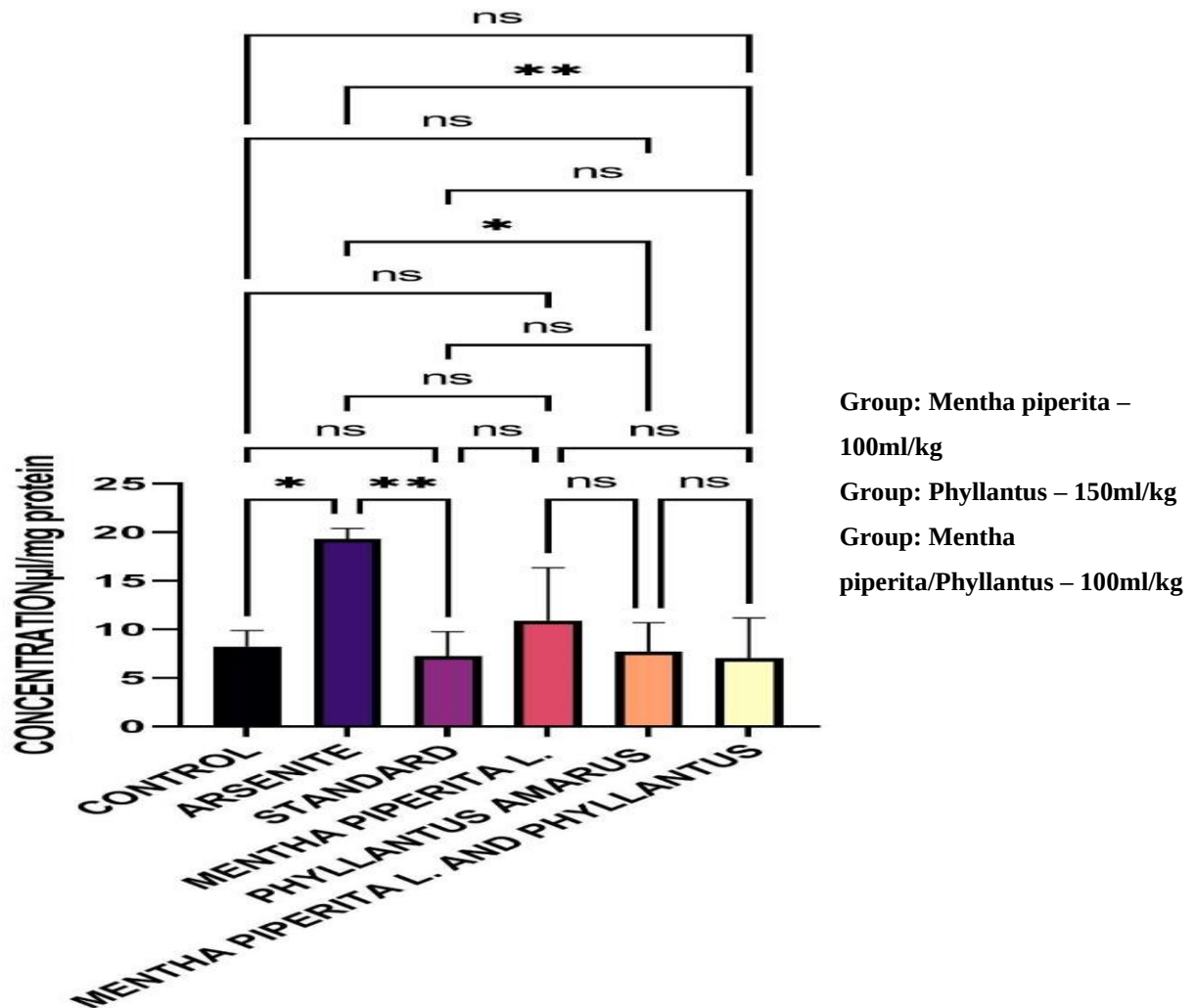


**Fig 3.8: ALP LIVER**

These values were reported as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in ALP (alkaline phosphatase) values ( $p < 0.05$ ) compared to the control, indicating probable liver injury. Treatment with Mentha piperita ( $p < 0.05$ ) and Phyllanthus amarus ( $p < 0.05$ ) indicates a drop in ALP levels compared to the arsenite group. However, combination therapies comprising Mentha piperita + Phyllanthus amarus exhibit no significant (ns) reduction, suggesting that the combined treatments may not have a strong hepatoprotective effect on ALP levels in the liver. Nonetheless, some individual treatments show minor improvements, indicating a mild impact on liver function.

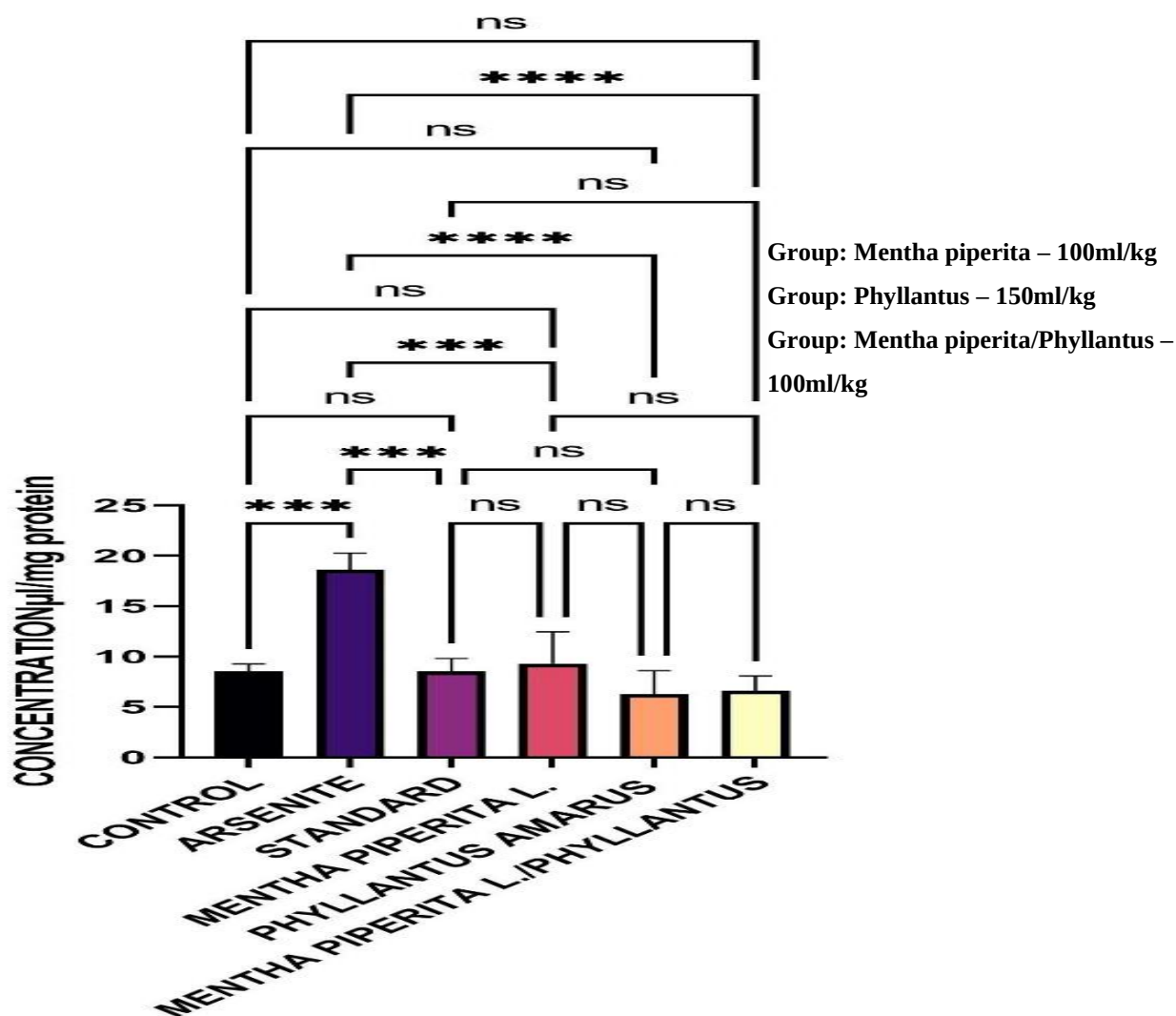


**Fig 3.9: ALP BRAIN**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group showed a substantial rise in ALP (alkaline phosphatase) levels ( $p < 0.01$ ) compared to the control, indicating probable brain toxicity. Treatment with Mentha piperita ( $p < 0.01$ ) and Phyllanthus amarus ( $p < 0.001$ ) dramatically lowers ALP levels in comparison to the arsenite group. Additionally, combination therapy involving Mentha piperita + Phyllanthus amarus ( $p < 0.001$ ) exhibits the most effective reduction, demonstrating a substantial synergistic neuroprotective impact against arsenic-induced brain damage.

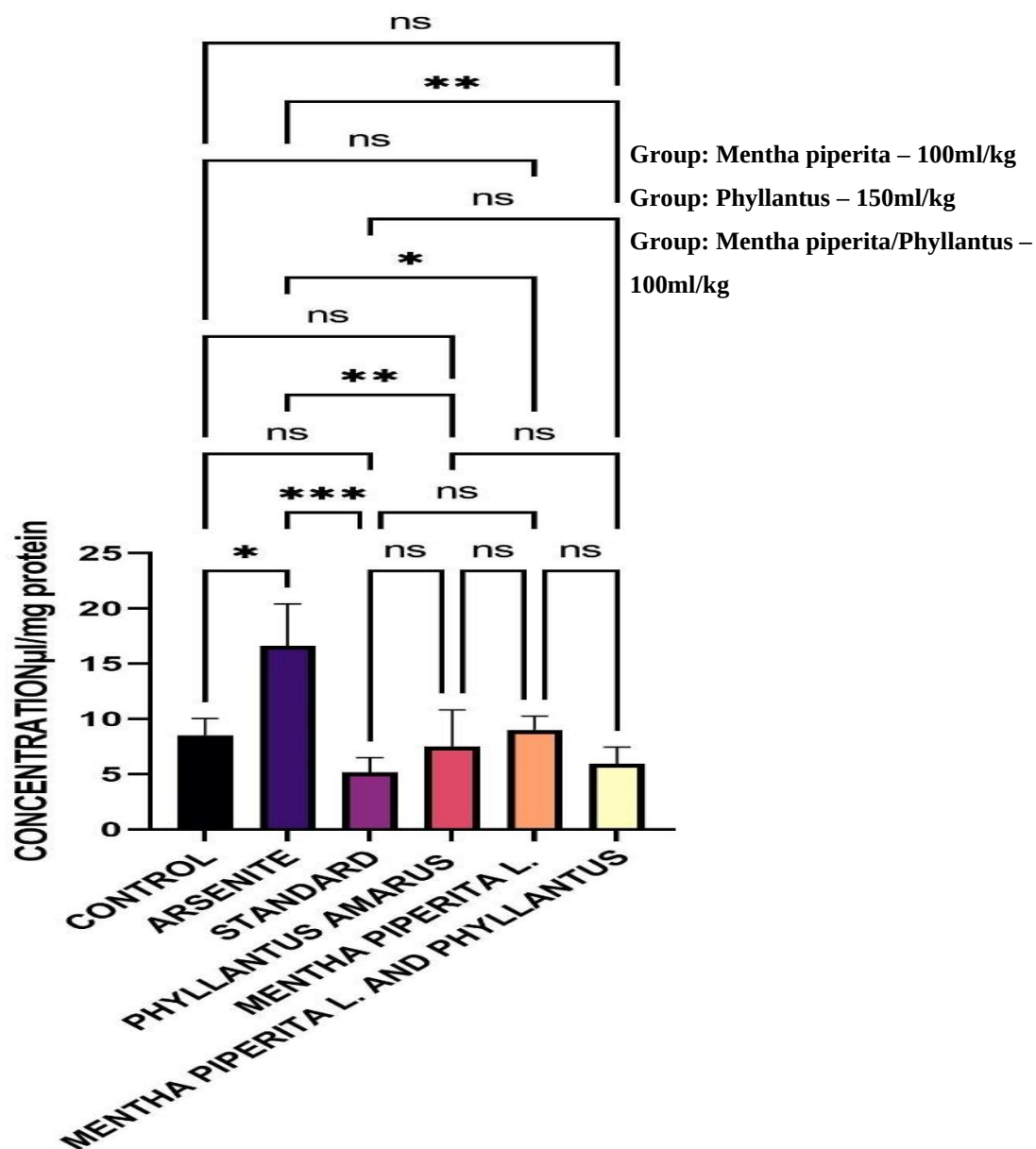


**Fig 3.10: ALP KIDNEY**

These values were reported as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a substantial rise in ALP (alkaline phosphatase) values ( $p < 0.0001$ ) compared to the control, indicating probable kidney injury. Treatment with Mentha piperita ( $p < 0.01$ ) and Phyllanthus amarus ( $p < 0.001$ ) dramatically lowers ALP levels in comparison to the arsenite group. Additionally, combination therapies involving Mentha piperita + Phyllanthus amarus ( $p < 0.05$ ) exhibit the most effective reduction, demonstrating a high synergistic nephroprotective impact against arsenic-induced kidney damage.

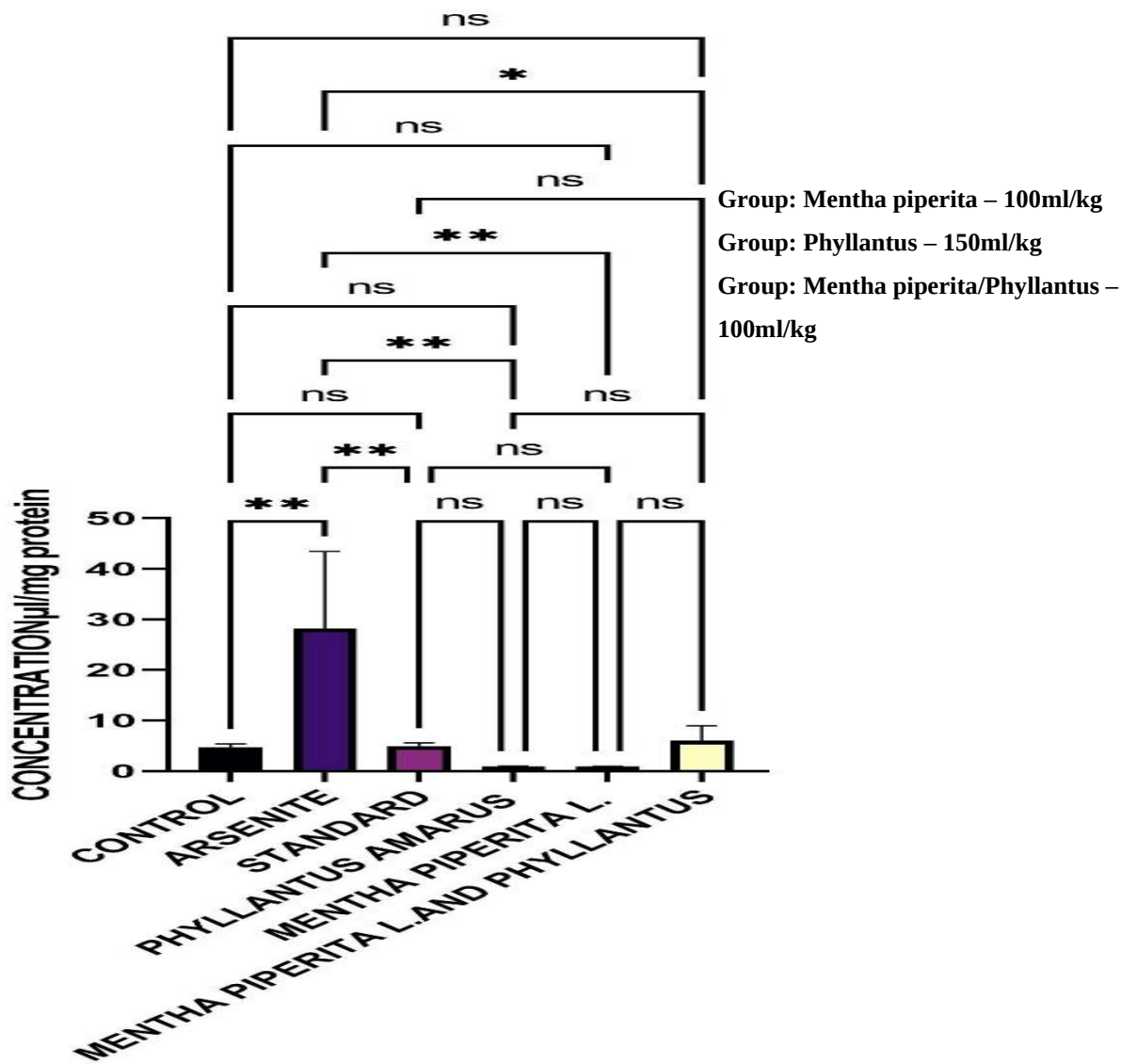


**Fig 3.11: DNA FRAGMENTATION**

The values were presented as mean  $\pm$  SD and judged significant at  $P < 0.05$ . The arsenite-exposed group demonstrates a considerable increase in DPA (perhaps deoxyypyridinoline or another oxidative stress marker) levels ( $p < 0.0001$ ) compared to the control, indicating severe toxicity and oxidative damage. Treatment with *Mentha piperita* ( $p < 0.0001$ ) and *Phyllanthus amarus* ( $p < 0.0001$ ) dramatically lowers DPA levels relative to the arsenite group. Additionally, combination therapies involving *Mentha piperita* + *Phyllanthus amarus* ( $p < 0.001$ ) exhibit the most effective reduction, demonstrating a substantial synergistic protective impact against arsenic-induced oxidative stress and damage.

#### 4.2 DISCUSSION

The haematology results offer important information on how various treatments affect blood parameters. The *Phyllanthus* Only group has the greatest red blood cell (RBC) count, closely followed by the *Mentha piperita* Only group. This suggests that both therapies may promote the generation of red blood cells. On the other hand, the RBC count significantly drops in the Arsenite group, indicating a serious adverse effect on blood health. Comparably, the Standard groups have the greatest hemoglobin (Hb) levels, but the Arsenite group has the lowest, suggesting that Arsenite exposure may have caused anemia. The Control group's white blood cell (WBC) count is significantly higher, which could indicate an infection or immunological reaction. However, the WBC count is much lower in the Arsenite group, suggesting that immunological activity may be suppressed. The neutrophil percentage likewise shows this pattern, with the *Phyllanthus* Only groups having the highest levels. This suggests that these therapies may strengthen

immunological responses. The notion of arsenite-induced immune suppression is further supported by the sharp drop in neutrophil numbers observed in the Arsenite group. The percentages of lymphocytes and monocytes show a similar pattern, with the Arsenite group showing a notable decrease and the Control Only and Phyllanthus Only groups maintaining rather consistent values. Eosinophil and basophil counts exhibit a similar trend, with the Arsenite group registering the lowest values and the *Mentha piperita* Only group displaying the highest eosinophil count. The combo group of *Mentha piperita* and *Phyllanthus* has the highest platelet count, suggesting a possible preventive effect against problems with blood coagulation. Arsenite, on the other hand, has the lowest platelet count, which may indicate hematotoxicity. With the exception of the Arsenite group, where it is considerably lower, the hematocrit (HCT) % stays constant across the majority of treatment groups. All things considered, these results imply that exposure to arsenite has a severe detrimental effect on hematological parameters, resulting in decreased hemoglobin levels, a decrease in red and white blood cell counts, and a suppression of immunological function. Treatments with *Phyllanthus* and *Mentha piperita*, on the other hand, seem to have protective benefits that may help immunological function and blood cell synthesis. The greatest effect appears to come from combining these three treatments, especially when it comes to platelet count, which may be a sign of better blood health overall. These findings demonstrate how these natural remedies may be able to lessen the negative effects of arsenite toxicity on the hematological system. The in-vivo study looks into how *Phyllanthus amarus* and *Mentha piperita* can protect the liver, kidney, and brain against arsenic-induced toxicity. According to the results, exposure to arsenite causes notable elevations in a number of toxicity markers, such as AST, ALB, BIL, ALP, and DNA FRAG, demonstrating its detrimental effects. Nonetheless, both single and combined therapies with these medicinal plants dramatically lower toxicity markers, indicating a potent nephroprotective, hepatoprotective, and neuroprotective effect. Arsenite exposure significantly raised AST (aspartate aminotransferase) levels in the liver ( $p < 0.001$ ), kidney ( $p < 0.001$ ), and brain ( $p < 0.01$ ), indicating organ damage. AST levels were dramatically lowered in all cases by treatment with *Mentha piperita* and *Phyllanthus amarus*; the most successful combination therapy was *Mentha piperita* + *Phyllanthus* ( $p < 0.0001$ ). This implies that triple combination therapy improves recovery more successfully than either treatment alone. Similarly, groups treated to arsenite showed a significant increase in ALB (Albumin) levels, especially in the kidney ( $p < 0.0001$ ) and brain ( $p < 0.01$ ). ALB levels were dramatically reduced by treatment with individual plant extracts; however, the combination treatments showed the largest reductions ( $p < 0.0001$ ), indicating their synergistic protective actions against damage caused by arsenic. Arsenite exposure caused a significant rise ( $p < 0.0001$ ) in bilirubin (BIL) levels in the kidney and brain, further demonstrating its harmful effects. Once more, BIL levels were dramatically lowered by treatment with *Phyllanthus amarus* and *Mentha piperita*; the triple combination therapy had the strongest protective effect ( $p < 0.0001$ ). After exposure to arsenite, there was a significant increase in ALP (Alkaline Phosphatase) levels in the kidney ( $p < 0.0001$ ), brain ( $p < 0.01$ ), and liver ( $p < 0.05$ ). Although ALP levels were somewhat lowered by separate treatments, combination therapy had no discernible impact on liver ALP levels, indicating that the plants may not have much of an impact on this specific indicator of liver impairment. In groups exposed to arsenite, the oxidative stress marker DPA also rose sharply ( $p < 0.0001$ ). *Phyllanthus amarus* and *Mentha piperita* treatments consistently decreased oxidative stress ( $p < 0.0001$ ), demonstrating their potent antioxidant qualities. Once more, combo therapies showed the most beneficial decreases. *Mentha piperita* + *Phyllanthus* (150 ml/kg) was the most effective treatment group when compared to all toxicity markers, demonstrating the medicinal plants' synergistic interaction. While some protection was offered by individual treatments, combination therapies continuously outperformed single treatments, indicating increased therapeutic potential. In summary, this study shows that *Phyllanthus amarus* and *Mentha piperita* considerably prevent arsenic-induced toxicity by lowering oxidative stress and toxicity indicators in the kidney, liver, and brain. With the most protective benefits, the triple combination therapy (150 ml/kg) is a potential approach to reducing organ damage caused by arsenic. Methanolic, ethanolic, and aqueous extracts are the best solvents for removing bioactive substances like tannins, flavonoids, glycosides, phenols, and terpenoids from the plants under study, such as *Phyllanthus amarus* and *Mentha* spp., according to the results of the phytochemical screening. While n-hexane and ethyl acetate/acetic extracts demonstrated low extraction efficiency, these solvents demonstrated a high content of the chemicals. While alkaloids were only found in n-hexane extracts from *Mentha* spp., they were significantly present in both methanolic and aqueous extracts of *Phyllanthus amarus*, with a high concentration in methanol and a moderate presence in aqueous extracts. Furthermore, none of the extracts contained steroids or saponins, indicating that either these plants did not contain them or that the solvents utilized had trouble extracting them. Overall, the results point to the superiority of polar solvents—such as methanol, ethanol, and water—over non-polar solvents, like n-hexane and ethyl acetate, in the extraction of bioactive chemicals.

## 4.0 CONCLUSION AND RECOMMENDATION

### 4.1 CONCLUSION

The hematological results provide significant information regarding the effects of various treatments on blood parameters. The *Phyllanthus* Only group has the maximum red blood cell (RBC) count, followed by the *Mentha piperita* Only group, demonstrating that these therapies may stimulate red blood cell development. In contrast, the Arsenite group had a considerable fall in RBC count, indicating a substantial detrimental influence on blood health. Similarly, hemoglobin (Hb) levels are highest in the standard groups and lowest in the arsenite group, indicating that arsenite exposure is likely to cause anemia. The white blood cell (WBC) count is considerably larger in the control group, indicating an immunological response or illness. However, the Arsenite group had a substantially lower WBC count, indicating that immunological activity may have been suppressed. This pattern is also visible in the neutrophil percentage, with the *Phyllanthus*-only groups having the greatest percentages, showing that these therapies may improve immunological responses. The arsenite group, on the other hand, exhibits a considerable decline in neutrophil counts, supporting the idea of arsenite-induced immune suppression. A similar tendency can be found in lymphocyte and monocyte percentages, where the Control Only and *Phyllanthus* Only groups maintain pretty consistent levels, but the Arsenite group exhibits a considerable reduction. Eosinophil and basophil counts follow a similar pattern, with the *Mentha piperita* Only group having the highest eosinophil count and the Arsenite group having the lowest. The highest platelet count was reported in the *Mentha piperita*/*Phyllanthus* combo group, indicating a potential prophylactic activity against blood clotting issues.

### 4.2 RECOMMENDATION

Based on the findings of this study, more investigations, including clinical trials, should be done to confirm the therapeutic potential of *Mentha piperita* L., and *Phyllanthus amarus* in alleviating arsenic-induced toxicity in humans. Because these plants have been shown to have strong protective effects, pharmaceutical formulations using their triple combination therapy should be created so that they can be used as a natural medicine to treat arsenic toxicity. Additionally, long-term toxicity and safety assessments should be undertaken to determine the optimum dosage and safety profiles, particularly in prolonged exposure scenarios. Further studies should examine the precise biochemical pathways involved in the protective effects of these plants, notably their involvement in controlling oxidative stress, inflammation, hematological stability, and cellular repair mechanisms. Since methanolic, ethanolic, and aqueous extracts proved most effective in extracting bioactive chemicals, future studies should enhance extraction procedures to maximize their medicinal potential. We should study advanced approaches like supercritical fluid extraction and nanoformulation to enhance bioavailability.

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