The Efficacy of Bio-synthesized Silver Nanoparticles with *Carica papaya* Extract against *Plasmodium berghei* Infected Mice.

ABSTRACT

Background: The evolution of antimalarial drug resistance increases malaria's burden and is a recurring concern in the global fight against malaria. Medicinal herbs have played an important role in the treatment of malaria and have been advised in reducing Plasmodium parasite resistance to standard antimalarial medications. Papaya contains antimalarial bioactive chemicals. Herbal medicines include limitations such as in vivo instability, poor solubility, limited bioavailability, poor absorption in the body, target-specific delivery issues, and tonic efficacy. Using nanotechnology and nanomedicine for malaria treatment can overcome these limitations.
Objective: This study was aimed to assess the potential of *Carica papaya* leaf extract with silver nanoparticles against *Plasmodium berghei*-infected mice.

Materials and Methods: Fresh *Carica papaya* leaves were collected in Oke-Arade, Ogbomoso, active contents of leaves were extracted and biosynthesized silver nanoparticles were passed into the infected experimental mice. For the investigation, 40 mice were utilized, and their parasitaemia level, haematological and histological parameters determined to assess safety effect of the nanoparticle as well as efficacy of the extract. Kidney and liver samples were analyzed for haematological, parasitological, and histological parameters.

Results: On days 3 and 5, Ag-NPs at 25mg/kg and 50mg/kg cleared malaria parasitemia better than plant extracts at 100mg/kg and 200mg/kg ($p<0.05$). The mice's weight loss improved on day 5($p>0.05$), as did PCV$p<0.05$).
Conclusion: he Ag-NPs synthesized with *C. papaya* showed some antimalarial activity against *Plasmodium berghei* as more of the infected red blood cells were cleared indicating a decrease in parasitemia coupled with the reduction of the malaria parasite density across the experimental periods. This process of synthesizing Ag-NPs with the extract is simple and cost effective, thereby it's a promising antimalarial alternative.

Keywords: *Plasmodium berghei, Carica papaya,* Ag-NPs, Malaria.

1. INTRODUCTION

Malaria is still a hazard to life in Sub-Saharan Africa, particularly in children under the age of five. Effective antimalarial drugs are crucial for malaria control and elimination. Increasing antimalarial drug resistance once again threatens effective antimalarial drug treatment, malaria control, and elimination [1]. Resistance to chloroquine and later to sulfadoxine-pyrimethamine have followed this route and have contributed to millions of excess malaria attributable mortality in African children [2,3]. However, only with the advent of artemisinin partial resistance were large-scale efforts made in the Greater Mekong Sub region (GMS) [4].

Malaria still remains a threat to life especially in children below the age of 5 in sub-Saharan Africa. According to the latest world malaria report, over 400,000 deaths were reported from over 200 million infections in 2019. The report also shows that the gains in the fight against malaria seem to have plateaued in recent times [5].

In Nigeria, malaria contributes up to 60 percent of all outpatient visits, 30 percent of all hospital admissions, 11 percent of maternal mortality, 30 percent of under-five mortality and 25 percent of infant mortality [6]. Nigeria alone contributes 29 percent of the over 80 percent global malaria burden [7].

Plasmodium berghei infection of laboratory mouse strain is frequently used in research as a model for human malaria, owing to their life cycle resemblance to the common *P. falciparum* and their ability to be easily manipulated in molecular research [8].

Medicinal plants have played significant roles in the treatment of malaria and have been recommended in curbing resistance posed by Plasmodium parasites to conventional antimalarial drugs [9]. According to a recent review of plant-derived drugs and their contribution to the global disease pandemic, it is believed that the future of drug discovery lies in plant-derived natural products [10].

Carica papaya commonly known as pawpaw belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [11]. Originally derived from the southern part of Mexico, *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *C. papaya* fruit circulates widely, and it is accepted as food or as a quasi-drug. The outcomes of a study indicate that *C. papaya* possess bioactive compounds responsible for antimalarial activity [12]. The study validated the folklore use of this plant for the management of malaria in ethnomedicine in Ethiopia [13].

However, challenges such as in vivo instability, poor solubility, low bioavailability, poor absorption in the body, problems with target-specific delivery, and tonic effectiveness are disadvantages along with the use of large scale-sized materials in the treatment process by herbal medicines. Therefore, using new treatment systems related to nanotechnology and nanomedicine for disease treatment can be an effective option to overcome these challenges [14]. Nanoscale sized particles exhibit unique structural, chemical, mechanical, magnetic, electrical, and biological properties [15]. The novelty of this work is to study the multifaceted types of synthesized nanoparticles (NPs) particularly based on biological materials. Nano-biotechnology can eradicate Malaria disease by directly targeting parasites, providing good therapeutic strategies for vector eradication [16,17].

This study is aimed at investigating the anti-plasmodial, cytotoxic and predatory efficiency of aqueous leaf extract of *C. papaya* and its mediated Ag-NPs.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

Fresh *Carica papaya* leaves were harvested from Oke-Arade Area, Ogbomoso, Oyo State, Nigeria.

Authentication of the plant was done by a botanist in the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, with herbarium number 18020**.**

They were washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and water as the solvent according to the method described by Airaodion et al. [18].

The extract was prepared by suspending 0.1g of the leaf powder in 10mL of distilled water. The suspension was heated in a water bath at 60^{o}C for 1h. It was then filtered using Whatman No 1 filter

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paper and then centrifuged for 20 min at 4,000 rpm. The clear filtrate was poured out and kept for further use while the residue was discarded.

2.2 *In vivo* Anti-plasmodial Investigation and Determination of Percentage of Parasitemia

The parasites were maintained by serial passage of blood from infected mice to non-infected ones. To infect the mice, the blood sample was collected through cardiac puncture of a donor mouse with a rising parasitemia of 24%, the blood sample was diluted with normal saline. Each mouse was passaged with 0.2 mL of the infected blood containing 1×10^7 *P. berghei* parasitized red blood cell via the intraperitoneal route (IP) [19].

Blood sample for microbiological parameters was collected from the mice tail. Parasitemia of the animal was detected before the experiment and then daily monitored by counting the number of infected erythrocytes per 1,000 erythrocytes under light microscope using Giemsa-stained thin blood smear. Survival rate of infected mice was also calculated. The parasite was kept alive by continuous Intra-peritoneal passage in naïve mice every six days.

2.3 Determination of Packed Cell Volume (PCV).

Packed cell volume (PCV) was determined using the capillary method. Intraperitoneal blood was collected into a heparinized hematocrit tubes using the Wintrobe method [20]. The tubes were sealed with plasticine seal and thereafter centrifuged for about 12,000 rpm for 5 minutes. The volume of cells was calculated based on the following formula: PCV = Erythrocyte volume /Total blood volume × 100.

2.4 Determination of Malaria Parasite Density

Parasite density of the animal detected before the experiment and then daily monitored by counting the number of malaria parasite against white blood cells under light microscope using Giemsa-stained thick blood smear. Survival rate of infected mice was also calculated.

Malaria Parasite density = Number of Malaria parasites x 8000/200

2.5 Experimental Design and Treatment of Mice.

The experimental study was carried out at the Institute of Medical Research and Training (IMRAT), University Teaching Hospital, Ibadan, Oyo state, Nigeria.

Chloroquine-sensitive strain of *P. berghei* NK65 was used in this study. Infection was initiated by intraperitoneal (IP) injection of 1×10^6 PbNK65 parasitized erythrocytes into ICR mice.

A total of 40 mice was used for the experimental study. In this study, Swiss albino mice weighing 18-24g were selected. The mice were randomly divided into 8 groups consisting of 5 mice in each group which include the experimental groups, positive and negative control.

The animals were kept and maintained in a temperature-controlled environment (25 ± 2^oC) with a 12-hour light cycle and made to acclimatize to the animal house condition for 5 days prior to the commencement of the experiment. The animals were fed with pelletised growers mash and water *ad libitum*. All animals received humane care in accordance to the ''Guide for the Care and Use of Lab animals'' [21].

Low and high doses of crude *C. papaya* extract (100 and 200 mg/kg), synthesized Ag-NPs of *C. papaya* extract (25 and 50 mg/kg), chloroquine (10 mg/Kg) and Artemether lumefantrine (0.16mg/Kg) was used for treatment among the different groups (Table 1).

2.6 Bio-Synthesis and Characterization of CPAg-NPs

One (1) mL of the leaf extract was reacted with 40mL of 1mM $AgNO_3$ at an ambient condition (30 ± 2^oC) for 20 min. The control consisted of 1mM $AgNO_3$ alone. The change in color was monitored for the photosynthesis of the CPAg-NPs [22].

The bio-fabrication of CPAg-NPs was visually monitored by the changes in color of the reaction mixture. The absorption spectra due to surface plasmon resonance (SPR) were determined using UV-Vis Spectrophotometer (Cecil, USA). Nanoparticles are characteristically categorized by their size, surface area, shape and dispersity nature. The common procedures implemented in their characterization are UV-visible spectrophotometry. The development of various metallic nanoparticles from their precursor metal salts gives representative peaks at different absorptions that can be examined using UV-visible spectrophotometry. For instance, noble metallic nanoparticles like Ag absorb strongly within the visible

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region producing λ-max of 400-450nm, as a function of SPR phenomenon occurring in metallic nanoparticles [23].

2.7 Lethality (LD_{50}) Test

The mean lethal dose (LD_{50}) of the aqueous extract and Ag-NPs was determined in mice (weighing 20-30g) using the arithmetic-geometric-harmonic (AGH) methods of rough estimation as modified by Saganuwan [24].

2.8 Cytotoxicity Screening

Cytotoxicity screening of *C. Papaya* extract was carried out by dividing the mice into 6 groups (2mice/group). Each group will contain mice that will receive 10mg/kg, 100mg/kg, 1000mg/kg of the plant extract respectively for phase 1 and 2000mg/kg, 4000mg/kg, 6000mg/kg of the plant extract for phase 2 to determine the lethal and the therapeutic dose orally according to the Lorke's method of toxicity screening.

Synthesized Ag-NPs toxicity screening was also carried out by dividing the mice into 3 groups (2mice/group). Each group will contain mice that will receive 5mg/kg, 50mg/kg, 300mg/kg of the Ag-NPs respectively to determine the lethal and the therapeutic dose orally using to the Organisation for Economic Co-Operation and Development (OECD) method of toxicity screening. Control mice were kept under the same condition without any treatment. The mice were then observed for signs of toxicity which include but not limited to salivation, paw licking, weakness, stretching of the entire body, respiratory distress, coma and death in the first 4 hours and subsequently daily for 7 days [25].

2.9 Treatment with *Carica Papaya* Crude Extract and Bio-synthesized Ag-NPs

Groups of naive ICR mice (5 mice/group) were inoculated with 1×10^6 *P. berghei* NK65 parasitized erythrocytes intraperitonially. The crude extract, Ag-NPs, artemether lumefantrine and chloroquine (for the control groups) were given orally using oral cannula once a day for 3 consecutive days.

2.10 Histological Study of Kidney and Liver

Kidney and liver were collected and fixed in 10% neutral buffered formalin, dehydrated in ethanol and embedded in paraffin and then sectioned in 5µm thickness. Sections were then stained with haematoxylin and eosin. Liver and kidney areas were assessed for the degree of liver and kidney damage respectively. The scoring of each section of the mouse liver and kidney tissue represented a mean score of five separate sections of high microscopic power fields (400x magnification).

2.11 Statistical Analysis

Data obtained from the result of the study was expressed as mean ± standard error of mean (SEM) for the control and test group. Student *t* test and analysis of variance (ANOVA) was used between all the groups. Significant differences were taken at $p<0.05$.

2.12 Ethical Approval

Ethical approval was obtained from the Ethical Committee of The Department of Medical Laboratory Science, Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria.

3. RESULTS AND DISCUSSION

A total number of forty (40) blood samples were collected from mice which were grouped into eight groups ranging from group 1 to group 8. Group 8 represent the control group and the other groups are test groups subjected to varying conditions. The body weight of the mice was determined, and their blood samples were examined for the estimation of packed cell volume (PCV), malaria parasite density (MPD) and infected red blood cell (IRBC).

The use of experimental animal model has contributed immensely both to the understanding of the pathophysiology of a disease condition and the development of drug candidates [26]. Not only is the direct use of plant extracts with potential antimicrobial activity invaluable in their contribution to treatment, but also their usage for synthesizing nanoparticles which can be engaged for the same purpose [27].

There are many bioactive constituents present in the leaf extract of *C. papaya* with potential antimalarial activity, but presently many of them have not been well classified to the extent that they can individually

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be linked to their specific effect and their role in preventing malaria infection. However, some reports have shown that flavonoids, tannins, saponins, and other phytoconstituents may play some roles in the inhibition of malaria parasites in infected animals [28]. The synthesis of silver nanoparticles using plant extracts is getting more attention, due to their application in biomedical sciences such as anti-parasitic, anti-malarial, bactericidal, fungicidal, and anti-viral activity [29].

C. papaya extracts did not cause any death within the 24 hours of cytotoxicity test. Moreover, 11 days observation of the mice did not reveal any behavioural characteristic changes of toxicity. This indicates that the extract may be safe for *in vivo* administration up to 200 mg/kg**.** On the contrary, up to 3000mg/Kg dose had been reported to show zero death tolerance in study carried out by Solikhah et al. (2020) [30].

Examination of Ag-NPs using UV-visible spectrophotometry showed that the nanoparticles were absorb strongly within the visible region producing λ-max of 280-500nm (Fig 1). As for Metwally et al. (2021), Ag-NPs formation was visible at a band of ~450 nm which implies agreement with our study [31].

The decrease in PCV of all the infected groups after infections are in conformity with previous reports, documenting reduced serum PCV in malarial subjects administered aqueous extracts of *Abrus precatorius* leaf in *Mus musculus* by Saganuwan et al. (2011) and in reports by Atanu et al. [32,12].

Our study is in agreement with the study by Okpe et al. (2016) where the administration of aqueous leaf extracts of *C. papaya* extracts and the synthesized Ag-NPs significantly cleared the infected RBC and reduced their percentage in the blood circulation of the mice which triggered an increase in PCV as a result of increased production of RBC, thus, suppressing hemolytic damage to RBC [33]. This effect of decreased PCV had shown to be statistical significance ($p<0.05$) (Table 1).

Findings from this study does not totally agree with that of Okpe et al. (2016) in which there was marked increase in body weight of the infected groups of mice and the noninfected group, because the change noticed in the body weight was negligible, and as such does not yet fully support the notion that *P. berghei* decreases body weight [33]. The increase in body weight observed in this study may be due to constant feeding of the animals during the experimental period and not accrued to the treatment choice of infected mice.

The results from this study shows that the administration of the Ag-NPs synthesized from *C. papaya* decreased parasite load in mice and would definitely aid their survival. As can be seen from the Fig 3, where the malaria parasite density (MPD) in infected mice decreased across the experimental periods following the administration of the Ag-NPs, this is opposed to the disease control (negative control in which the mice are infected but not treated) which is characterized by excessive parasite growth, exaggerated immune reactions, or a combination of both causing severe pathology and death, which is detrimental to both parasite and host. This association of MPD among the infected mice was statistically significant ($p<0.05$). Although this decrease can also be seen when ordinarily the plant extract (*C. papaya*) was administered but the clearance of malaria parasitemia on administration of the Ag-NPs was found to be higher. Thus, the findings of Saganuwan et al. (2014) that many Nigerian plants can be used for the treatment of malaria and that infection with *P. berghei* is almost fatal with death occurring within 1–3 weeks are corroborated [34].

The level of parasitemia decreases when observed on days seven, nine, and eleven of treatment in the treated groups in comparison with the untreated group in mice infected with *P. berghei* treated with crude extracts of *C. papaya*, bio-synthesized Ag-NPs, Chloroquine and Artemeter lumefantrine displayed many normal RBC and few parasitized erythrocytes (Fig 1). As artemether lumefantrine and chloroquine are known to have antimalarial activity, our study also confirms this in that there was a decrease in the parasitemia on their administration to infected mice. On the contrary, observations by Georgewill et al. (2020) had correlation with improved antimalarial activity when Ketotifen was co-administered with CQ [35].

Histological examination of the liver cells (Fig 4) in the treated groups showed the inability of the extract to clear the entire parasite inclusion from circulation and repair the hepatocyte, which agrees with the work of Stenad et al. (2013), who stated that hepatic inclusions are defined as intracellular aggregates of stainable substances which represent established hallmarks of their respective human disorder [36]. Signs of regeneration/repairs of tissues using medicinal plants have been reported in other works by

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Shittu et al. (2011) and Okpe et al. (2014), which are consistent with the present study [37,38]. Thus, tissue repairs or therapy may offer therapeutic benefit in disease conditions.

Malaria is known to cause kidney damage especially is severe cases. In fact, it is the first parasitic infection to be clearly associated with glomerular diseases in the tropical region [39]. Histological examination of the kidney cells (Fig 4) in the treated groups shows that the bio-fabricated silver nanoparticles of *C. papaya* extract show no distortion to the histo-architecture of the kidney tissue despite the high level of malaria clearance and giving it edge over chloroquine and artemether lumefantrine. Knowing fully that injuries inflicted on the kidney tissues are irreversible [40]. The findings are in keeping with Nikkon et al. (2009) [41], but contradict the study of Woodard et al. (2007) [42].

This study shows that low dose of Bio-synthesized Ag-NPs of *C. papaya* leave extract is effective against *P. berghei* infected mice with additional advantage of no toxicity compared to synthetic drug. This process of synthesizing Ag-NPs is simple and cost effective, thereby it's a promising antimalarial alternative.

We recommend that further studies should be made on implementing nanomedicine into our health care system.

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Table 1: Experimental Design and Treatment

Group	Treatment	No of Animal
1	Infected mice + 0.2ml of 100mg/Kg (Low dose) of *C. papaya* crude extract only	5
2	Infected mice + 0.2ml of 200mg/Kg (High dose) of *C. papaya* crude extract only	5
3	Infected mice + 0.2ml of 25mg/Kg (Low dose) of Synthesized Ag-NPs of *C. papaya* extract.	5
4	Infected mice + 0.2ml of 50mg/Kg (High dose) of Synthesized Ag-NPs of *C. papaya* extract.	5
5	Infected mice + 0.2ml of 10mg/Kg of chloroquine (Positive control 1)	5
6	Infected mice + 0.2ml of 0.16mg/Kg of Artemether	5

	lumefantrine (Positive control 2)	
7	Infected mice + distilled H_2O (Negative control)	5
8	Uninfected mice + distilled H_2O (Positive control 3)	5

Table 2: The Packed Cell Volume of mice infected with *P. berghei* and treated with aqueous leaf extracts of *C. papaya*.

Parameters	Groups	Pre-infection	Day 7	Day 9	Day 11
PCV (%)	Group 1	47.6±2.60	42.6±7.80	23.6±2.19	20.6±3.80
	Group 2	47.2±2.95	43.4±4.04	26.75±5.32	23±2.83
	Group 3	48±3.54	41.4±15.96	31.5±14.85	24.3±5.69
	Group 4	45.6±1.82	44.6±13.90	24.2±13.33	21.25±6.24
	Group 5	44.8±3.77	44.75±8.88	29.4±8.26	25.25±7.59
	Group 6	45.4±2.41	48.8±3.19	30.8±2.28	31.2±3.90
	Group 7	47.4±3.36	46.2±6.83	28±7.94	22±8.49
	Group 8	46.6±1.82	46.6±1.82	46.6±1.82	46.6±1.82
	p-value	0.556	0.929	0.002	0.000

Table 3: The mean body weight of mice infected with *P. berghei* and treated with aqueous leaf extracts of C. *papaya*.

Parameters	Groups	Pre-infection	Day 7	Day 9	Day 11
Body Weight	Group 1	18.2±2.17	18.6±1.82	17.4±1.82	15.8±1.48
	Group 2	17.8±1.92	17.4±3.65	16.5±2.52	16±0.0
	Group 3	20.4±3.78	20±2.74	20±2.65	17±2.0
	Group 4	18±1.0	18.5±1.29	18.2±1.64	17±3.37
	Group 5	16.8±2.95	18.6±3.44	18.8±2.78	17.25±3.59
	Group 6	17.6±1.14	19.4±0.89	20±1.58	20.2±1.92
	Group 7	20.8±2.05	22±2.55	20±4.36	19.5±7.78
	Group 8	17.8±1.10	17.8±1.10	17.8±1.10	17.8±1.10
	p-value	.082	.128	.256	.336

UNDER PEER REVIEW

Table 4: The malaria parasite density of mice infected with *P. berghei* and treated with aqueous leaf extracts of *C. papaya*.

Parameters	Groups	Pre-infection	Day 7	Day 9	Day 11
Malaria Parasite Density	Group 1		308250±97068.275	147333.33±5033.22	147500±4769.70
	Group 2		308250±97068.28	142000±5291.50	140000±2000
	Group 3		240920±64735.09	43566.67±10480.62	9000±1414.21
	Group 4		196000±73430.24	68250±9456.04	38100±7275.30
	Group 5		108400±53085.59	3812.5±1463.087	0±0.0
	Group 6		162500±21683.33	3812.5±1463.09	0±0
	Group 7		155000±31358.15	211750±8838.84	
	p-value		.004	0.000	0.000

UNDER PEER REVIEW

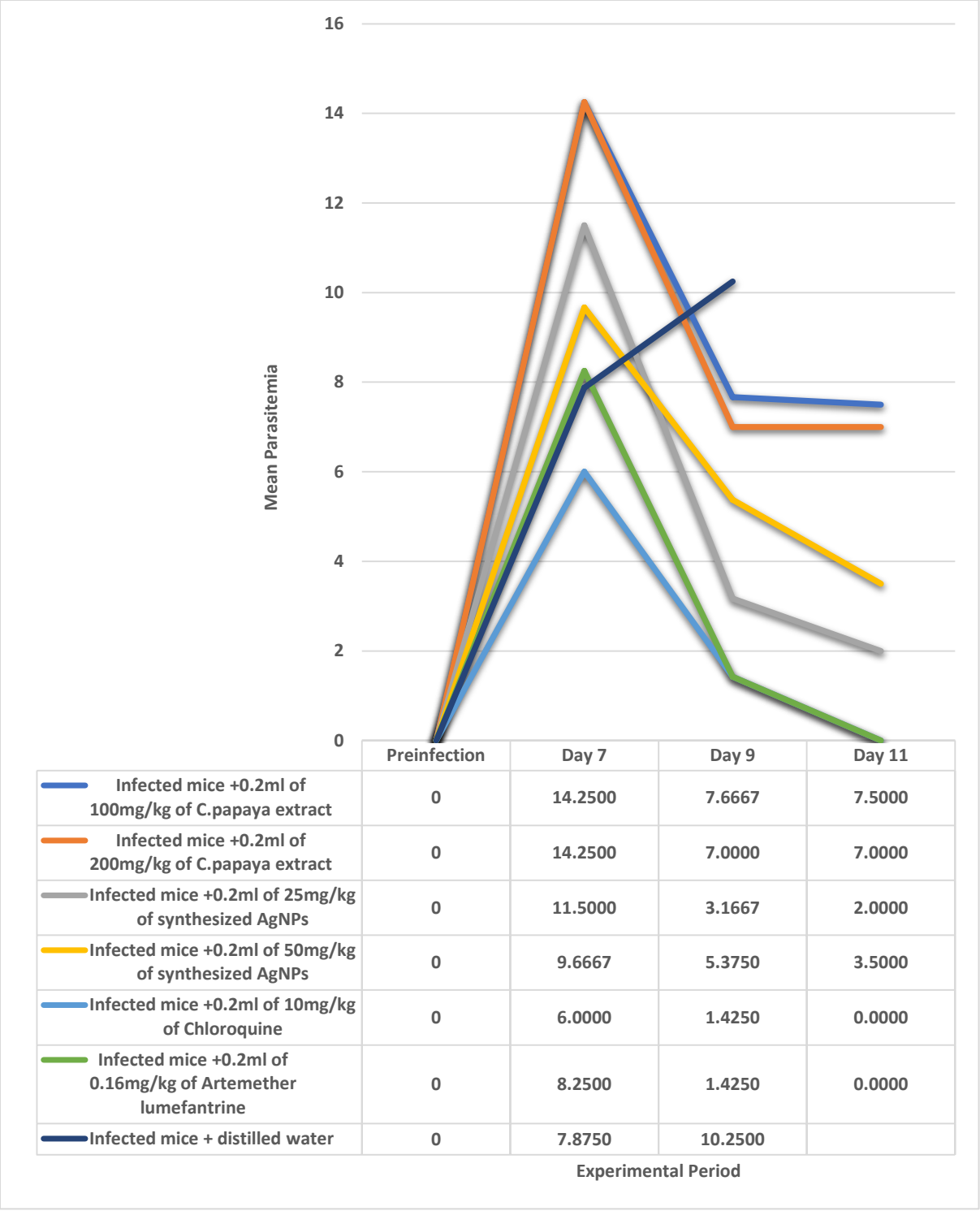

Fig 1: The mean of parasitized erythrocytes in mice infected with *P. berghei* and treated with aqueous leaf extracts of *C. papaya,* bio-synthesized Ag-NPs, Chloroquine and Artemether lumefantrine.

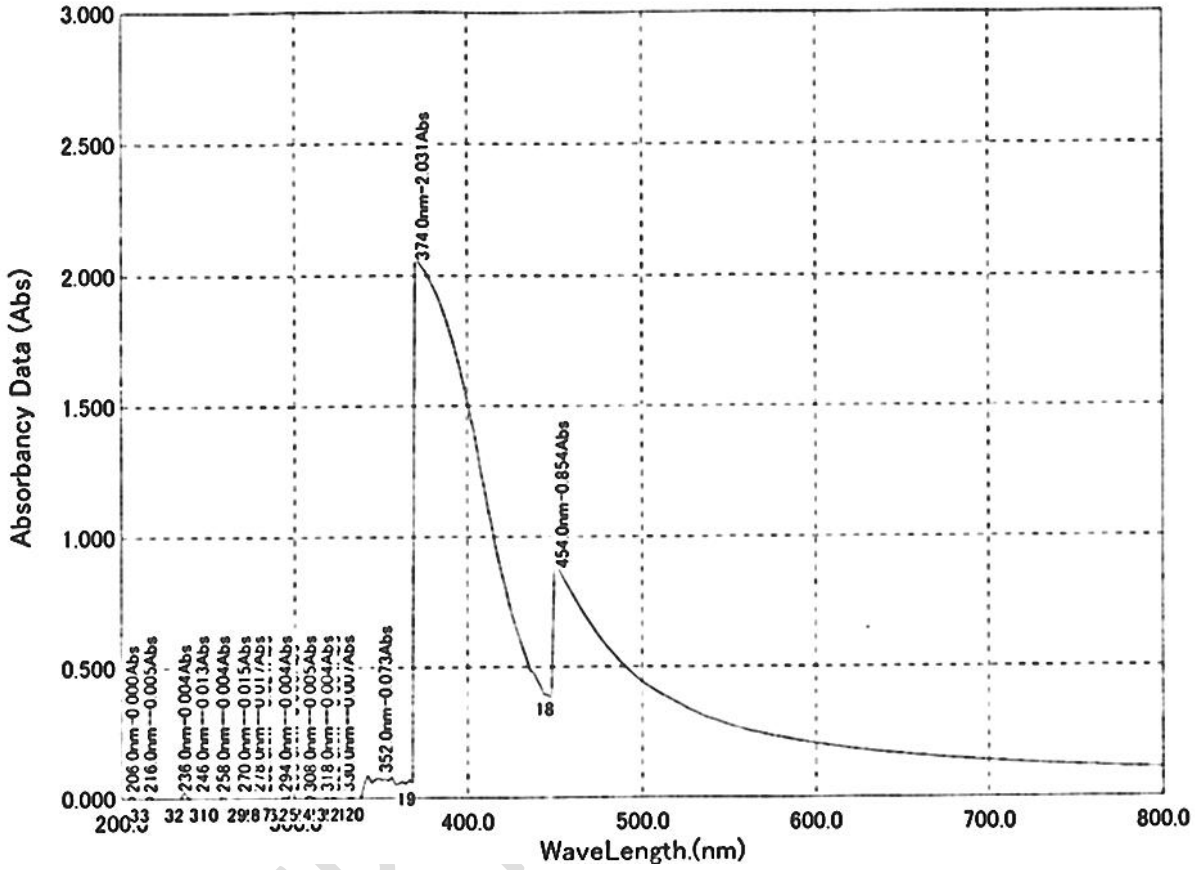

Fig 2: UV-Visible Spectrophotometry of Bio-Fabricated Ag-NP(s) of *C. papaya*

UNDER

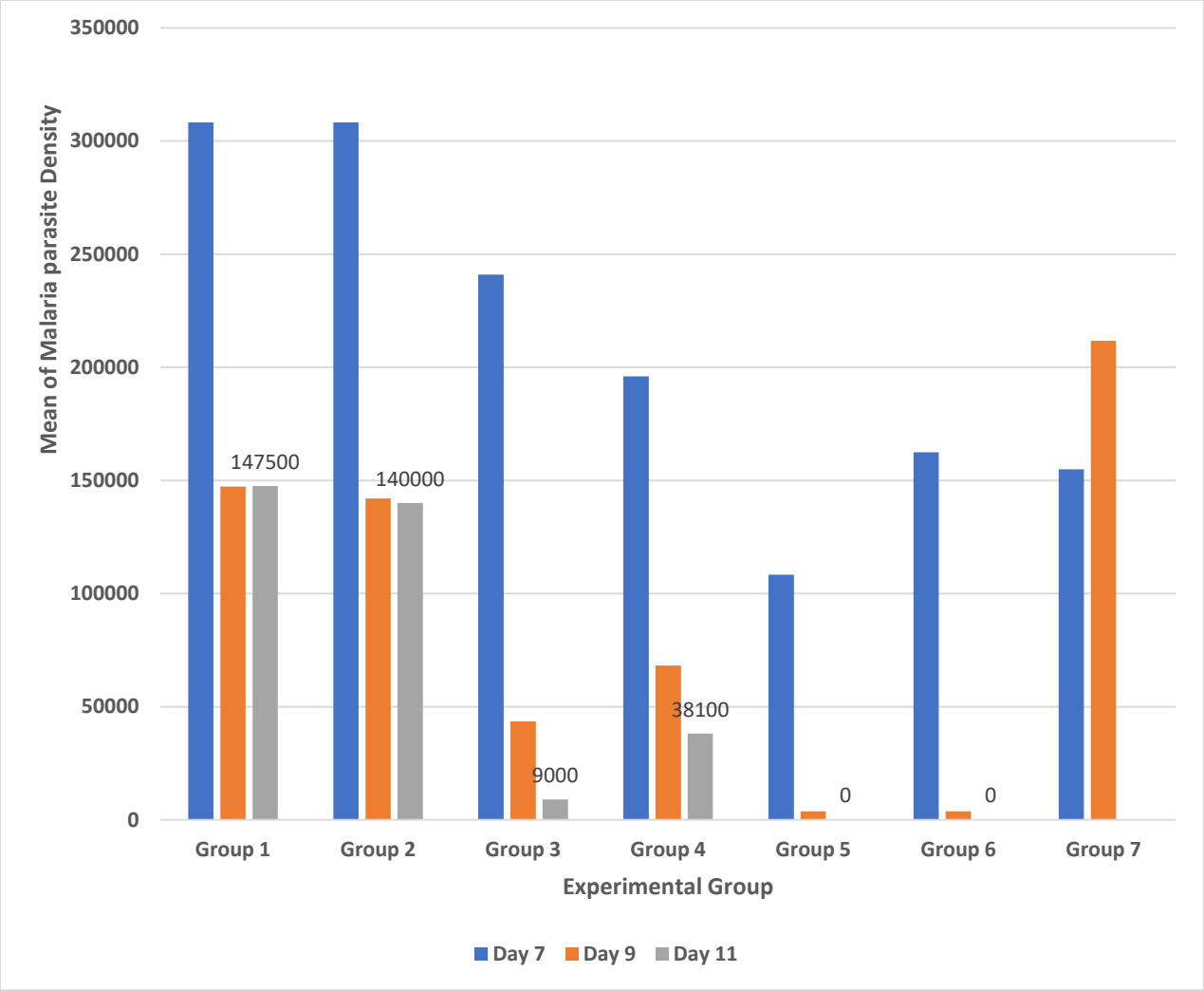

Fig 3: Comparison of Malaria parasite density across experimental groups

UNDER

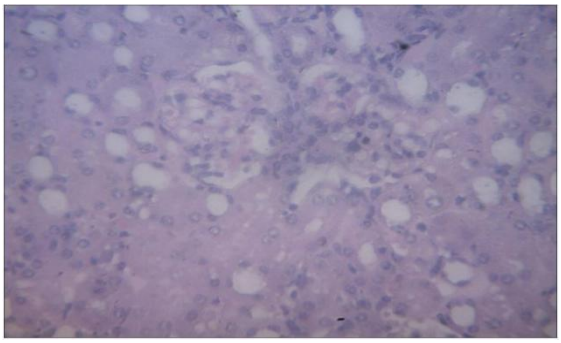

Fig 4.1(a) 100mg *C. papaya* crude extract (Kidney).
No visible lesion seen.

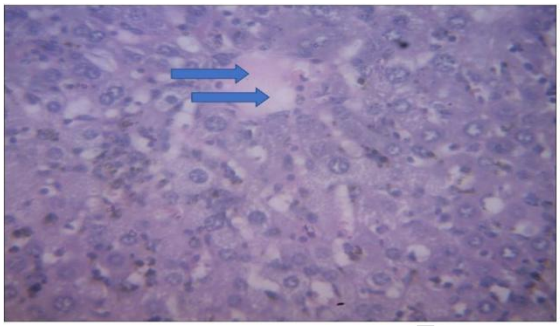

Fig 4.1(b) 100mg *C. papaya* crude extract (Liver).
The sinusoids appear congested (arrows).

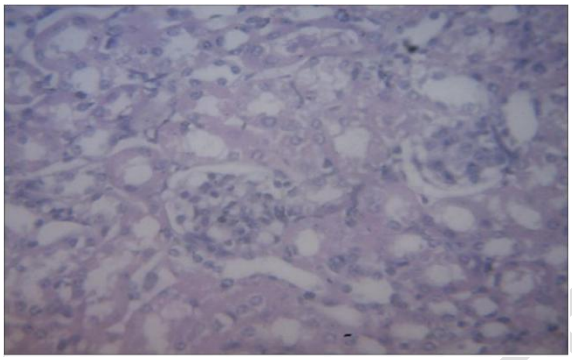

Fig 4.2(a) 200mg *C. papaya* crude extract (Kidney).
No visible lesion seen.

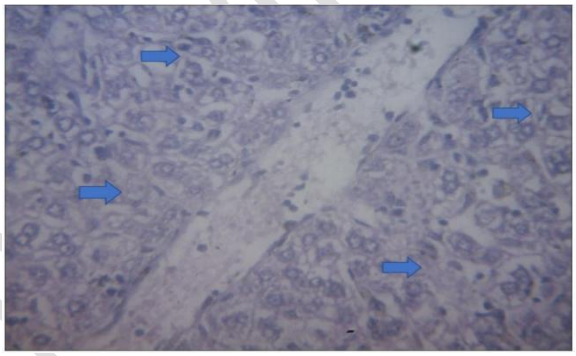

Fig 4.2(b) 200mg *C. papaya* crude extract (Liver).
Severe diffuse vacuolar degeneration and necrosis of hepatocytes (arrows).

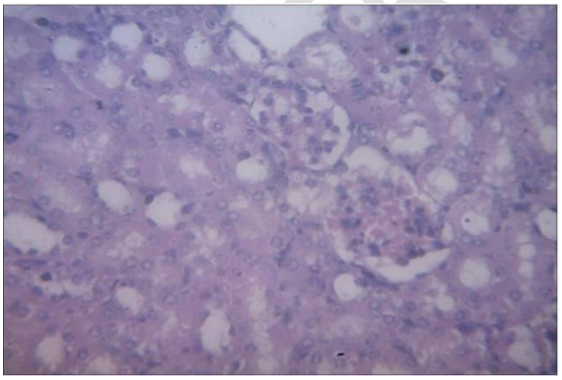

Fig 4.3(a) 25mg Ag-NPs(s) *C. papaya* extract (Kidney).

No visible lesion seen.

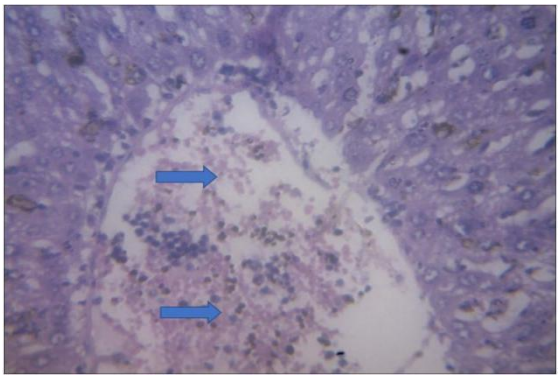

Fig 4.3(b) 25mg Ag-NPs(s) *C. papaya* extract (Liver).

Severe central venous congestion (arrows).

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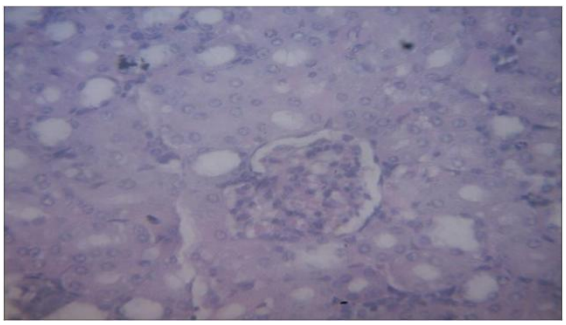

Fig 4.4(a) 50mg Ag-NPs(s) *C. papaya* extract (Kidney).

No visible lesion seen.

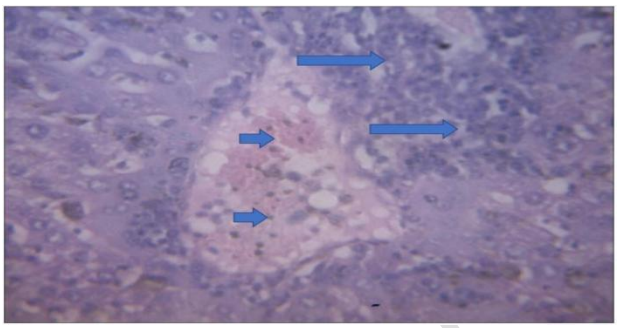

Fig 4.4(b) 50mg Ag-NPs(s) *C.papaya* extract (Liver).

There is a mild portal congestion (short arrows), with a moderate periportal cellular infiltration (long arrows)

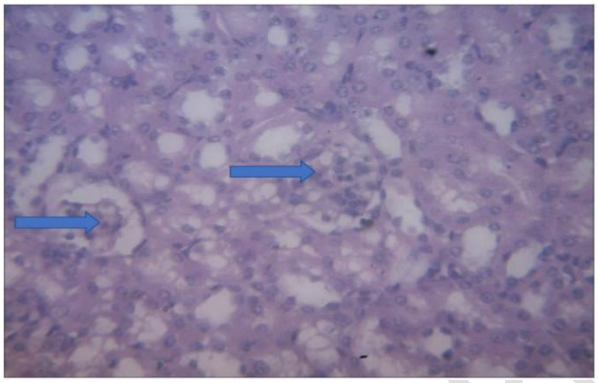

Fig 4.5(a) C.Q (Positive Control 1) (Kidney).

Moderate glomerular degeneration and necrosis(arrows).

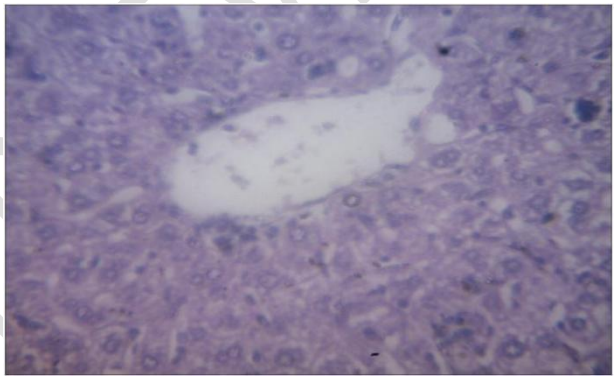

Fig 4.5(b) C.Q (Positive Control 1) (Liver).

No visible lesions seen.

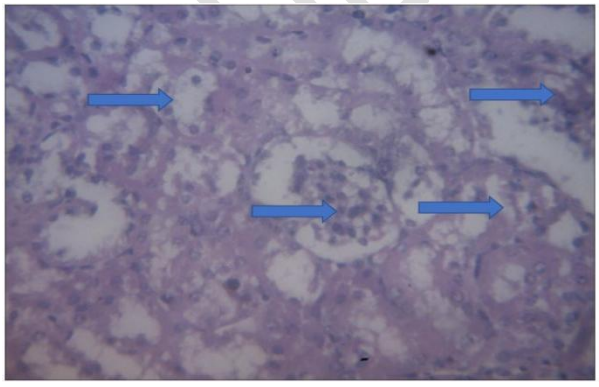

Fig 4.6(a) AL (Positive Control 2) (Kidney).
Moderate to severe glomerular and tubular degeneration and necrosis (arrows).

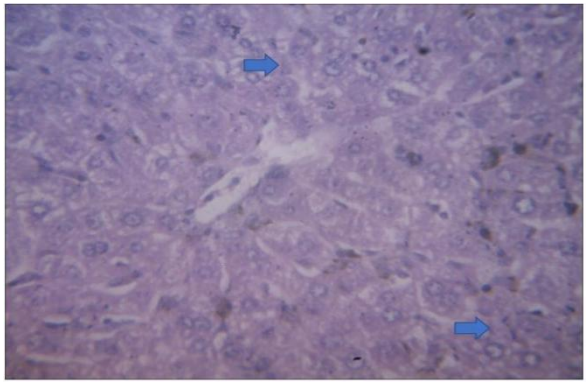

Fig 4.6(b) AL (Positive Control 2) (Liver).
Very mild hydropic degeneration of hepatocytes (arrows)

UNDER PEER REVIEW

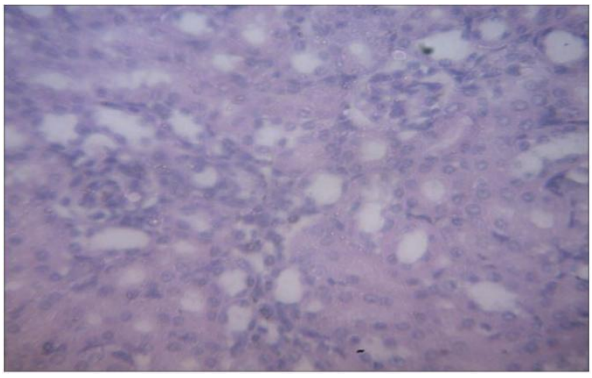

Fig 4.7(a) Distilled H_2O (Negative Control) (Kidney).

No visible lesions seen.

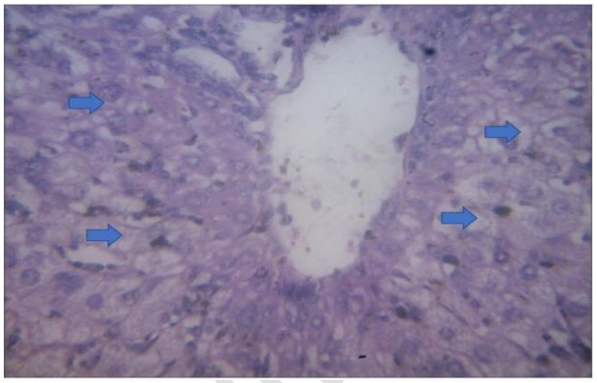

Fig 4.7(b) Distilled H_2O (Negative Control) (Liver).

Mild diffuse vacuolar degeneration of hepatocytes (arrows)

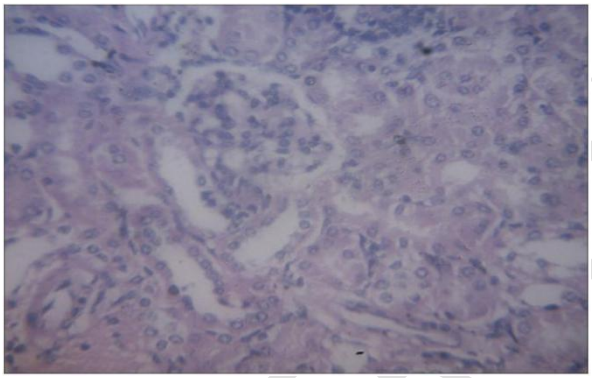

Fig 4.8(a) Uninfected (Positive Control 3) (Kidney).

No visible lesions seen.

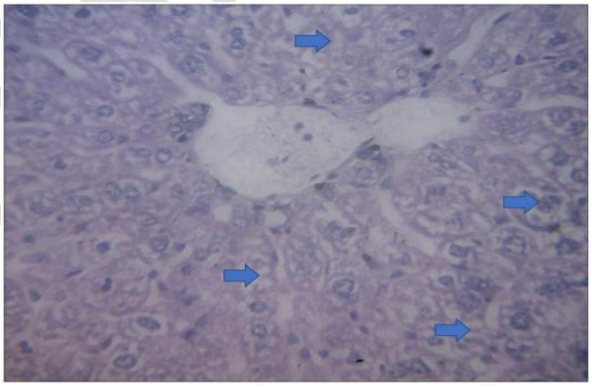

Fig 4.8(b) Uninfected (Positive Control 3) (Liver).

Diffuse vacuolation of the hepatocytes (arrows), the nuclei are centralised, likelihood a nutritional issue.

Fig 4: Micrograph of H&E-Stained Sections of Kidney (a) and Liver (b)

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