

## The Effects of Co-Administration of *Azadirachta indica* and *Gongronema latifolium* on the Liver of *Plasmodium beighei* Infected Swiss Albino Mice

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### Abstract

The effects of co-administration of *Azadirachta indica* and *Gongronema latifolium* on the liver of *Plasmodium beighei* infected Swiss albino mice was evaluated. Thirty mice divided into 6 groups of 5 animals each were used for this study. Healthy control group was not infected with. Other group was infected by intraperitoneal injection of *P. beighei*. Once parasitaemia was confirmed, treatment groups were assigned; Group A received distilled water at 10ml/kg body weight. Group B was not given any extracts. Groups C was given *G. latifolium* extract at 500mg/kg body weight. Groups D was given *A. indica* extract at 500 mg/kg body weight. Group E received both extracts at 500 mg/kg each. Group F received Artemether at 1.6mg/kg body weight intraperitoneally. The extracts were administered orally for 5 days. The animals were sacrificed after blood was obtained for serum liver enzymes estimation. The liver were processed for histological study using H and E. Histology of the liver showed sinusoidal congestion and hepatocyte necrosis in the diseased control and steatosis, loss of normal sinusoidal architecture, necrosis of hepatocytes and portal tract inflammation in the *A. indica* only group. The groups administered *G. latifolium*, both singly and in combination with *A. indica* had normal liver histology. The liver enzyme ALT was significantly ( $p < 0.05$ ) raised in *A. indica* treated group while it was normal in the *G. latifolium* groups. It will thus appear that *G. latifolium* ameliorated the hepatotoxicity of *A. indica* in *Plasmodium beighei* infected mice.

**Keywords:** Co-Administration; *Azadirachta indica*; *Gongronema latifolium*; *Plasmodium beighei*; Swiss albino mice.



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### 1. Introduction

In Nigerian indigenous communities, plant based treatment of malaria have been the main stay of treatment from time immemorial and continue to be so even today. The World Health Organization estimates that in Nigeria, 80% of the population use herbal remedies as first line of treatment for any illness and specifically 60% of children with fever due to malaria receive herbal drugs as first line care [1]. Two of such plants commonly used in Nigeria include neem plant (*Azadirachta indica*) popularly known as Dongoyaro and *Gongronema latifolium* (Utazi).

*Azadirachta indica* is a tree in the Mahogany family Melicaceae [2]. It is typically grown in tropical and semi-tropical regions and thrive in arid/ savanna regions in Nigeria. Neem and neem products have been used in ethnomedicine as an antihelminth, antifungal, antidiabetic, antibacterial, antiviral, contraception and as an antimalarial. The antimalarial effect of neem has been documented and is thought to act by redox perturbation in the form of imposition of substantial oxidant stress during malaria infection [3-7].

*Gongronema latifolium* (Utazi) is a climbing plant of the family Asclepiadaceae. It is widely used in Africa for nutritional and medicinal purposes. It is used to treat cough, intestinal worms, dysentery, dyspepsia and malaria [8].

The liver is an important organ in the pathophysiology of malaria infection. It serves an important part of the life cycle of the parasite, being the organ where asexual reproduction (schizogony) of the mosquito injected sporozoites takes place. In addition, liver damage secondary to parasitaemia or the immune response to the parasite account wholly or partly for a number of clinical features associated with severe malaria, including hypoglycemia, hyperparasitaemia, jaundice and bleeding diathesis [1, 9, 10]. Migration damage to hepatocytes, clustering of necrotic hepatocytes adjacent to structurally intact, sporozoite-infected hepatocytes, increased numbers of nonparenchymal cells lining the sinusoids, large numbers of proliferating nonparenchymal cells and hepatocytes, focal deposition of collagen in some spaces of Disse and focal increase in the concentration of smooth muscle actin has been reported in liver sections with elevated serum alanine aminotransferase (ALT) activity [11]. Significant increase of the ALT serum levels however is dependent on the number of injected sporozoites [11]. Elimination of infected parasites is likely mediated by direct recognition of infected hepatocytes by antigen-specific CD8<sup>+</sup> T cells [12].

*Gongronema latifolium* and *Azadirachta indica* herbal mixtures are used to treat malaria in Local Nigerian communities but no record exist on studies of the effects of combination of the two herbal extracts on liver histology and biochemistry hence the need for this study.

## 2. Materials and Methods

### 2.1. Experimental Animals

A total of thirty (30) Swiss albino mice of body weight 20-30g were purchased from the Animal House of Faculty of Basic Medical Sciences, University of Uyo for this study. They were acclimatized for two weeks and fed standard laboratory feeds and clean water. The animals were housed in clean, well ventilated wooden cages and exposed to 12 hours light and dark cycles at room temperature. All the experimental animals were handled according to the guidelines on the use of laboratory animals of the National Institute of Health (NIH) of the United States of America [13].

### 2.2. Procurement of *Azadarichta Indica* and *Gongronema Latifolium* Leaves

Mature leaves of *A. indica* and *G. latifolium* were collected from the medicinal farm of Faculty of Pharmacy, University of Uyo. The plants were identified and authenticated by the Principal curator, Mrs Emanuella G. Udoma at the Pharmacognosy herbarium of the Faculty of Pharmacy, University of Uyo and specimen voucher numbers UUPH 9(a) and UUPH 49(a) were issued for *G. latifolium* and *A. indica* samples, respectively. Ethical approval was obtained from Postgraduate Ethics committee number 14/PG/BMS/AN/003. The leaves were washed clear of any impurity and air dried for two weeks under a shade.

### 2.3. Preparation of *A. Indica* and *G. Latifolium* Leaf Extracts

The dried leaves of both plants were separately pulverized into powder using a kitchen blender. They were preserved in separate containers with plastic coverings at room temperature. Seven hundred gram (700g) of each powdered leaf were mixed with 2.5L of absolute ethanol for 72hrs at room temperature. The extracts were filtered with cheesecloth and later with filter paper and allowed to stand for 30 minutes, after which it was evaporated to dryness using water bath at 40°C. The extracts were then kept in a closed container and refrigerated at 2-8 degree Celsius until use.

### 2.4. Infection of Mice with *P. Beighei* and Administration of Leaf Extracts

The 30 mice were divided into 6 groups of 5 animals each. The mice were infected with  $10 \times 10^6$  *P. berghei* parasitized erythrocytes intraperitoneally, exception of the healthy control group. The course of the infection was followed by a daily determination of parasitaemia through a thin blood smear prepared from tail blood and stained with 3% Giemsa stain. Once parasitaemia has reached 5% of the initial inoculation, treatment groups were assigned. Group A was the healthy control and was administered with distilled water at 10ml/kg body weight. Group B was not administered with any extracts. Group C was administered with *G. latifolium* extract at 500mg/kg body weight. Group D was administered with *A. Indica* extract at 500mg/kg body weight. Group E was administered with both extracts at 500mg/kg each. Group F was administered with the standard drug Artemether at 1.6mg/kg body weight intraperitoneally, dissolved in olive oil [7]. All extracts treatment was given orally and daily for 5 days.

### 2.5. Termination of the Experiment

The animals were anaesthetized using 50 mg/kg body weight of ketamine hydrochloride (Rotex Medica, Germany) intraperitoneally. The thoraco-abdominal wall was dissected to assess the heart, and blood was aspirated from the left ventricle of the heart. The blood obtained was centrifuged at 3000xg for 10 minutes. The serum obtained was processed as a single batch for determination of AST and ALT levels within 12 hours using Biosystem A25 Random Access Analyzer. Intra-cardiac perfusion of phosphate-buffered saline (PBS, 2M, pH 7.0), was carried out on the animals by means of a cannula and then perfused-fixed using 10 % buffered formalin. On complete perfusion, the liver of all the animals were removed and fixed in 10 % buffered formalin for 48 hours.

### 2.6. Tissue Processing and Data Analysis

The fixed liver was processed and stained with hematoxylin and eosin. One-way analysis of variance (ANOVA) was carried out, and post-hoc Tukey honestly significant difference (HSD) test was used to determine the significance. Data was expressed as Mean  $\pm$  Standard Error of Mean (S.E.M). Values were considered to be statistically significant at  $p < 0.05$

## 3. Results

### 3.1. Effect on Liver Enzymes

There was no significant difference in the serum levels of AST between the controls and the groups receiving only *A. indica*. There was a statistically significant ( $p < 0.05$ ) elevation of ALT in the only *A. indica* groups. There was also observed a significant reduction in both liver enzymes in the group treated with *G. latifolium* and in the combination therapy groups. There was a significant reduction in serum AST levels of mice treated with Artemether (Table 1).

**Table-1.** Effect of *Gongronema latifolium* and *Azadirachta indica* on serum levels of AST and ALT

Groups*	AST( IU/L)	ALT(IU/L)
A	180 ±9.5	41 ±11.0
B	180 ±27.0 <sup>NS</sup>	51 ±10.0 <sup>NS</sup>
C	130 ±7.6 <sup>#,b</sup>	27 ±5.5 <sup>#,b</sup>
D	190 ±7.5 <sup>NS</sup>	134 ±38.0 <sup>#,b</sup>
E	110 ±24.0 <sup>#,b</sup>	28 ±17.0 <sup>#,b</sup>
F	120 ±7.7 <sup>#,b</sup>	36 ±9.3 <sup>NS</sup>

Data are presented as Mean ± Standard deviation of Mean

<sup>#</sup> - Significantly different from the Group A at p<0.05

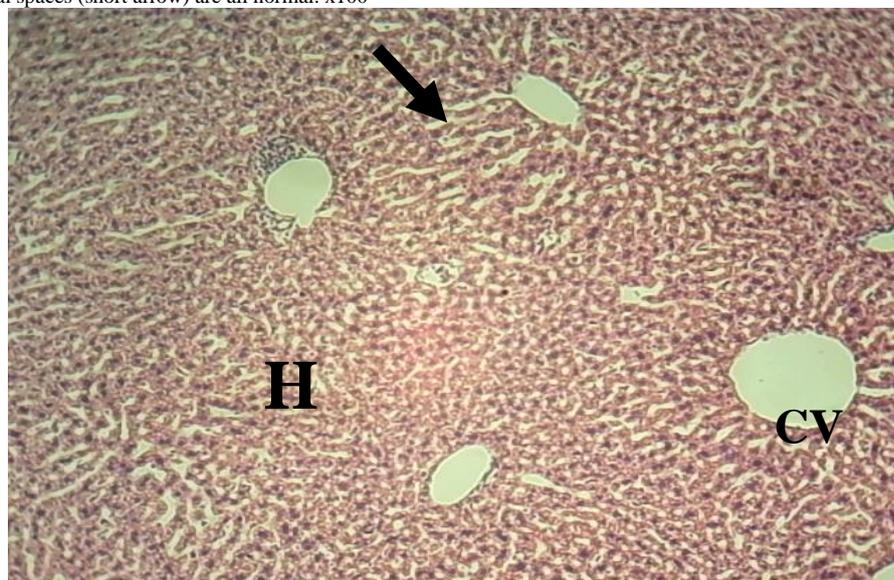
<sup>b</sup> - Significantly different from the Group B at p<0.05

<sup>NS</sup> - Not significantly different from the Group A and B at p<0.05

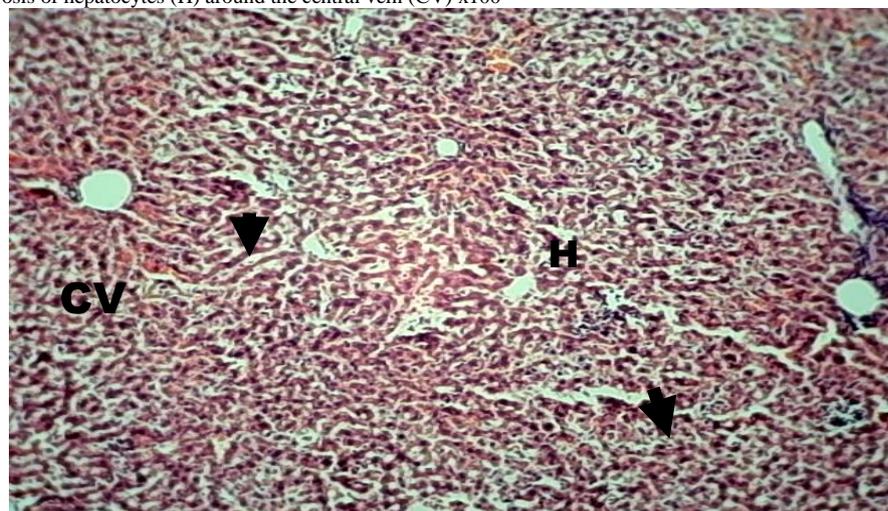
\***Group A:** Distilled water 10ml/kg; **Group B:** *P. beighei* only; **Group C:** *G. latifolium* 500mg/kg; **Group D:** *A. indica* 500mg/kg **Group E:** *G. latifolium* + *A. indica* 500mg/kg; **Group F:** Artemeter 1.6mg/kg

The section of the Liver of the normal control group showed a normal liver cytoarchitecture. Hepatocytes were normal with uniform nuclei and granular cytoplasm. The central veins and sinusoidal spaces all appeared normal. The liver of the *P. beighei* only group showed sinusoidal dilation and congestion with necrosis of hepatocytes around the central vein. In the group administered 500mg *A. indica*, there was Steatosis, loss of normal sinusoidal architecture, necrosis around the central veins and portal tract inflammation. The groups receiving *G. latifolium*, both singly and in combination with *A. indica* showed no adverse changes in the liver histology. There was dilation and congestion of the central veins in the artemether group, but hepatocytes appeared not to be affected.

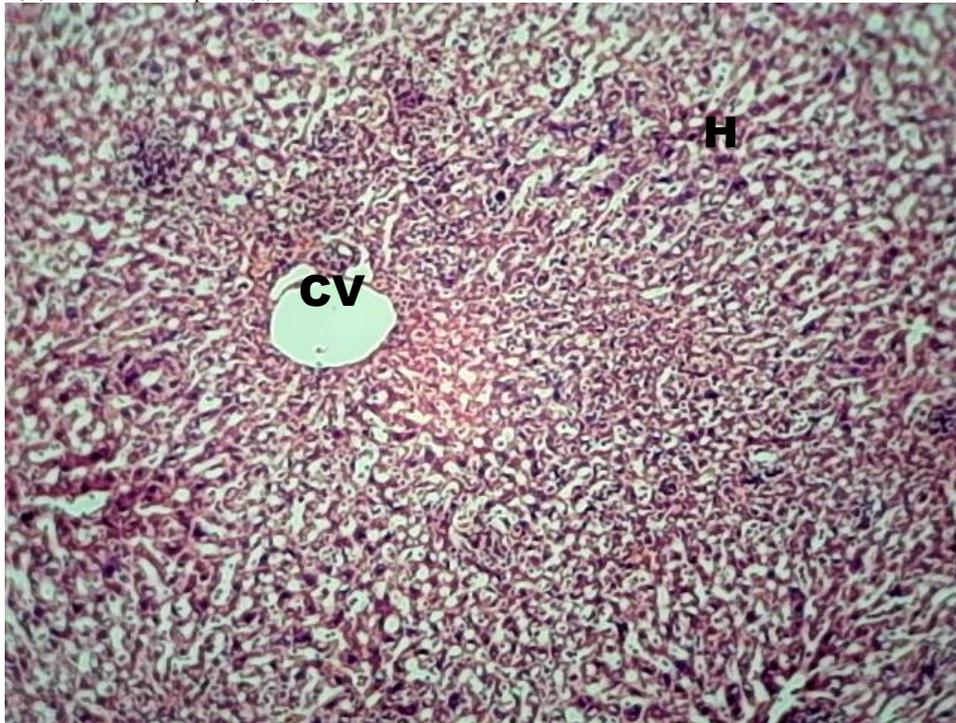
**Fig-1.** Section of the liver of group A administered 10ml/kg distilled water showing normal liver architecture; the central vein (V), hepatocytes plates (H), sinusoidal spaces (short arrow) are all normal. x100



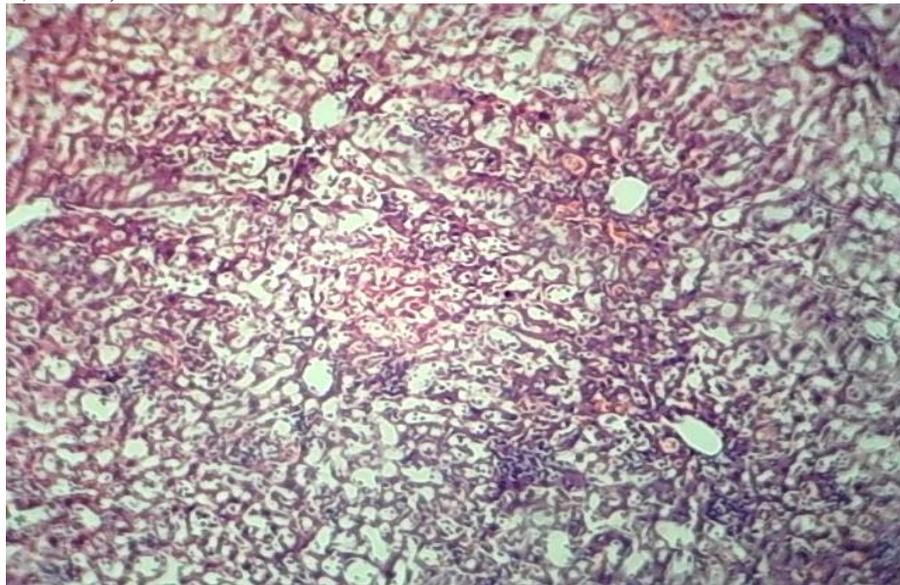
**Fig-2.** Section of the liver of group B animals infected with *Plasmodium beighei* parasites showing sinusoidal (arrows) dilatation and congestion, and necrosis of hepatocytes (H) around the central vein (CV) x100



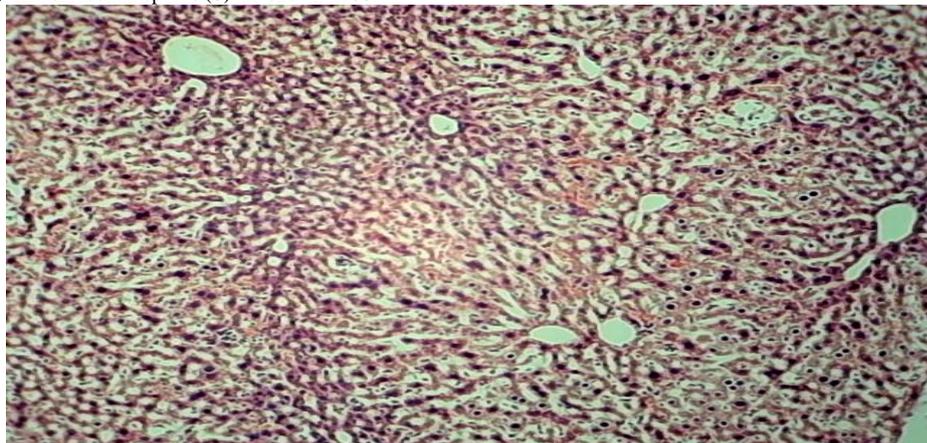
**Fig-3.** Section of the liver of group C animals given 500mg/kg of *G. latifolium* showing normal liver architecture; the central vein (CV), hepatocytes plates (H) and sinusoidal spaces (S) are shown x100



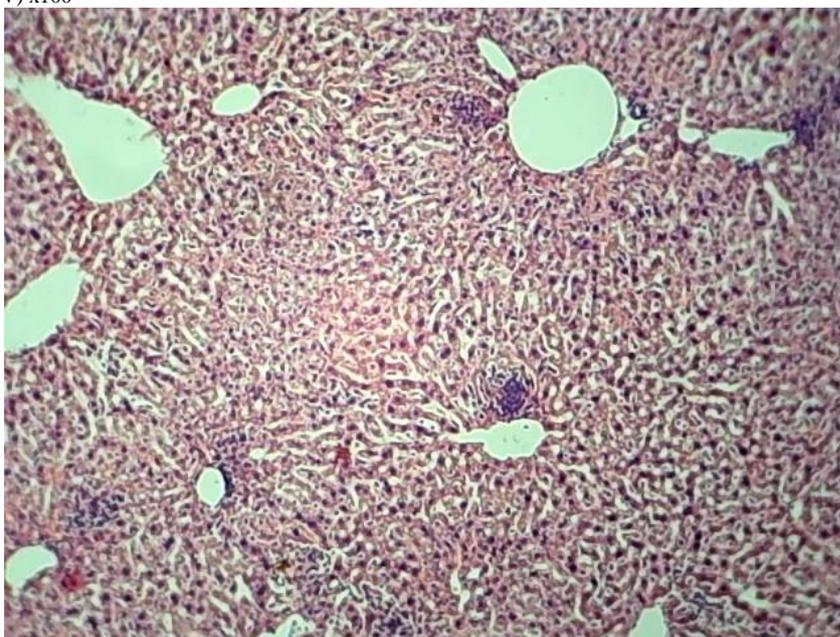
**Fig-4.** Section of the liver of group D animals given 500mg/kg of *A. indica* showing loss of normal sinusoidal architecture of the liver, steatosis (fatty change), necrosis of hepatocytes around the central veins and inflammation of the portal tract. The central vein (CV), hepatocytes plates (H) are swollen, (E1 x100, E2 x 400)



**Fig-5.** Section of the liver of group E animals given 500mg/kg of both *A. indica* and *G. latifolium* normal liver architecture; the central vein (CV), hepatocytes plates (H) and sinusoidal spaces (S) are shown x100



**Fig-6.** Section of the liver of group F animals given 1.6mg/kg of Artemether showing a normal Hepatocytic plates (H) with dilated and congested central veins (CV) x100



#### 4. Discussion

*Azadirachta indica* and *Gongronema latifolium* are two plants regularly combined as herbal mixtures for the treatment of malaria in local Nigerian communities. This study evaluated the effects of co-administering extracts of both plants on the liver enzymes AST and ALT as well of the histology of the liver of *Plasmodium beighei* infected mice.

The liver enzymes AST and ALT are usually raised in cases of inflammation or damage to hepatocytes. When compared to the healthy control, it was observed that animals receiving only the parasite had similar AST values, but with a statistically insignificant elevation in ALT. The histology of the liver of animals treated with *P. beighei* showed sinusoidal congestion, evidence of inflammatory changes and necrosis of hepatocytes. Thus it can be seen that the parasite has a deleterious effect on the liver. Similar findings showing alterations in liver morphology and liver enzymes by the parasite have been documented [11, 14, 15]. The group treated with 500mg/kg of *A. indica* extract had elevated AST and a markedly and statistically significant elevation of ALT. Severe deleterious effect on liver histology was also observed in this group, including fatty change, intraparenchymal hemorrhage in addition to inflammatory changes and loss of normal liver cytoarchitecture. The liver damage was more extensive than that observed in the diseased control, indicating that the extract at this dose may be toxic to the liver, or potentiate the toxicity induced by the parasite. This observation is in contrast to other works that have shown that neem leaf extracts are protective against several chemical induced hepatotoxicity and significantly reduce both AST and ALT [16-18]. All groups treated with *G. latifolium* (500mg/kg alone and in combination with 500mg/kg of *A. indica*) had a normal liver histology with significant reduction in the liver enzymes when compared with the controls and the neem only group. This indicates that *G. latifolium* may have ameliorated the liver damage induced by the parasite and *A. indica*.

#### 5. Conclusion

This study shows that both *Plasmodium beighei* and *A. indica* induces elevation of liver enzymes AST and ALT and produces a deleterious effect on the microstructure of the liver of Swiss albino mice and that co-administering *G. latifolium* reverses these changes, thus justifying their combination for ethnomedical use.

#### Reference

- [1] WHO, 2014. "Malaria fact sheet." Available: <http://www.Who.ir1t/mediacentre/factsheets/fs094/en/>
- [2] Girish, K., 2008. "Neem-a green treasure." *Electronic Journal of Biology*, vol. 4, pp. 102-111.
- [3] Iwu, M. M., Obidoa, O., and Anazodo, M., 1986. "Biochemical mechanism of the antimalarial activity of *Azadirachta indica* leaf extract." *Pharmacological Research Communications*, vol. 18, pp. 81-91.
- [4] Gianotti, R. L., Bomblies, A., Dafalla, M., Issa-Arzika, Duchemin, J. B., and Eltahir, E. A., 2008. "Efficacy of local neem extracts for sustainable malaria vector control in an African village." *Malaria Journal*, vol. 7, p. 138.
- [5] Anyaehie, U. B., 2009. "Medicinal properties of fractionated acetone/water neem (*Azadirachta indica*) leaf extract from Nigeria: A review." *Nigerian Journal of Physiological Sciences*, vol. 1, p. 241.
- [6] Dua, V. K., Pandey, A. C., Raghavendra, K., Gupta, A., Sharma, T., and Dash, A. P., 2009. "Laivicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes." *Malaria Journal*, vol. 8, p. 124.

- [7] Farahna, M., Bedri, S., Khalid, S., Idris, M., Pillai, C. R., and Khalil, E. A., 2010. "Anti- plasmodial effects of Azadirachta indica in experimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a marker." *North American Journal of Medical Sciences*, vol. 2, pp. 518-525.
- [8] Ogbonnaya, E. A., Uanseje, S. O., and Ojeaburu, S. I., 2014. "Effect of gongronema latifolium leaves ethanolic extract on paracetamol-induced hepatotoxicity in rats." *Journal of Physiology and Pharmaceutical Advances*, vol. 4, pp. 337-341.
- [9] White, N. J., Warrel, D. A., Chanthavanich, P., Looareesuwan, S., Warrel, M. J., Krishna, S., Williamson, D. H., and Turner, R. C., 1983. "Severe hypoglycemia and hyperinsulinemia in falciparum Malaria." *New England Journal of Medicine*, vol. 309, pp. 61-66.
- [10] Ogetti, G. N., Akech, S., Jemutai, J., Boga, M., Kiyaya, E., Fegan, G., and Maitland, K., 2010. "Hypoglycaemia in severe malaria, clinical associations and relationship to quinine dosage." *BMC Infectious Diseases*, vol. 10, p. 334.
- [11] Frevert, U., Engelmann, S., Zougbedé, S., Stange, J., Ng, B., Matuschewski, K., Liebes, L., and Yee, H., 2005. "Intravital observation of plasmodium berghei sporozoite infection of the liver." *PLOS Biology*, vol. 2, pp. 345-347.
- [12] Cockburn, I. A., Tse, S. W., and Zavala, F., 2014. "CD8+ T cells eliminate liver-stage plasmodium berghei parasites without detectable bystander effect." *Infection and Immunity*, vol. 82, pp. 1460-1464.
- [13] National Institute of Health, 2010. *Guide for the care and use of Laboratory animals*. 8th ed. Washington DC: National Academies Press.
- [14] Haque, A., Best, S. E., Amante, F. H., Ammerdorffer, A., de Labastida, F., Pereira, T., Rainm, G. A., and Engwerda, C. R., 2011. "High parasite burdens cause liver damage in micen following plasmodium berghei anka infection independently of CD8(+) T cell- mediated immune pathology." *Infection and Immunity*, vol. 79, pp. 1882-1888.
- [15] Adachi, K., Tsitsui, H., Kashiwamura, S., Seki, E., Nakano, H., and Takeuchi, O., 2001. "Plasmodium berghei infection in mice induces liver injury by an IL-12- and Toll-like receptor/myeloid differentiation factor 88- dependent mechanism." *The Journal of Immunology*, vol. 167, pp. 5928-5934.
- [16] Bhanwra, S., Singh, J., and Khosla, P., 2000. "Effect of azadirachta indica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats." *Indian Journal of Physiology and Pharmacology*, vol. 44, pp. 64-68.
- [17] Yanpallewar, S. U., Sen, S., Tapas, S., Kumar, M., Raju, S. S., and Acharya, S. B., 2003. "Effect of azadirachta indica on paracetamol-induced hepatic damage in albino rats." *International Journal of Phytotherapy and Phytopharmacology*, vol. 10, pp. 391-396.
- [18] Dkhil, M. A., Saleh, A., Ahmed, M. A., Mohamed, S. O., Kamal, M. E., and Ahmed, E. A., 2013. "The potential role of azadirachta indica treatment on cisplatin-induced hepatotoxicity and oxidative stress in female rats." *Oxidative Medicine and Cellular Longevity*, vol. 2013, pp. 741-817.