Original Research Article

SYNERGISM OF *AZADIRACHTA INDICA* AND *NIGELLA SATIVA* ON *PLASMODIUM FALCIPARUM*: STUDY ON WISTAR RATS

ABSTRACT

Azadirachta indica has a long-time history of antimalarial activity with equivalent to half the therapeutic dose of Chloroquine Sulphate on dry weight basis. Extracts from different parts of the plant have shown inhibition of *Plasmodium berghei* and *Plasmodium falciparum* in mice. *Nigella sativa* is one of the plants of therapeutic potential that have recently drawn great attention for extensive studies in natural medicine due to its less toxic properties and having little or no side effects as compared to synthetic drugs. It has been used used to treat various ailments including parasitic infections.

AIM; To Determine the Phytochemical contents of *A. indica* and *N. sativa* and their synergistic therapeutic effect on indices of clinical importance in malaria-induced male wistar rats.

METHODS; *A. indica* and *N. sativa* seeds were extracted with distilled water and the lethal doses (LD50) were determined on the rats. *Plasmodium* infected rats were divided into 3 groups of 6 rats each and a normal control group of 4 rats was left uninfected. Each plant extract and the mixture of both extracts were administered at 100, 200, and 300mg/kg body weight of the rats. After first 4 days, 7 days, and at 14 days of treatment each rat's blood sample was taken for analysis.

RESULTS; the plant extracts lethal dose (LD50) was considered safe for *A. indica* at 5000mg/kg and 2000mg/kg for *N. sativa* respectively. Qualitative phytochemical screening reavealed high concentration of saponins, alkaloids and steroids in *A. indica* extract and high concentration of resins, alkaloids and steroids in *N. sativa* extract. Treatment with a mixture of both extracts showed

an restoration red blood cell count and also an increase in the packed-cells volume (PCV) with no significant change in the hemoglobin concentration after 7 days.

CONCLUSION; The combination of both *A. indica* and *N.sativa* is well tolerated and safe for *Plasmodium* parasites effects on wister rats at concentration of 400mg/kg of body weight which showed highest values of restoration of RBC count as compared to the normal control group. Group A treated with only *N. sativa* also showed RBC count higher than that obtained in group B treated with only *A. indica*. PCV values compared with the normal control group showed an 8% increase at concentration of 400mg/kg at the end of the experiment in group C treated with the mixture of *A. indica* and *N. sativa.* At 200mg/kg concentration, the mixture of *A.indica* and *N. sativa* gave a 25% increase in PCV values. This shows that the synergistic effect of A. indica and *N. sativa* has better therapeutic effects against *Plasmodium* parasites than either *N. sativa* or *A. indica* as a single therapy. This study provides a basis for the development of a cheaper plant-based antimalarial combination therapy.

INTRODUCTION

Malaria is a protozoal parasitic infection that is transmitted by the female *Anopheline* mosquito (Osanaiye, et. al., 2013). It is caused by the parasite *Plasmodium,* four strains of which infect humans; *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax,* and *Plasmodium malariae*. It is one of the world's most important public health issues, causing millions of deaths annually, especially in the developing countries. In sub-Saharan Africa, 2/5 of the global 300 million deaths caused by malaria occurs, which leads to poor health, poverty and obstructs social and economic development (Sabina K., 2017). Malaria spread can be reduced using mosquito repellent, treated mosquito nets, clearing of bushes and stagnant water. Malaria has been treated using herbal drugs for thousands of years. The oldest and pioneer Antimalarial to be extracted from Cinchona tree is 'Quinine', after which 'Artemisinin' was later discovered. As at 2005, the Artemisinin Combination Therapy (ACT) was adopted in Nigeria. Over the last decade however, there has been increasing multi-drug resistance of the *Plasmodium* parasite (Yimer, et al., 2019).

The difficulty of creating efficient vaccines as well as the increasing multi-drug resistance of the *Plasmodium falciparum* strains has aggravated the problem of treatment failure. The high economic impact of the malaria infection is directed mostly towards high infant mortality, pregnant women and immunocompromised individuals. Adverse drug reactions posed by commercially available antimalarials also highlights the need for a novel and well-tolerated antimalarial drug (Modupe, 2017). Scientists all over the world have embarked on a journey to find an alternative antimalarial, testing different plants and plant parts for Antimalarial potency, some of which have been used as traditional remedy for many ailments. Amongst these plants are the Neem (*Azadirachta indica*) and Black Seeds (*Nigella sativa*) (Yimer et al., (2019).

Azadirachta indica (*A. indica*) commonly known as 'Neem' or 'Darbejiya' (Hausa) is an evergreen plant abundant in Africa, America and parts of India. It has a long time history of traditional remedy against many ailments, due to its antibacterial, antifungal, anti-inflammatory and anti-parasitic properties. It is also rich in therapeutics like nimbin, nimbidin, gedunin, salannin, etc, but the most active ingredient is azadirachtin (Kumawat, et. al., 2018). Different parts of the Neem plant have been tested for their antimalarial properties i.e. the leaves, seeds, stem bark, using aqueous, ethanolic and methanolic extracts. Several tests have proven the Neem extracts to be active against the chloroquines resistant malaria strains (Mehta, et. al., 2016).

Nigella sativa which is also known as 'Black Seeds' is a plant of ancient traditional medicine, since the time of Prophet Muhammad (PBUH), who described it as 'the cure for all diseases except death'. (Amalia, 2016). Its most active ingredient is thymoquinone and has antifungal, antiviral, antibacterial and anti-parasitic properties (Molla, et. al., 2019). Studies using its Ethanolic, aqueous and chloroform extracts have also demonstrated high percentages of parasitemia suppression (Oyweri, et. al., 2019). Since both plant extracts have proven to be effective against malaria infection, this research work focused on investigating the synergistic effect of a combination of these two (2) plant extracts against malaria parasite, the result of which will form a basis for further research study for a novel potent antimalarial.

LITERATURE REVIEW

In these trying times of increasing multi-drug resistant organisms, the ancient traditional healing methods are becoming more and more acceptable because people’s perception towards

traditional therapy has changed encouragingly. The World Health Organization (WHO) has estimated that about 80% of people living in the developing countries exclusively use traditional medicine for their health care, the use of which has been shown to be even safer as compared to modern allopathic medicines (Sharmin, et al., 2019).In many parts for Africa, particularly Nigeria, the use of herbal preparations as remedy for malaria is very common. Neem is one of such common therapies, and has proved to be effective against resistant *Plasmodium* strains. Due to high cost and unavailability of the commercial Antimalarial, this seems to be the only reliable form of treatment (Ucheya, et. al., 2011).

Azadirachta indica belongs to the Mahogany family, used to treat incurable diabetes, has antiviral, antibacterial, antifungal, antipyretic, antiseptic, anti-inflammatory and antiparasitic properties. It can also be used to treat pneumonia, bronchitis and other respiratory problems (Sharmin et. al., 2019). All parts of the Neem plant are commonly used as traditional remedy against many infections. It has many biologically active compounds i.e. alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Its most active ingredient is 'Azadirachtin', (a mixture of seven (7) isomeric compounds labeled Azadirachtin A-G, with Azadirachtin E being the most effective (Navjeet, et. al., 2019). The Neem seeds contain high amount of lipid and a lot of bitter principles (azadirachtin, azadiradione, fraxinellone, nimbin, salannin, salannol, vepinin, vilasinin, etc). Azadirachtin is effective against about 200 insect species including mosquitoes, and has proved to be nontoxic to humans (Ucheya, et. al., 2011). Neem kernel lipids include oleic aic (50-60%), palmitic acid (13-15%), stearic acid (14-19%), linoleic acid (8-16%), and arachidic acid (1-3%).This extract appears as a yellow non-drying oil, with an acrid taste and an unpleasant odour (Navjeet, et. al., 2019).

Antimalarial activity of Neem was reported by (Bakare et. al., 2015) to be equivalent to half the therapeutic dose of Chloroquine Sulphate on dry weight basis. Extracts from different parts of the plant showed inhibition of *Plasmodium berghei* and *Plasmodium falciparum* in mice (Bakare et. al., 2015). Another study, using the Neem leaf extract (ethanolic) in Wister rats infected with *Plasmodium berghei*, revealed the safety of *A. indica* for use as an antiplasmodial. The results showed increase in total cholesterol, HDL and LDL ($p<0.05$) in both groups treated with *A. indica* leaves extracts and Lumartem. Increase in body weight of rats treated with *A. indica* was dependent on the concentration of extract administered (Achi, et. al., 2018).

Fig. 1: ***Azadirachta IndicaSeed***

TAXONOMIC CLASSIFICATION OF AZADIRACHTA INDICA

Order	Rutale
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Meliae
Genus	*Azadirachta*
Species	*Indica*

Nigella sativa or Black Seeds is one of the plants of therapeutic potential that have recently drawn great attention for extensive studies in natural medicine due to its less toxic properties and having little or no side effects as compared to synthetic drugs (Elnour, et. al., 2019). It has been used to treat various ailments including parasitic infections (Abdulelah, et. al., 2007). Black Seeds comprises of 1.5% unstable oil and 37.5% fixed oil, nigellin, melanthin and Arabic acid, carvene, carvone, cymene, thymohydroquinone, and thymoquinone (which is the most active compound of its volatile oil, about 30-80%). It is also an immune system enhancer, and several scientific studies have confirmed the use of *N. sativa* oil and its extracts safe for use due to its minor/negligible toxological effects (Ashfaq, et. al., 2021). In a study by (Abdulelah, et. al., 2007), ethanol, chloroform and aqueous extracts of the Black Seeds were tested against *Plasmodium berghei* in

mice, administered both intraperitoneally and orally. Both treatments showed significant parasitemia suppression, with highest values observed in mice treated with ethanol extracts of 100 and 200µL/kg and Chloroform extracts of 100µL/kg which ($p<0.05$) reduced parasitemia and increased survival rate of the mice. In 2018, Ashcroft, et al., tested aqueous and methanolic extracts of *N. sativa* in *Plasmodium berghei* infected mice, which revealed parasitemia suppression significantly ($p<0.05$) in all groups treated with 25mg/kg, 50mg/kg and 100mg/kg. This result of antimalarial potency of *N. sativa* paves way for further investigation for a novel plant based antimalarial, as it indicated that higher doses of the extracts do not necessarily cause higher degree of parasitemia suppression.

Fig. 2: *Nigella Sativa Seed*

TAXONOMIC CLASSIFICATION OF NIGELLA SATIVA

Kingdom	Plantae
Subkingdom	Tracheobionata (vascular plant)
Supervision	Spermatophyte
Order	Ranunculales
Family	Ranunculaceae (buttercup family)
Genera	*Nigella*
Species	*Sativa*

Most antimalarials like Quinine and Artemisinin were all isolated from plants. Hence, investigating more traditional plants for anti-parasitic properties will serve as a basis for the development of a novel potent antimalarial. Usage of traditional medicine without a standard dosage has raised concerns about the possibility of toxicity. The lethal dose (LD50) of the Neem extract has been estimated to be up to 5000mg/kg, hence qualifying it as safe, non toxic and well-tolerated at the administered doses (Achi, et. al., 2018).

MATERIALS AND METHODS

Plant collection, identification and extraction

Neem seeds were collected from Neem trees in Abubakar Tafawa Balewa University, Bauchi, while Black Seeds of Egyptian origin were purchased from Al-Jazeera Bookshop, Anguwan Rogo, in Jos. Both seeds samples were taken to the NVRI Biology laboratory for authentication.

Seeds Extraction

The two seed samples were air-dried separately at room temperature in the laboratory and ground into powder using mortar and pestle. 100g of each powder was weighed on a weighing balance and placed into two different conical flasks. 500ml of distilled water was added to each conical flask and allowed to stand overnight (24hours). Each mixture was filtered separately using Whatman filter paper 1 and each of the filtrates was evaporated to dryness in a hot air oven at 40°C for 72hrs to obtain the extracts, and then each extract was stored in a sterile airtight container until use.

Preliminary Qualitative Phytochemical Analysis

Phytochemical studies for the two extracts was carried out as follows:

Preliminary Qualitative Phytochemical Screening of Neem Seeds Extract: as described by Oyweri *et al.,* in 2021;

Detection of Alkaloids: dilute hydrochloric acid was added to 1g of Neem seeds extract for Wagner's test. Four drops of Wagner's reagent (iodine in potassium iodide) was added to the prepared sampleto test for the presence of alkaloids (appearance of a brown/reddish precipitate).

Detection of Terpenoids: 1g of Neem seeds extract was mixed with 2ml chloroform. 3ml concentrated sulphuric acid was added gradually to form a layer. A reddish brown interface indicated the presence of terpenoids.

Detection of Saponins: for thefoam test, 2ml of water was added to 1g of neem seeds extract and shaken well. The persistence of foam for about 10 minutes indicated the presence of saponins.

Detection of Sterols: 2ml chloroform and 2ml concentrated sulphuric acid were added to 1g of neem seed extract and shaken well. The appearance of greenish-yellow fluorescence at the red layer of chloroform and acid indicated the presence of sterols.

Detection of Glyosides: 1ml of water was added to 1g of neem seeds extract and shaken well. 2ml of aqueous solution ofsodium hydroxide was added. Appearance of yellow colour indicated the presence of glycosides.

Detection of Phenols: for ferric chloride test, 1g of neem seeds extract was treated with 4 drops of ferric chloride solution. Formation of bluish-black colour indicated the presence of phenols.

Detection of Tannins: for gelatin test, 2ml of 1% gelatin solution encompassing sodium chloride was added to 1g of neem seeds extratct. Formationof white precipitate indicated the presence of tannins.

Detection of Flavonoids: alkaline reagent test; 1g of neem seeds extract was treated with 4 drops of sodium hydroxide solution. Establishment of intense yellow colour, which turned colourless on addition of diluted hydrochloric acid indicated the presenc of flavonoids.

Preliminary Qualitative Phytochemical Screening of Black Seeds Extract: following the procedure adopted by Abdurohaman, 2015**:**

Test for Alkaloids: Wagner's; 1.5ml of 1% HCL was added to 2ml of Black seeds extract. The solution was heated in a water bath, and then 6 drops of Wagner's reagent was added. An orange precipitate indicated the presene of alkaloids.

Test for Steroids: 5ml of chloroform and 2ml of acetic nhydride were added to 2ml of black seeds etract, followed by concentrated sulphuric acid. Reddish-brown coloration of the interface indicated the presence of steroids.

Test for Phenols: to 2ml of Black seeds extract, 5% ferric chloride solution was added.deep blue-black colour indicated the presence of phenols.

Test for Terpenoids: following the Salkowski's test procedure, 1ml of Black seeds extract was mixed with 2l of choloroform. 3ml concentrated sulphuric acid was added gradually to form a layer. A reddish-brown interface indicated the presence of terpenoids.

Test for Flavonoids: a few drops of ferric chloride solution was added to the test solution. Intense green colour showed the presence of flavonoids.

Test for Tannins: 2ml of Black seeds extract was dissolved in distilled water and then 2l of 5% ferric chloride solution was added. The formation of blue-green colour indicated the presence of tannins.

Test for Saponins: 2ml of Black seeds extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of a layer of foam indicated the presence of saponins.

Test for Glycosides: to 2ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride was added in a test tube. 0.5ml of concentrate sulphuric acid was carefully added by the side of the test tube. Formation of blue colour in the acetic acid layer indicated the presence of glycosides.

Animal Ethical Considerations, **Animals Used and Parasite Inoculation:** Animal care and use guideline was provided by the National Veterinary Research Institute (NVRI) Vom. Male wistar rats weighing between 110-125g were used as test animals. The rats were purchased from the NVRI Vom and kept under optimum conditions of temperature, and feeding in the laboratory in cages of 6 mice per cage at 25-28°C room temperature. Relative humidity was maintained at 60-70%.

Plasmodium falciparum infected blood was used to inoculate the mice intraperitoneally

Infecting and Dosing of test Animals: To 2ml of parasitized blood was added 4ml of distilled water as diluent. Then each rat labeled from 1-18 was infected with 0.2ml of diluted parasitized blood (2×10^7 parasitized erythrocytes) intraperitoneally and divided into 3 groups of 6 rats each, and 1 normal control group of 6 uninfected rats were labeled rats 19-24..

Determination of Packed Cells Volume (PCV): Protocol highlighted by Mekonnen was followed to determine the PCV. It aided in evaluating the efficacy of the test extracts in inhibiting hemolysis due to malaria. Blood was collected from the vein of each mouse in heparinized capillary tube (¾ of its volume) before infection and on day 4 post treatment, centrifuged at 12,000 rpm for 10min. The PCV was determined using the formular:

$$\text{PCV} = \frac{\text{VOLUME OF RBCCs IN GIVEN VOL.OF BLOOD}}{\text{/TOTAL BLOOD VOLUME}} \times 100$$

Statistical Analysis: one way analysis of variance (ANOVA) was utilized to establish statistical significance for assessment of in vivo assay. A p-value of less than 0.05 was considered statistically significant

RESULTS

Qualitative Phytochemical screening of the *A. indica* extract showed high concentrations of saponins, alkaloids and steroids, tannins, with moderate glycosides and terpens. Phytochemical screening of *N. sativa* revealed high concentrations of saponins, resins, alkaloids, steroids, with moderate flavonoids, glycosides and terpenes. This is in line with previous studies that revealed that alkaloids and terpenes in most medicinal plants have antimalarial activity.

Table 1: Phytochemical analysis of the plants extracts.

Test Parameters	**Sample A: *A. indica***	**Sample B: *N. sativa***
Saponins	9.10±0.03	5.95±0.01
Tannins	5.74±0.04	4.70±0.05
Resins	2.74±0.04	11.65±0.41
Alkaloids	9.10±0.03	6.33±0.03
Flavonoids	0.11±0.02	2.18±0.02

Glycosides	4.12±0.08	3.09±0.05
Steroids	12.20±0.15	11.65±0.27
Terpenes	5.85±0.03	4.27±0.10
Cardiac glycosides	4.70±0.05	5.95±0.02

Symptoms of malaria infection were observed three days after infection. One of the rats in group D died after day 8 but no deaths from the treatment groups was recorded after 8 days of treatment. At the end of the treatment, the infected rats were still alive

Table 2: Report AFTER FIRST 4 DAYS OF TREATMENT

Group		Weight(g)	RBC(10^12/L)	WBC (10^9/L)	PCV(%)	HB(g/L)
group 1	Mean	132.8933	5.9500	12.5333	36.1667	12.0333
	N	6	6	6	6	6
	Std. Error of Mean	7.70854	.53213	1.78562	2.22736	.75085
group 2	Mean	132.7647	5.7833	11.8000	33.0000	11.8667
	N	6	6	6	6	6
	Std. Error of Mean	2.69409	.21042	1.35769	1.26491	.37387
group 3	Mean	146.8480	5.6833	12.4517	31.5000	11.5667
	N	6	6	6	6	6
	Std. Error of Mean	6.80675	.21042	.25613	1.83938	.23190
group 4	Mean	127.9900	6.5600	10.2000	37.7500	11.6000
	N	4	4	4	4	4
	Std. Error of Mean	1.91256	.08083	.64161	.47871	.04082
Total	Mean	135.7725	5.9427	11.8868	34.3182	11.7818
	N	22	22	22	22	22
	Std. Error of Mean	3.12667	.16974	.61319	.96112	.22588

Table 3: Report after 7 days of treatment.

Group		RBC	WBC	PCV	HB
group 1	Mean	6.0667	12.1500	34.5000	11.9500
	N	6	6	6	6
	Std. Error of Mean	.45509	1.75988	2.04532	.65358
group 2	Mean	5.1833	11.7167	31.5833	11.7333
	N	6	6	6	6
	Std. Error of Mean	.22423	1.35804	1.28073	.31798
group 3	Mean	5.5333	10.5000	31.0833	11.4833
	N	6	6	6	6
	Std. Error of Mean	.17062	2.10840	1.98014	.21512
group 4	Mean	6.4700	10.3000	37.7500	11.6000
	N	4	4	4	4
	Std. Error of Mean	.07821	.67206	.47871	.04082
Total	Mean	5.7536	11.2455	33.3636	11.7000
	N	22	22	22	22
	Std. Error of Mean	.17127	.80606	.96476	.19717

Table 4: Report after 14 days of treatment.

Group		RBC	WBC	PCV	HB
group 1	Mean	7.3000	11.8667	37.5650	13.0000
	N	6	6	6	6
	Std. Error of Mean	.60992	.64377	.80334	.48922
group 2	Mean	6.1667	11.6417	39.1683	12.5633
	N	6	6	6	6
	Std. Error of Mean	.24313	.73801	.33456	.57629
group 3	Mean	6.5167	12.0500	38.9367	13.3750
	N	6	6	6	6
	Std. Error of Mean	.37275	.49783	.37134	.41585
group 4	Mean	8.0250	14.3750	40.4125	14.1850
	N	4	4	4	4
	Std. Error of Mean	.21747	.22500	.24291	.08302
Total	Mean	6.9091	12.3114	38.8941	13.1986
	N	22	22	22	22
	Std. Error of Mean	.24529	.35474	.32181	.25119

The body weights of the rats investigated in this study are shown in table 5 and chart 1. There was no significant weight difference observed in all treatment groups.

Table 5: Body weights of rats before infection across treatment .

Group		Wieght of rat before infection	Weight of infected rats	Weights of rats after treatment
group 1	Mean	111.3140	132.9457	120.3197
	N	6	6	6
	Std. Error of Mean	23.53011	7.66173	5.75425
group 2	Mean	132.7647	132.7528	138.3095
	N	6	6	6
	Std. Error of Mean	2.69409	2.69301	14.54295
group 3	Mean	146.0030	145.9893	128.4700
	N	6	6	6
	Std. Error of Mean	7.14203	7.14243	6.77126
group 4	Mean	129.1997	129.1997	133.8110
	N	3	3	3
	Std. Error of Mean	2.09504	2.09504	1.45455
Total	Mean	129.9090	136.0822	129.7156
	N	21	21	21
	Std. Error of Mean	7.24791	3.23373	4.82032

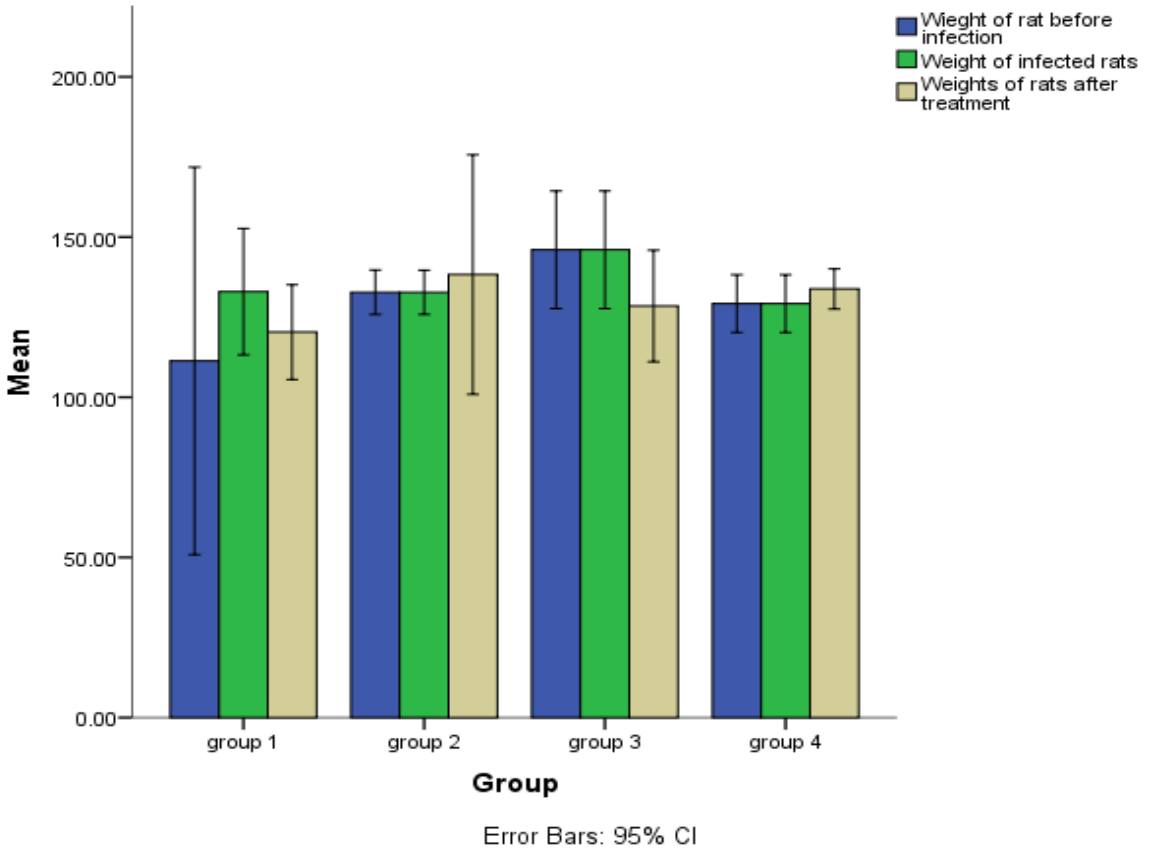

Fig.3: Hematological Parameters of The Rats Investigated

A reduction in all hematological values was observed in the treatment groups after administration of plant extracts compared to the normal control. Malaria infection caused a non-significant decrease in PCV and WBC with a significant ($p>0.05$) increase in RBC. There was no significant decrease in hemoglobin concentration observed across all treatment groups.

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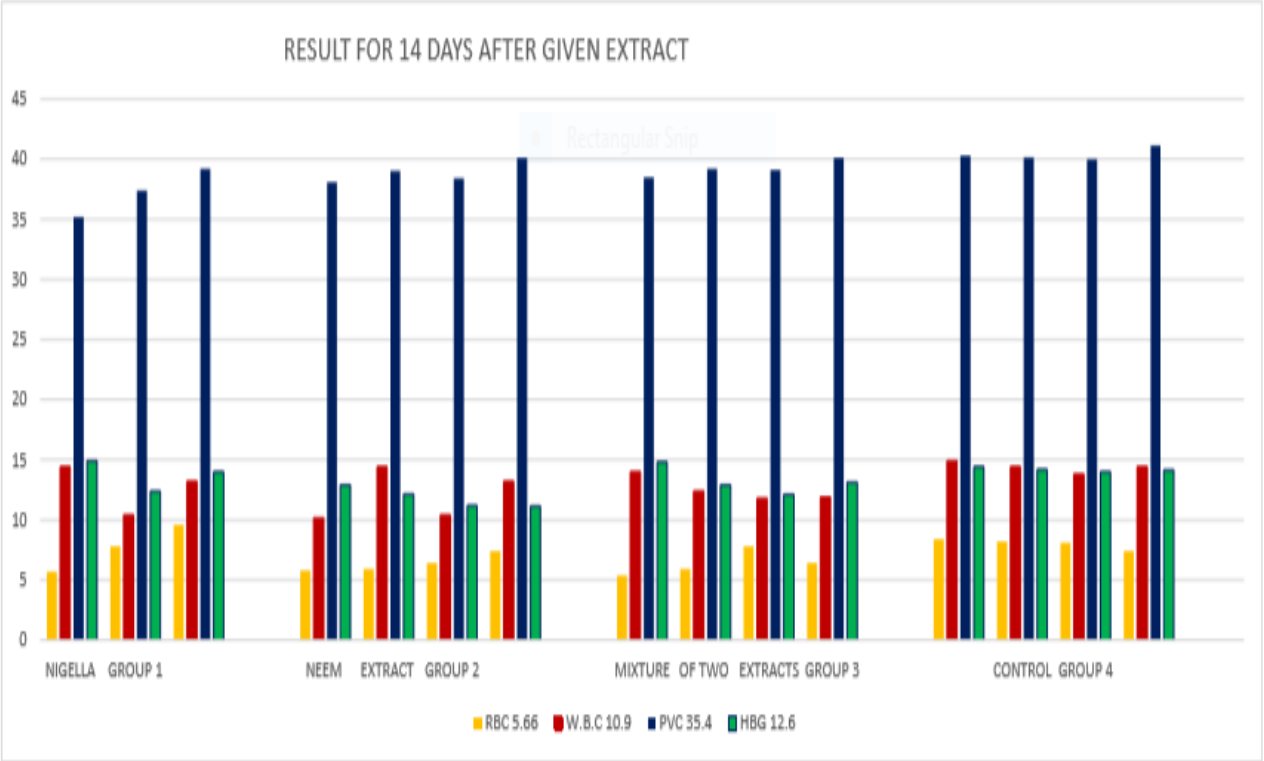

Fig.4: Graph Showing Hematological Changes During Treatment

DISCUSSION

Malaria is characterized by anemia which is as a result of the destruction of RBCs both infected and non infected which leads to a decrease in erythroid precursors and erythropoiesis inhibition resulting in the death of the patient (Achi *et al.*, 2018). Anemia level is directly proportional to the severity of the infection (Oyweri et al, 2021). PCV levels are inversely proportional to anemia due to malaria infection. The onset of anemia results from decrease in hemoglobin i.e. decrease in RBCs. The administration of *A. indica* extract in combination with *N. sativa* extract was practically safe and well tolerated at the administered doses. The results indicate increased PCV values in the

test groups, with highest values in group C treated with mixture of *A. indica* and *N. sativa*, which shows the extracts endure hemolysis of RBC due to infection causing anemia. This agrees with Ashcroft et al., 2018 who reported that N. sativa extracts elevate PCV values (Yimer et al., 2019).

Medicinal plants with anti-anemic properties are used to manage severe anemia by restoration of the RBC count (Onyeabo et al., 2017). The hematological values in this study were restored with the administration of the plant extracts.during the treatment, rats in group C treated with a mixture of A. indica and N. sativa extracts exhibited higher restoration of the RBC count. The study also proved that the rats treated with only either of the extracts responded to treatment as only 2 deaths were recorded at the end of the experiment. The prevention of weight loss by both the Neem and Black seeds extracts in the treated rats was also observed as compared to the weights of the normal control group.

Various medicinal plants that have antimalarial activity such as chloroquine, artemisinin products and derivatives were discovered using the rodent models. With such drug discovery investigations, invivo tests are considered to be more practical, faster and cheaper than the invitro experiments. A potential antimalarial drug exhibits therapeutic activity without having any toxic effects on the host animal. Acute toxicity test for both Neem and Black seeds proved no death at doses as high as 5000mg/kg and 2000mg/kg respectively for the duration of the 14 days period. The successful completion of the 4-day suppressive test without any death records indicates the safety of the two extracts.

N. sativa has been found to be active against various strains of *Plasmodium*, with high parasite clearance and restoration of altered hematological parameters. In this study, *N. sativa* showed higher RBC count at concentrations of 200mg/kg and 400mg/kg. These results indicated that Neem seeds and Black seeds extracts are safe for use as a form of malaria treatment.

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NOTE:

The study highlights the efficacy of "Herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.