NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., January 2024 Vol 44 (4): 1 - 16.

https://dx.doi.org/10.4314/nvj.v45i1.1 **ORIGINAL ARTICLE**

Effect of Treatment of Malaria and Diabetes with Aqueous Extract of *Anthocleista Djalonensis* in Mice

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SUMMARY

Anthocleista djalonensis is a medicinal plant that has been used to treat malaria, jaundice, abscesses and diabetes. The study assessed the effects of aqueous extract of *A. djalonensis* leaves on some biochemical indices in the treatment of malaria and diabetes. Twenty-four mice used were randomly assigned into six groups numbering four each and provided with feed and water *ad libitum*. Group 1 (control) received water and feed only. Groups 2, 3, 4 5 and 6 received 200 mg/kg body weight of extract; 5 mg/kg body weight of chloroquine, 400 mg/kg body weight extract, 2 mg/kg body weight glibenclamide 5 mg/kg body weight chloroquine and 2 mg/kg body weight glibenclamide respectively for seven days. Phytochemical screening of *A. djalonensis* leaves revealed the presence of alkaloids, phenols, saponins, steroids, flavonoids and tannins at 6.76 ± 0.03, 0.61 ± 0.01, 2.11 ± 0.02, 0.89 ± 0.01, 3.10 ± 0.03 and 1.13 ± 0.01 % respectively. Alanine aminotransferase and aspartate aminotransferase activity of Groups 5 and 6 were significantly ($p<0.05$) higher compared to the control. There were no visible histological changes in liver and kidney. Results of this study suggest that *A. djalonensis* is relatively safe for the treatment of diabetes and malaria at the administered doses.

Keywords: *Anthocleista djalonensis*, Diabetes, Extract, Malaria, Mice

INTRODUCTION

Malaria is a common disease whose treatment has been challenging especially in tropical Africa. Malaria, a parasitic disease caused by the

Plasmodium species through anopheline mosquito bite has been a major cause of death both for pregnant women and children under the age of five in developing countries of the world. Although a world-wide disease, malaria is highly prevalent in the tropics particularly in Sub-Sahara Africa (Nguta *et al.*, 2010; WHO, 2015). In 2018, WHO reported an estimated value of over 200 million cases of malaria infection globally, of which about 405,000 deaths occurred and 272,000 (67 %) number of children under the age of five were affected by this deadly infection (WHO, 2019). In Africa, the region with highest number of cases of malaria infection is Nigeria seconded by the Democratic Republic of Congo (WHO, 2017). Among the four infectious *Plasmodium* (*P. falciparium, P. vivax, P. malariae* and *P. ovale*), *P. falciparium* is the deadliest of them all causing majority of deaths experienced by human as a result of its cerebral and anaemic complications (Wiese *et al.*, 2008; Caraballo and King, 2014). Signs and symptoms of malaria are severe headache, flu-like illness, fever, nausea and chills (Trampuz *et al.*, 2003). Malaria has been a burden to both economic and emotional aspects of human being (CDC, 2018). Wide spread resistance of *P. falciparium* to most used antimalarial drugs is at its peak in Africa (Zofou *et al.*, 2011) leading to failure of malaria treatment with the drugs and re-occurrence of disease transmission (Titanji *et al.*, 2008).

Diabetes is a metabolic disorder known and characterized by increase in blood glucose level due to lack of insulin secretion and resistance (WHO, 2019). It is a common disease globally, that affects body organs such as the heart, pancreas, liver and so on (Basit *et al*., 2018). Over 400 million adults were reported to be diabetic in 2017 and a prediction has been made stating that more than 600 million adults will be diabetic by 2045 (Younk *et al*., 2011). Diabetes is classified into type 1 and 2; type 1 is characterized with total or near total lack of insulin while type 2 is characterized with various levels of insulin resistance and defect in insulin secretion. In 2021, it was reported that about 24 million Africans between the age of 20 and 79 years were suffering from diabetes (IDF, 2021), while the global death rate as a result of diabetes is estimated to be about 3.8 million in 2013 (Azandjeme *et al.*, 2013). Researchers have reported a range of 2 to 12 % prevalence in Nigeria (Nyenwe *et al*., 2003; Puepet and Ohwovoriole, 2008; Sabir *et al*., 2011; Gezawa *et al*., 2015). In a recent report, the prevalence of diabetes in Nigeria was reported to be approximately 6 % (Uloko *et al.*, 2018). Over the years, management and treatment of diabetes has been a challenge because most of the adverse side effects associated with the drugs used for treatment and also the fact that such drugs can only manage the diabetic complications. There is therefore continuous search for new sources of therapeutic agents that could be used to manage diabetes (Marshall, 2017).

Anthocleista djalonensis A. Chev., 1908 (Gentianales: Loganiaceae) occurs from Guinea Bissau east to Cameroon and is a shrub-like plant that grows up to 20 feet high. It has a blunt spine on the unbranched pale grey trunk and wide spread crown (Dalziel, 1954) and produces fruits between October and November in Nigeria. *A. djalonensis* is commonly known as cabbage tree and widely found in tropical Africa and Madagascar (Hosseinkhani *et al.*, 2017). In Nigeria, it is known as *Okpokolo* by the Igbos, *Sapo* by the Yorubas and *Kwarii* by the Hausas (Anyanwu *et al*., 2015). Extracts from some parts (stem, leaves, root and bark) of the plant have

been reported to have different medicinal potentials in the treatment of malaria, jaundice, abscesses and diabetes (Dalziel, 1954). Okokon *et al.* (2012) reported the antidiabetic potentials of the root extracts. Researchers have reported wound healing and antibacterial activities of *A. djalonensis* leaves (Chah, *et al.*, 2006; Ugwu *et al.*, 2019). *In-vivo* antimalarial activities of the leaf and stem bark of *A. djalonensis* has also been reported (Bassey *et al.*, 2009; Bla *et al.*, 2020).

Malaria and diabetes are on the increase in Nigeria and in sub-Saharan Africa at large due to western life styles and genetic predisposition. There are concepts stating that diabetes could be a risk factor for malaria (Oni and Unwin, 2015; Van Crevel *et al.*, 2017; Carrillo-Larco *et al.*, 2020). Researchers have shown the use of *A. djalonensis* in the treatment of malaria and diabetes separately (Bassey *et al*., 2009; Okokon *et al*., 2012). However, this study is aimed at studying the effect of the aqueous extract of *A. djalonensis* in both disease condition and to ascertain some biochemical and histological changes associated with treatment of malaria and diabetes.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol (absolute), Giemsa stain powder, Immersion oil, Chloroquine, Glibenclamide and other reagents used were of analytical grade and prepared using deionized distilled water.

Animals

Twenty-four male albino mice weighing 22.50 ± 2.50 g obtained from Animal Breeding Unit, College of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria were used for the study. The mice were housed in standard cages and acclimatized for a period of 14 days. The mice were maintained under standard laboratory conditions, according to guideline for the use of mice in biomedical research (NRC, 2011), and were given free access to rat pellets (Vital Growers Mesh produced by Grand Cereals Limited, crude protein – 14.5 %, metabolizable energy – 2450 kcal/kg) and tap water *ad libitum.* The experiment protocol for this research work was approved by the Animal Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202121).

Parasites

A chloroquine-sensitive strain of *Plasmodium berghei* obtained from the University of Nigerian Nsukka, Enugu State, Nigeria was used for this study.

Plant Material and Extract Preparation

Fresh matured leaves of *A. djalonensis* were collected from a farm in Michael Okpara University of Agriculture Umudike, Abia State, Nigeria, identified (Llamas, 2003) and authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Voucher specimen (MOH 0155) was kept in the departmental herbarium for referral purposes. The leaves were sorted and cleaned. Afterward, the leaves were cut into smaller sizes, dried in the shade at room temperature, and then pulverized to fine powder using an electric blender. The powdered plant (100 g) was weighed using a top loading balance (Scout Pro SPU402) and soaked in 1000 ml of water for approximately 72 hours after which it was filtered using a muslin cloth. The filtrate was poured into a pre-weighed beaker and allowed to

evaporate at 50^{o}C in a water bath. After proper evaporation the extract concentrate was preserved in a refrigerator until it was ready for use.

Phytochemical Screening

Phytochemical screening of *A. djalonensis* leaf extract for the presence of secondary metabolites which include: alkaloids, flavanoids, phenols, tannins, saponins and steroids was done using the methods described by Harborne (1973) and AOAC (2006).

Toxicity Assay

The acute toxicity of *A. djalonensis* leaf extract was determined by the method of Lorke (1983).

Experimental Design

Animals were randomly divided into 6 groups of four mice each. Animals in control group were not inoculated with the parasite nor induced with diabetes, while the other groups were inoculated from the same donor mouse and also induced with diabetes. The percentage parasitaemia and the blood glucose were first determined and appropriate dilutions of the infected blood with normal saline were done.

Animal Inoculation and treatment

Each mouse in the infected groups was inoculated on day 0 intraperitoneally with 0.2 ml of infected blood containing about 1 x 10^{7} *P. berghei* parasitized red blood cells, after which hyperglycemia was induced in the mice by intraperitoneal injection of 0.3 ml of alloxan. Parasitaemia was established by screening for malaria parasites in both thick and thin film made from tail blood of infected animals after fixing in methanol and staining with Giemsa stain (Ryley and Peters, 1970), likewise glucose level was monitored by cutting the tail tip of a conscious mouse and using blood glucose on call plus strip inserted in the glucometer (code 131, LOT390028). Treatment commenced when both conditions were established. The mice were treated daily from day 1 (two days after infection). Aqueous preparations of the extract corresponding to 200 and 400 mg/kg of the extract, chloroquine (corresponding to 5 mg/kg body weight) and glibenclamide (corresponding to 2 mg/kg body weight) were made before administering orally to the mice. The administration of the different doses which lasted for six days were as follows: Group 1 (control) (*plasmodium* uninfected and non-diabetic mice): received an appropriate volume of sterile distilled water corresponding to the highest volume of extract administered. Group 2 (*plasmodium* infected and diabetic mice): received the aqueous preparation of the extract (200 mg/kg body weight daily). Group 3 (*plasmodium* infected and diabetic mice): received the aqueous solution of chloroquine (5 mg/kg body weight daily). Group 4 (*plasmodium* infected and diabetic mice): received the aqueous preparation of the extract (400 mg/kg body weight daily). Group 5 (*plasmodium* infected and diabetic mice): received the aqueous solution of glibenclamide (2 mg/kg body weight daily). Group 6 (*plasmodium* uninfected and non-diabetic mice): received the aqueous solutions of chloroquine (5 mg/kg body weight) and glibenclamide (2 mg/kg body weight) daily.

Sample Collection and Analyses

Daily blood films were made from tail blood of all the infected animals (Groups 1, 2, 3, 4 and 5), the percentage parasitaemia and blood glucose level were obtained through microscopic determination

and glucometer respectively. Percentage chemo-suppression was calculated by subtracting the average percentage parasitaemia in the treated group from the average percentage parasitaemia in the control group and the value obtained was expressed as a percentage of the average parasitaemia in the control group. Animals were sacrificed via cardiac puncture after seven days of daily administration of the various treatments. The mice were euthanized with chloroform before sacrificing. Alanine aminotransferases (ALT), alkaline phosphate (ALP) and aspartate aminotransferase (AST) activities were determined by the methods described by Reitman and Frankel (1957). Total bilirubin was determined by colorimetric method as described by Jendrassik and Grof (1938). The concentration of serum urea was determined using the method described by Weatherburn (1967). Serum creatinine concentration was determined by the kinetic enzymatic method described by Moss *et al.* (1975). Serum bicarbonate was determined as described by Den Boer *et al.* (1974). Serum chloride was determined using the colorimetric method of Schoenfeld and Lewellan (1964). Serum potassium was determined using the sodium tetraphenylboro method described by Terri and Sesin (1958). Serum sodium was determined using the colorimetric methods described by Maruna (1957). Histopathology technique was carried out on the liver and kidney as described by Drury and Wallington (1976) and Bancroft and Stevens (1990). Photomicrographs of the organs were taken using Motic camera (Optika, Germany).

Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA). Significant difference between group means was separated using Duncan New Multiple Range Test of the same statistical package. Significant difference was accepted at $p<0.05$. All data analyses were done using SPSS version 20. Results were expressed as mean ± standard error of mean.

RESULTS

The phytochemical and toxicity screening of the pulverized leaves of the plant *A. djalonensis* revealed that it contains alkaloids, phenols, saponins, steroids, flavonoids and tannins (Table I) with LD_{50} value of 5 g/kg.

TABLE I: Phytochemical composition of the pulverized leaves of *A. djalonensis*

Phytochemical	Composition (%)
Alkaloids	6.76 ± 0.03
Phenols	0.61 ± 0.01
Saponin	2.11 ± 0.02
Steroids	0.89 ± 0.01
Flavonoids	3.10 ± 0.03
Tannins	1.13 ± 0.01

Values are mean ± standard error of triplicate determination.

Parasitaemia counts in group (Group 1) treated with 200 mg/kg body weight of extract showed significant reduction ($p<0.05$) from 11.67 ± 2.08 % on second day to 4.00 ± 1.00 % on seventh day of treatment showing about 65.72 % decrease within 4 days (Table 2). The group (Group 2) treated with chloroquine decreased from 3.33 ± 2.08 % on the second day to 0.33 ± 0.58 % on day seven indicating a significant reduction ($p<0.05$) of 90.09 %. Group 3 that was treated with a higher dose (400 mg/kg) of plant extract showed significant ($p<0.05$) reduction from 6.67 ± 5.03 %

TABLE II: Effect of treatment on the percentage parasitaemia from day 1 to 7 of *Plasmodium berghei* infected diabetic mice treated with various concentrations of *Anthocleista djalonensis* leaf aqueous extract

Group	Parasitaemia (%)							
	Baseline	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
200 mg/kg extract	8.33 ± 3.51^{ab}	11.67 ± 2.89^{b}	11.67 ± 2.08^{c}	11.33 ± 1.15^{c}	10.00 ± 2.00^{b}	9.00 ± 1.73^{c}	5.33 ± 1.15^{b}	4.00 ± 1.00^{b}
Chloroquine (CH)	2.67 ± 2.08^{a}	3.33 ± 2.08^{a}	2.00 ± 2.00^{a}	1.67 ± 1.53^{a}	1.33 ± 1.53^{a}	0.67 ± 1.15^{a}	0.33 ± 0.58^{a}	0.33 ± 0.58^{a}
400 mg/kg extract	3.67 ± 3.79^{a}	4.67 ± 3.21^{a}	6.00 ± 2.65^{b}	$6.67 \pm 5.03b^{c}$	5.00 ± 5.00^{ab}	2.67 ± 2.52^{a}	1.33 ± 1.53^{a}	1.00 ± 1.00^{a}
Glibenclimide (GL)	3.00 ± 3.00^{a}	7.00 ± 3.00^{ab}	9.00 ± 1.73^{cd}	10.33 ± 0.58^{c}	8.33 ± 0.58^{b}	6.00 ± 1.00^{b}	4.00 ± 1.73^{b}	3.33 ± 1.15^{b}
Chloroquine+ Glibenclimide	10.00 ± 2.00^{b}	11.00 ± 1.00^{b}	9.67 ± 0.58^{cd}	4.67 ± 1.53^{ab}	2.00 ± 2.00^{a}	1.00 ± 1.00^{a}	1.33 ± 1.53^{a}	1.00 ± 1.00^{a}

Values are mean ± standard error of triplet determination. Values within a row having different superscripts are significantly different at $p<0.05$.

on the third day of treatment to 1.00 ± 1.00 % on the seventh day indicating 85.01 % decrease (Table 2).

The mean blood glucose levels of the groups (Groups 1 to 4) induced with hyperglycemia ranged from 106.33 ± 53.20 mg/dL to 129.00 ± 28.21 mg/dL body weight confirming that the animals were diabetic (Table 3). The group treated with 200 mg/kg body weight of plant extract decreased from 129.00 ± 28.21 mg/dL on the day of induction to 105.00 ± 2.00 mg/dL on the fifth day of treatment indicating 18.61 % reduction in blood glucose level of the mice. The group treated with 400 mg/kg body weight of plant extract had 29.20 % decrease between the day of induction and day five, this reduction was significant ($p<0.05$), while the group treated with standard antidiabetic drug had the highest reduction of 36.36 % on same day. These reductions were significant ($p<0.05$) when compared with the control on day five (Table III).

There was no significant difference ($p>0.05$) in ALP values between the groups and the control.

The groups treated with standard antidiabetic drug alone and together with chloroquine (Glibenclimide and Chloroquine + Glibenclimide) had significantly higher ($p<0.05$) AST and ALT activity respectively. Total bilirubin concentration of rats administered 200 mg/kg extract, Chloroquine (CH) and 400 mg/kg extract were significantly ($p<0.05$) lower compared to the control, while the other groups (Glibenclimide (GL) and Chloroquine + Glibenclimide) were statistically similar ($p>0.05$) (Table IV).

TABLE III: Effect of treatments on blood glucose level of *Plasmodium berghei* infected diabetic mice treated with various concentrations of *Anthocleista djalonensis* leaf aqueous extract

Day	Blood glucose level (mg/L)					
	Control	**200mg/kg extract**	**Chloroquine (CH)**	**400mg/kg extract**	**Glibenclimide (GL)**	**Chloroquine+Glibenclimide**
Day 0	114.66 ± 1.08^{a}	129.00 ± 28.21^{a}	125.67±25.11^{a}	121.33 ± 17.95^{a}	106.33 ± 53.20^{a}	125.67 ± 37.87^{a}
Day 1	113.67 ± 1.18^{a}	133.67 ± 15.04^{a}	132.33 ± 6.03^{a}	114.00 ± 25.71^{a}	119.67 ± 27.59^{a}	183.67 ± 46.01^{b}
Day 2	109.67 ± 2.33^{bc}	126.67 ± 19.66^{c}	57.00 ± 19.29^{a}	89.33 ± 17.16^{b}	110.00 ± 17.44^{bc}	114.67 ± 15.04^{bc}
Day 3	112.33 ± 1.51^{ab}	126.67 ± 24.58^{b}	104.33±12.50^{ab}	101.00 ± 16.37^{ab}	96.00 ± 13.86^{a}	104.33 ± 4.73^{ab}
Day 4	113.33 ± 2.85^{a}	133.00 ± 12.12^{b}	122.67 ± 6.43^{ab}	120.67 ± 17.16^{ab}	119.33 ± 10.02^{ab}	153.00 ± 2.00^{c}
Day 5	112.00 ± 1.87^{cd}	105.00 ± 2.00^{c}	116.67± 10.02^{d}	86.00 ± 7.21^{b}	67.67 ± 3.10^{a}	120.00 ± 3.00^{d}
Day 6	110.66 ± 0.70^{a}	113.00 ± 21.79^{a}	132.67 ± 7.51^{a}	123.67 ± 11.24^{a}	140.33 ± 25.11^{a}	112.33 ± 25.89^{a}
Day 7	114.66 ± 1.78^{a}	122.33 ± 6.11^{a}	115.67± 18.90^{a}	112.67 ± 9.29^{a}	124.00 ± 7.00^{a}	117.00 ± 2.00^{a}

Values are mean ± standard error of triplet determination. Values within a row having different superscripts are significantly different at $p<0.05$.

TABLE IV: Effect of treatment on liver function indices of *Plasmodium berghei* infected diabetic mice treated with various concentrations of *Anthocleista djalonensis* leaf aqueous extract

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TB (mg/dL)
Control	27.00 ± 2.83 ^{a}	16.00±1.41 ^{cd}	166.50±21.92 ^{bc}	0.87±0.13 ^{c}
200 mg/kg extract	32.67 ±3.79 ^{a}	12.33±1.53 ^{d}	140.00±16.64 ^{c}	0.62±0.10 ^{b}
Chloroquine (CH)	32.00±2.83 ^{a}	16.50±2.12 ^{cd}	149.00±2.82 ^{c}	0.39±0.01 ^{a}
400 mg/kg extract	28.33±3.51 ^{a}	17.67±2.08 ^{bc}	199.67±18.15 ^{ab}	0.50±0.07 ^{b}
Glibenclimide (GL)	31.67±2.08 ^{a}	23.67±2.52 ^{a}	231.33±10.70 ^{a}	1.45±0.14 ^{d}
Chloroquine+Glibenclimide	25.00±1.31 ^{a}	21.50±2.12 ^{ab}	228.00±22.63 ^{a}	0.88±0.15 ^{c}

Values are means ± standard error of triplet determinations; values within a column having different superscripts are significantly different at $p<0.05$.

ALP – Alkaline phosphatase, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, TB – Total bilirubin

TABLE V: Effect of treatment on kidney function indices of *Plasmodium berghei* infected diabetic mice treated with various concentrations of *Anthocleista djalonensis* leaf aqueous extract

Group	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Bicarbonate (mmol/L)
Control	37.33 ± 6.81^{b}	1.45 ± 0.25^{d}	205.33 ± 7.37^{b}	42.00 ± 3.46^{a}	4.13 ± 0.55^{b}	74.67 ± 10.79^{a}
200 mg/kg extract	34.00 ± 1.73^{b}	0.88 ± 0.30^{c}	202.00 ± 8.72^{ab}	40.00 ± 3.00^{a}	3.83 ± 0.32^{b}	77.00 ± 10.54^{ab}
Chloroquine (CH)	32.00 ± 7.00^{b}	1.37 ± 0.24^{d}	182.67 ± 13.43^{a}	41.67 ± 4.62^{a}	2.47 ± 0.58^{a}	90.00 ± 3.61^{b}
400 mg/kg extract	20.00 ± 2.65^{a}	0.23 ± 0.02^{a}	190.33 ± 7.64^{ab}	39.00 ± 3.61^{a}	2.83 ± 0.81^{a}	76.00 ± 6.00^{ab}
Glibenclimide (GL)	29.67 ± 5.03^{b}	0.68 ± 0.12^{bc}	208.00 ± 9.17^{b}	46.33 ± 6.66^{a}	1.93 ± 0.06^{a}	76.00 ± 1.00^{ab}
Chloroquine+ Glibenclimide	34.67 ± 2.08^{b}	0.49 ± 0.13^{ab}	205.67 ± 14.43^{b}	48.33 ± 7.64^{a}	4.40 ± 0.62^{b}	79.67 ± 7.51^{ab}

Values are means ± standard error of triplet determinations; values within a column having different superscripts are significantly different at $p<0.05$.

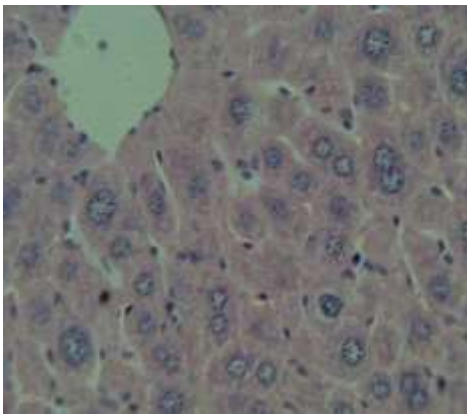

Plate A: Photomicrograph of liver section from control group administered distilled water having normal liver hepatocytes (× 400) H & E

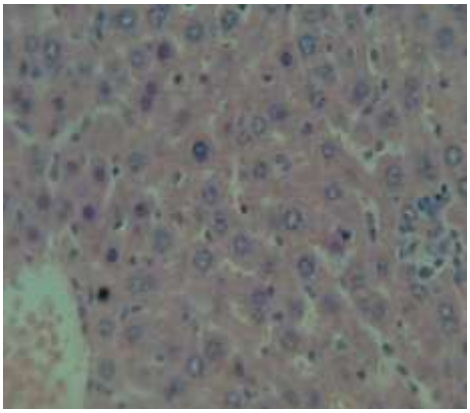

Plate B: Photomicrograph of liver section from group administered with 200 mg/kg aqueous extract of *A. djalonensis* showing normal liver hepatocytes (× 400) H & E

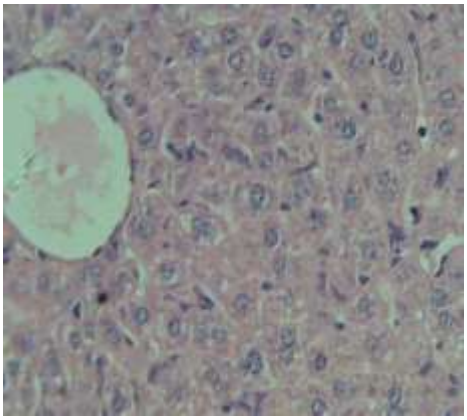

Plate C: Photomicrograph of liver section from group administered with 5 mg/kg of chloroquine showing slight fatty degeneration (× 400) H & E

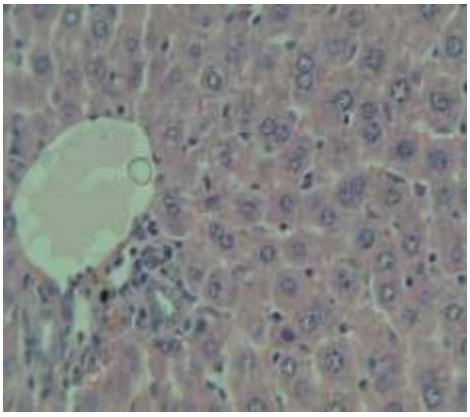

Plate D: Photomicrograph of liver section from group administered with 400 mg/kg aqueous extract of *A. djalonensis* showing normal liver hepatocytes (× 400) H & E

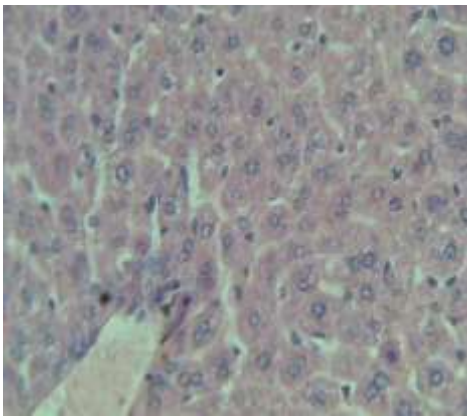

Plate E: Photomicrograph of liver section from group administered with 2 mg/kg glibenclamide showing normal liver hepatocytes (× 400) H & E

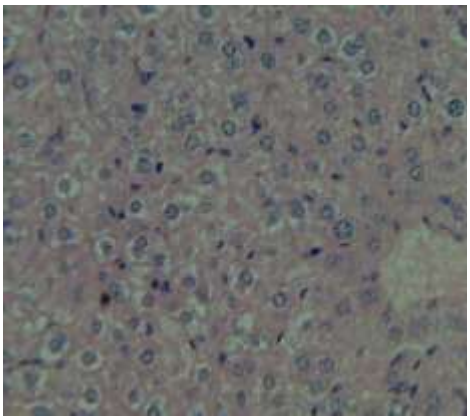

Plate F: Photomicrograph of liver section from group administered with 2 mg/kg glibenclamide showing normal liver hepatocytes (× 400) H & E

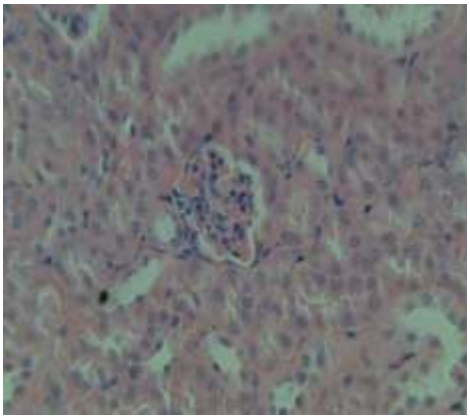

Plate G: Photomicrograph of kidney section from control group showing normal kidney tubules and glomeruli (× 400) H & E

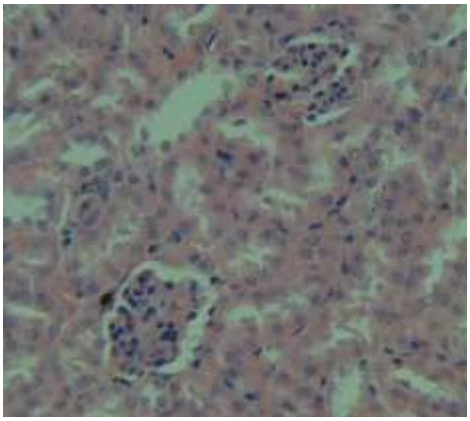

Plate H: Photomicrograph of kidney section from group administered 200 mg/kg of *A. djalonensis* extract showing normal tubules and glomeruli (× 400) H & E

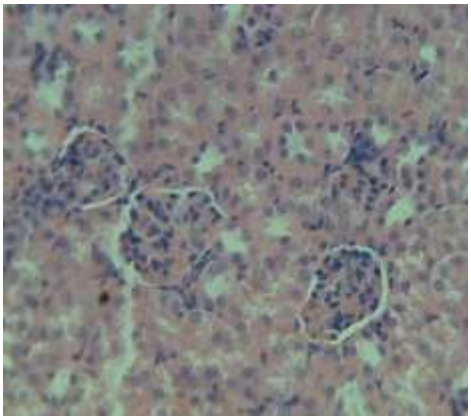

Plate I: Photomicrograph of kidney section from group administered 5 mg/kg of chloroquine showing normal kidney tubules and glomeruli (× 400) H & E

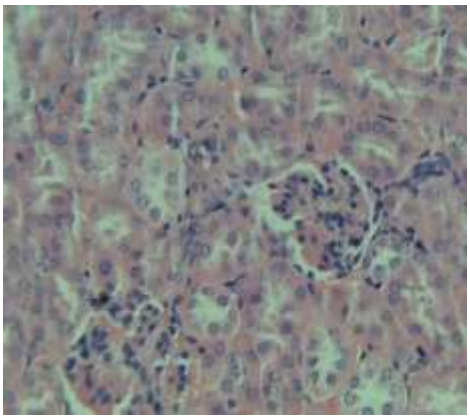

Plate J: Photomicrograph of kidney section from group administered 400 mg/kg of *A. djalonensis* aqueous extract showing normal tubules and glomeruli (× 400) H & E

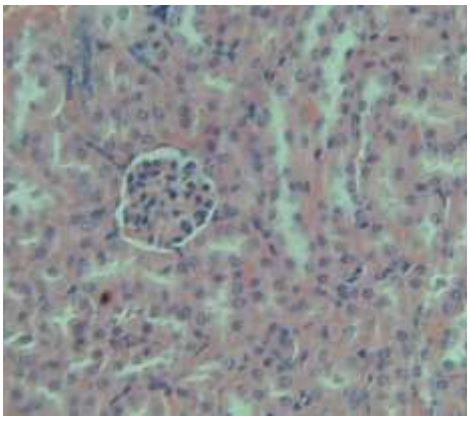

Plate K: Photomicrograph of kidney section from group administered 2 mg/kg glibenclamide showing normal tubules and glomeruli (× 400) H & E

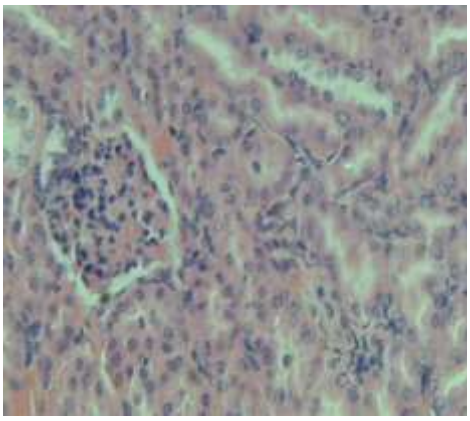

Plate L: Photomicrograph of kidney section from group administered 5 mg/kg chloroquine and 2 mg/kg glibenclamide showing normal tubules and glomeruli (× 400) H & E

Results for some kidney function test are shown in Table V. Urea concentration for all the treatment groups were similar ($p>0.05$) except the group treated with 400 mg/kg extract that was significantly ($p<0.05$) lower. Creatinine concentration of 400 mg/kg extract, Glibenclimide (GL) and Chloroquine + Glibenclimide were significantly lower ($p<0.05$), while sodium and bicarbonate concentrations were similar to the control ($p>0.05$).

Chloride ion concentration for all the treatment groups were similar ($p>0.05$) compared to the control. Potassium ion concentration of the groups administered Chloroquine (CH), 400 mg/kg extract and Glibenclimide (GL) were significantly ($p<0.05$) lower when compared to the control while mice in Chloroquine + Glibenclimide and 200 mg/kg extract groups were similar ($p<0.05$) to the control.

Histopathological effects observed in the photomicrographs of liver and kidney sections of treated and untreated mice are presented in Figures 1 and 2 (Plates A to L). Photomicrographs of liver sections of one mouse in each group treated with the aqueous leaf extract of *A. djalonensis* and glibenclamide alone showed no visible histopathological changes as compared to the control group while those treated with chloroquine alone and co-treated with both standard drugs showed fatty degeneration which may be as result of steatosis caused by untreated diabetes.

DISCUSSION

The result of phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolics and steroids. This agreed with the reports of Okokon *et al.* (2012) and Ibukunoluwa *et al.* (2015). The presence of these bioactive agents indicated that the plant could confer chemo-protective advantage to users (Enechi and Odonwodo, 2003). Plant extracts with LD_{50} values that are equal to or more than 5 g/kg are said to possess minimal toxicity, therefore *A. djalonensis* leaf extract could be said to have low toxicity (Homburger, 1989).

Increase in blood glucose concentration was observed in all mice after a single intraperitoneal injection of alloxan monohydrate which confirmed the induction of insulin-dependent diabetes mellitus (IDDM) in the mice. This agreed

with the work of Ghosh *et al*. (2004) who reported increase in blood glucose concentration after a single injection of alloxan monohydrate. Alloxan destroys the Beta-cells of the pancreas, hindering it from synthesizing and secreting adequate amount of insulin required for carbohydrate metabolism. All treated groups had a positive response both with the plant extract and glibenclimide. Within the treatment period, observable reduction was seen especially in day five for the groups treated with standard antidiabetic drug, high and low doses of plant extract. This finding was similar to the reports of Olubomehin *et al.* (2013) and Gnagne *et al.* (2018). Higher reduction (36 %) in the blood glucose level of the groups treated with standard drugs than in the groups treated with the plant extract, was as a result of glibenclamide; a sulphonylureas, that is effective in mild diabetic state and ineffective in severe diabetic animals where pancreatic β-cells are destroyed (Sekiou *et al*., 2021).

Group treated with chloroquine had their parasitaemia decreased up to 87 % while the groups treated with the extract had decrease in parasitaemia with above 50 %. It was observed that the parasitaemia in the group treated with glibenclamide increased all through the course of the experiment. Parasitaemia of the groups treated with chloroquine started decreasing from day two of treatment while the plant extract started reducing the parasitaemia between the fifth and sixth day of treatment. The exhibited antimalarial effect of *A. djalonensis* in this study could be attributed to the fact that liphophilic phytochemical – alkaloids, flavonoids and tannins were predominantly abundant in the extract (Adia *et al.,* 2016; Balogun *et al.,* 2019).

Liver is a vital organ of animals has a wide range of functions such as detoxification of various metabolites, protein synthesis and production of necessary digestion biochemicals. The liver is rich in alanine aminotransferase (ALT) and aspartate aminotransferase (AST).f These enzymes are released into blood circulation when there is liver damage and their concentration reveals the extent of damage (Gao *et al.,* 2012). The increase in AST and ALT activity obtained in groups treated with glibenclamide and chloroquine together and glibenclamide alone could be an indication of liver damage in the mice.

Urea is produced as a bye-product protein and amino acid metabolism. The low urea concentration observed in the group administered 400 mg/kg *A. djalonensis* plant extract could be an indication of possible renal protective effect by the extract. Elevated urea concentrations are caused by some kidney diseases such as chronic nephritis and tubular necrosis. Imo *et al.* (2019) reported an elevated urea concentration in albino rats treated with leaf, seed and fruit of *Datura metel.*

Photomicrographs of the kidney from all the groups showed normal kidney tubules and glomeruli. Liver photomicrographs of the mice showed normal hepatocytes except for groups treated with Chloroquine and that co-treated with glibenclimide that showed slight fatty degeneration when compared to the control group. This observation could be a direct consequence of the diabetic condition of the rats (Zafar *et al.,* 2009). This finding is in contrast to that of Imo *et al*. (2019) who reported glomerular extrusion, collapse and dilated tubules in the kidney of rats treated with *Datura metel.* El-Said *et al.* (2022) reported necrotic hepatocytes in the liver of rats under cadmium toxicity. The presence of

appreciable quantities of some phytochemicals such as alkaloids, flavonoids and saponins in the extract-treated groups could have conferred some protective effect on the kidney and liver of the mice.

CONCLUSION

Aqueous leaf extract of *A. djalonensis* exhibited antimalarial and antidiabetic potentials at the tested doses. The results of biochemical and histological studies suggest that the treatment of both malaria and diabetic with leaf extract of *A. djalonensis* at the tested doses may not have caused any visible negative effect on both the liver and kidneys of the mice.

ACKNOWLEDGEMENTS

The authors are thankful to the staff of the Plant Taxonomic Unit of the Department of Plant Science and Biotechnology for the identification of the plant sample used in this study.

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