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*Original Article*

# Biochemical and histological changes associated with treatment of malaria and diabetes mellitus in mice with extracts of *Mormodiaca charantia*

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ABSTRACT: Diabetes and malaria are prevalent diseases in the tropics. In spite of the availability of various independent therapies for each of these diseases, treatments of patients with both diseases have been quite challenging. *Mormodiaca charantia* is used in Nigeria separately to treat malaria and diabetes. This present study was aimed at investigating the effects of the methanolic extract of *Mormodiaca charantia* leaves (100 mg/kg body weight) on diabetic mice infected with malaria. Animals were infected with malaria and induced with diabetes by intraperitoneal injection of 1 x $10^7$ *Plasmodium berghei* and 100 mg/kg body weight alloxan monohydrate respectively. Animal subjects were treated with 5 mg/kg body weight of chloroquine phosphate, 10mg/kg body weight of glibenclamide and 100 mg/kg body weight of the extract daily for five days. Parasitemia, packed cell volume (PCV) and blood glucose level were monitored in the course of the treatment: Antioxidant status, kidney function test and histology of the kidney and pancreas section were examined. There was a decrease in the parasitemia level with a concomitant increase in the PCV, heamoglobin (Hb) and red blood cell count (RBC) level in groups treated with *M. charantia* and chloroquine. Blood glucose of all the treated groups decreased significantly ($p<0.05$) at the end of the experiment. The creatinine, uric acid and urea values in the group treated with the plant extract was significantly increased with values 1.23 mg/dl, 2.68 mg/dl and 30.15 mg/dl respectively, however, the group treated with both standard drugs had the highest urea value (51.55 mg/dl). Photomicrograph of the section of the pancreas and kidney of the infected group showed treated with 100 mg/kg of the plant extract showed mild degeneration of the islet of langerhan and mild atropy of the glomeruli respectively. These results support further studies on the use of *Mormodiaca charantia* as a single treatment for both diseases.

KEYWORDS: Biochemical, Histological, *Mormodiaca charantia*, malaria, diabetes



## INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites (genus *plasmodium*) widespread in the tropical and subtropical regions including parts of the America, Asia and Africa. Each year, there are approximately 5.5 million cases of malaria, causing the death of about one to three million people, the majority of whom are under 5 year-old and pregnant women (Lothar *et al*., 2008; Dapper *et al*., 2007; Murnigsih *et al*., 2005). Four species of *plasmodium* are infectious to humans and these include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale. Plasmodium falciparum* is responsible for the vast majority of deaths from malaria (Lothar *et al*., 2008). This is mainly due to the two major complications; cerebral malaria and anaemia (Lothar *et al*., 2008; Mohapatra, 2001). Other symptoms include fever, chills, nausea and flu-like illness. Spleenomegaly, severe headache, hepatomegaly, hemoglobinuria with renal failure may occur (Trampuz *et al*., 2003). Severe malaria can progress extremely rapidly and cause coma or even death within hours or days (Trampuz *et al*., 2003).

Diabetes is a disease characterized by abnormally high blood glucose and the excretion of excess glucose in the urine (Ghosh *et al*., 2004). Diabetes is subdivided on clinical grounds into Insulin dependent and non-insulin dependent diabetes milletus i.e IDDM and NIDDM or Type-1 and Type-2 respectively (Elased *et al*., 1995). NIDDM is characterized by hyperglycemia in both the fed and fasted state, variable degrees of hyperinsulinemia and obesity (Elased *et al*., 1995). Treatment of patients with malaria and diabete mellitus has been challenging (Mohapatra, 2001). This is because treatment with quinine dihydrochloride (one of the commonest antimalarial drugs) has been reported to result in hypoglycaemia (Mohapatra, 2001). White *et al.* (1983) posited that quinine

stimulates the release of insulin, which consequently results in hypoglycaemia. Hence treatment of malaria infected diabetic patients undergoing insulin therapy with quinine would hold grievous physiological implications. Mohapatra (2001) reported that the treatment of such cases is so challenging because of the effect of the parasites and antimalarials particularly quinine on glucose homeostasis.

To the best of our knowlege, there is limited information in the literature on the interaction of malaria with diabetes. One of such report is that of Shalev *et al.* (1992) who reported hypoglycaemia in a diabetic patient infected with *P. Falciparum*. It has been reported that of the severe manifestations of occurrence of malaria and diabetes mellitus, cerebral malaria is the commonest form encountered, followed by multi organ dysfunction (Mohapatra, 2001). In a metabolic disease like diabetes mellitus, functional impairment of organs like kidney and heart are frequently found due to vascular involvement, thus *Plasmodium falciparum* aggravates the organ dysfunction by interfering with microcirculation. This may probably be the explanation for the higher incidence of cerebral and multiple organ involvement in diabetes mellitus with malaria (Mohapatra, 2001).

Increasing problems of occurrence of both diabetes and malaria in some patients calls for the need to explore the therapeutic potential of some local herbs that have been reportedly used separately for the treatment of diabetes mellitus and malaria, so as to investigate their efficacy in suitation of occurrence of both malaria and diabetes mellitus. One of such plant is *Mormodica charantia* (Bitter melon or balsam pear); a tropical vegetable, widely cultivated in Asia, Africa and South America, and has been used extensively in folk medicine as remedy for diabetes (Girron *et al.*, 1991), constipation, malaria and as antibiotics (Day *et al.*, 1990). It is therefore of interest to investigate the therapeutic potential of *Mormodiaca charantia* in occurrence of malaria and diabetes mellitus and to study the effects of treatment on the functionality of some organs. Elased *et al.* (1995) reported slow multiplication of the malaria parasite in diabetes induced mice, a report which is in concordance with the findings of Mohapatra, (2001).

One of the major complications of malaria infection is hypoglycaemia (WHO, 1990). An unexplained hypoglycaemia caused by *P. falciparum* malaria has been reported in Diabetes mellitus patients even before the initiation of quinine therapy (Jikki and Krishnamurrty, 1997; Mohapatra, 2001; Shalev *et al.*, 1992), likewise Elased *et al.* (1995) reported that the induction of malaria parasite or an extract of the parasite to a mouse model of NIDDM dramatically lowers the blood glucose level and resulted in reduced voluntary food intake, with consequent weight loss. Taylor *et al.* (1992) reported that injection of parasite supernatant caused a significant hypoglycaemia in normal mice. Infection of mice with blood-stage *Plasmodium yoelii* and *P. chabaudi* malaria induced hypoglycaemia in normal mice and normalized the hyperglycaemia of mice made moderately diabetic with streptozotocin (Elased *et al.*, 1995). It has also been shown that hypoglycaemia during infection with *P. Yoelii* and *P. chabaudi* was associated with hyperinsulinaemia, only occurring with parasitaemia above 50% (Elased *et al.*, 1995), although there was recovery when the parasitemia fell in the control group while in the diabetic mice, the blood glucose again fell to normal values. It was noted that the drop was greatest in the mice with the highest parasitaemia (Elased *et al.*, 1995).

Although this plant has been used separately for the treatment of diabetes and malaria, there is no documented study of its use in treating together both diabetes and malaria, this study was designed to explore the antiplasmodial and antidiabetic potentials of *Mormodiaca charantia* on combined infection of malaria and diabetes mellitus.

## MATERIALS AND METHODS

### Chemicals

Methanol used was product of Eagle Scientific Limited, Nottingham. Giemsa stain was obtained from Eagle Anosantec Laboratories, UK. Immersion oil was obtained from Panzonar Laboratories supplies, Button road, Canada. Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, Mo USA. Other reagents used were of analytical grade and were prepared using distilled water.

### Animals

Adult male mice, 4-6 weeks old, weighing between 20 $\pm$ 2 g obtained from Nigerian Institute of Medical Research (NIMR) Lagos was used for the study. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad-libitum* (NH publication #65-23, revised in 1985).

### Parasites

A chloroquine-sensitive strain of *Plasmodium bergei* (NK-65) was obtained from the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos state.

### Plant material and extract preparation

Fresh leaves of *Mormodiaca charantia* was harvested from a farm in Obantoko area of Abeokuta Ogun state and autenticated at the Department of Forestry and Wildlife of the University. The extract of the plant was prepared according to a method of Adewole *et.al.*, (2011). Fresh leaves of the plant were dried in the shade at room temperature and pulvrised to powder using an electric blender. 200 g of the powdered plant was percolated in 1 L of absolute methanol for 72 hours after which it was filtered. The filtrate was poured into a beaker and allowed to evaporate at room temperature to yield the extract concentrate, the percentage yield was 4.6% w/w.

### Phytochemical screening

The powedered sample of *Mormodiaca charantia* leaves was tested for the presence of secondarymetabolites such as alkaloids, flavanoids, phenolics and steroids following the methods described by Trease and Evans (1978).

### Experimental Design

Animals were randomly divided into 6 groups of eight mice each. Animals in group 1 were not innoculated with the parasite nor induced with diabetes, while the other groups innoculated from the same donor mouse and also induced with diabetes. The percentage parasitemia and the red blood cell count was first determined and appropriate dilutions of the infected blood with isotonic saline was done. Each mouse in the infected groups was innoculated on day 0 intraperitoneally with 0.2 ml of infected blood containing about 1 x $10^7$ *P. Berghei* parasitized red blood cells. Treatment was witheld for 5 days to allow for establishment of infection after which hyperglycemia was induced in the mice by intraperitoneal injection of 100mg/kg body weight of alloxan monohydrate. Treatment commenced when both conditions was established. Parasitemia was established by screening for malaria parasites in tail blood of infected animals after fixing in methanol and staining with Giemsa stain (Ryles 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).

Aqueous preparations of the extract corresponding to 100 mg/kg body weight, an optimum dose arrived at from studies of dose-dependent evaluation of hyperglycemia using 100 mg/kg, 200 mg/kg and 300mg/kg of the extract, chloroquine (corresponding to 5 mg/kg body weight) and glibenclamide (corresponding to 10 mg/kg body weight) were made before administering orally to the mice. The administration of the different treatment which lasted for five days is as follows:

Group 1 (uninfected and non-induced mice): received an appropriate volume of sterile distilled water corresponding to the highest volume of extract administered.

Group 2 (infected and induced mice): received an appropriate volume of sterile distilled water.

Group 3 (infected and induced mice): received the aqueous preparation of the extract (100 mg/kg body weight daily).

Group 4 (infected and induced mice): received the aqueous solution of chloroquine (5 mg/kg body weight daily).

Group 5 (infected and induced mice): received the aqueous solution of glibenclamide (10 mg/kg body weight daily).

Group 6 (infected and induced mice): received the aqueous solutions of chloroquine (5 mg/kg body weight) and glibenclamide (10 mg/kg body weight) daily.

### Sample colletion and analyses

Daily blood films were made from tail blood of all the infected animals (Groups 2, 3, 4, 5 and 6), the percentage parasitemia and blood glucose level obtained through microscopic determination and glucometer. Percentage chemosuppression was calculated by subtracting the average percentage parasitemia in the treated group from the average percentage parasitemia in the control group and the value obtained was expressed as a percentage of the average parasitemia in the control group. Animals were sacrificed under slight diethyl ether anesthesia.

Heamatological evaluation was done using the automated heamatological analyzer SYSMEX Corporation, Japan) wherein the red blood cell count (RBC), packed cell volume (PCV) heamoglobin concentration (Hb), mean corpuscular volume (MCV) were determined. The concentration of plasma urea was determined according to Fawcett and Scott (1960) whereas plasma creatinine and uric acid were determined spectrophotometrically as described respectively by Kaplan (1996) and Fosseti *et al.* (1980). Oxidative stress was evaluated by determining the total antioxidant potential (TAP), the total peroxide potential and the oxidative stress index was calculated using the method described by Harma *et al.*, (2003). Photomicograph of the histology of the pancreas and kidney sections of the animals were also evaluated.

### Statistical Analysis

Values are expressed as Mean ± Standard Error Mean. The data were statistically analyzed using analysis of variance (ANOVA) and Duncan Multiple Range test (Mahajan, 1997). Data from the test groups were compared with their respective controls and differences at $p<0.05$ were considered to be significant.

## RESULTS

Some of the phytochemicals present in the methanolic extract of *M. charantia* leaves are shown in Table 1. Table 2 shows the mean percentage parasitemia of infected mice before and during treatment. Parasitemia counts in the group treated with 100mg/kg body weight showed significant reduction ($p<0.05$) from 18.25% on day 2 to 7.42% on day 6 indicating 59.3% decrease within three days. Group MDQ treated with chloroquine also showed significant reduction ($p<0.05$) from 9.44% to 3.43% on day 6 indicating over 60% reduction while group MDB showed a reduction of 59.4% in parasitemia level within the same period of time. Group MDG treated with a standard antidiabetic drug glibenclamide revealed significant increase ($p<0.05$) in parasitemia from 11.98% to 23.31%.

TABLE 1 **Pytochemicals of the methanolic extract of *Mormodiaca charantia* leaves**

| Name | Qualitative | Quantitative(/%) |
|---|---|---|
| Alkaloids | + | 0.23±0.01 |
| Phenolics | + | 1.09±0.02 |
| Steroids | + | 0.52±0.01 |
| Flavonoids | + | 0.45±0.01 |

Each of the values was a mean of triplicate determinations ± standard deviation.

### Blood glucose level

Table 3 shows variations in blood glucose level of the animals during the experiment. The mean blood glucose level of the mice ranged from 62 mg/dl in group MDG to 70.66 mg/dl in group NC prior to induction with diabetes using a single dose of 100 mg/kg of alloxan monohydrate. Blood glucose during this period was within

TABLE 2 Effect of treatment on the parasitemia level of the groups induced with *P. berghei*.

| Group | Before Treatment | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | D O S |
|---|---|---|---|---|---|---|---|
| MDC | 6.55±0.62$^{a}$ | 5.83±0.26$^{a}$ | 5.31±0.60$^{a}$ | 7.64±0.66$^{ab}$ | 10.19±1.33$^{ab}$ | 14.4±2.68$^{bc}$ | 19.79±6.62$^{b}$ |
| MDP | 12.25±3.9$^{b}$ | 13.83±3.0$^{b}$ | 18.25±2.93$^{b}$ | 15.17±2.45$^{bc}$ | 12.50±4.49$^{ab}$ | 9.61±3.65$^{ab}$ | 7.42±1.95$^{a}$ |
| MDQ | 9.44±1.16$^{b}$ | 8.29±1.26$^{a}$ | 7.22±1.46$^{a}$ | 5.91±1.62$^{a}$ | 4.96±0.51$^{a}$ | 4.26±0.45$^{a}$ | 3.43±0.36$^{a}$ |
| MDG | 11.98±4.41$^{b}$ | 13.07±4.50$^{ab}$ | 14.72±4.88$^{ab}$ | 16.55±4.39$^{c}$ | 18.60±3.84$^{b}$ | 21.49±3.56$^{cd}$ | 23.31±3.51$^{b}$ |
| MDB | 6.99±0.92$^{a}$ | 8.32±1.18$^{a}$ | 7.88±1.39$^{a}$ | 5.53±0.74$^{a}$ | 5.44±0.78$^{a}$ | 4.77±0.69$^{a}$ | 3.29±0.42$^{a}$ |

Values are mean±S.E.M. n=4
Values within a column having different superscripts are significantly different at p<0.05
DOS- day of sacrifice

TABLE 3 Effect of treatment on blood glucose level in the different groups.

| GRP | BEFORE | CONFIRM | DAY2 | DAY3 | DAY4 | DAY5 | DAY6 |
|---|---|---|---|---|---|---|---|
| NC | 70.66±2.85$^{a}$ | 75.33±2.02$^{a}$ | 72.00±2.00$^{a}$ | 70.00±0.58a | 69.66±1.76$^{a}$ | 71.00±1.00$^{ab}$ | 71.66±0.67$^{ab}$ |
| MDC | 63.33±3.17$^{b}$ | 270.00±22.60$^{b}$ | 253.66±25.67$^{b}$ | 249.00±27.75$^{c}$ | 228.33±23.67$^{d}$ | 219.00±26.01$^{e}$ | 211.67±23.97$^{c}$ |
| MDP | 64.33±2.90$^{b}$ | 284.00±10.78$_{b}$ | 265.66±14.05$^{bc}$ | 202.66±11.55$^{b}$ | 137.66±12.02$^{b}$ | 89.00±3.21$^{abc}$ | 86.66±3.84$^{ab}$ |
| MDQ | 63.66±2.91$^{b}$ | 275.66±13.38$_{b}$ | 259.33±9.39$^{bc}$ | 243.00±11.55$^{c}$ | 232.00±19.92$^{d}$ | 204.66±7.86$^{e}$ | 199.33±3.53$^{c}$ |
| MDG | 62.00±3.79$^{b}$ | 287.00±12.85$_{b}$ | 268.00±6.08$^{b}$ | 226.66±4.33$^{bc}$ | 176.00±7.93$^{c}$ | 131.66±7.69$^{d}$ | 100.66±3.76$^{b}$ |
| MDB | 68.33±1.45$^{a}$ | 301.66±7.88$^{b}$ | 270.66±13.91$^{bc}$ | 217.66±6.57$^{bc}$ | 156.66±8.83$^{bc}$ | 111.33±10.48$^{cd}$ | 90.66±11.57$^{ab}$ |

Values are mean±S.E.M. n=4
Values within a column having different superscripts are significantly different at p<0.05

TABLE 4 Effect of Treatment on some kidney function test.

| Group | Creatinine (mg/dl) | Uric acid (mg/dl) | Urea (mg/dl) |
|---|---|---|---|
| NC | 0.79±0.07$^{a}$ | 2.05±0.10$^{ab}$ | 7.05±0.51$^{a}$ |
| MDC | 0.54±0.03$^{a}$ | 1.28±0.17$^{ab}$ | 24.63±2.16$^{b}$ |
| MDP | 1.23±0.09$^{ab}$ | 2.68±0.29$^{bc}$ | 30.15±8.46$^{b}$ |
| MDQ | 0.47±0.11$^{a}$ | 1.33±0.17$^{ab}$ | 29.90±1.85$^{b}$ |
| MDG | 0.57±0.12$^{a}$ | 0.78±0.12$^{a}$ | 26.75±4.69$^{b}$ |
| MDB | 0.98±0.30$^{ab}$ | 1.35±0.35$^{ab}$ | 51.55±10.40$^{c}$ |
| PO | 1.21±1.08$^{ab}$ | 3.87±1.23$^{c}$ | 13.10±1.59$^{a}$ |

Values are mean±S.E.M. n=4; Values within a column having different superscripts are significantly different at p<0.05

NC- Normal control, MDC- Malaria diabetic control, MDP- Malaria diabetic treated 100mg/kg body weight of methanolic extraction of M.charantia, MDQ- Malaria diabetic co- infected treated with 5mg/kg body weight of Chloroquine phosphate, MDG- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide, MDB- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide and 5mg/kg body weight of Chloroquine phosphate

normal range for all the animals. All groups induced with hyperglycemia (MDC, MDP, MDQ MDG and MDB) had about three to four fold increase in their mean glucose values having values between 270 mg/dl and 301 mg/dl which confirmed them as being diabetic.

MDC had its mean glucose level decreased from 270 mg/dl to 211.67 mg/dl (21.85%) on day 6. MDB had the highest percentage decrease in mean glucose level (70.10%). The group treated with the plant extract MDP had decreased mean glucose values indicating a percentage decrease of 69.72%. There was significant difference ($p<0.05$) in blood glucose value of the treated groups compared to the control group. The group treated with a standard antidiabetic drug glibenclamide had a decrease from 287.00 mg/dl to 100.66 mg/dl indicating a percentage decrease of 65.15% after the 5 days of treatment while group MDQ recorded a mean blood glucose reduction from 275.00 mg/dl to 199 mg/dl indicating a 27% decrease although the value was not significanly different at $p>0.05$ from the control group (MDC).

**Kidney function test**

Table 4 shows the values of some biochemical parameters indicating the kidney function test. The normal control group had a value of 0.79 mg/dl. MDP treated with the plant extract had increased creatinine value of 1.23 mg/dl which was significantly different ($p<0.05$) from the normal control group. MDQ and MDG groups treated with the standard drugs chloroquine and glibenclamide had reduced creatinine having values of 0.47 mg/dl and 0.57 mg/dl respectively although not significantly different ($p>0.05$) when compared with MDC and NC.

The uric acid value was significantly ($p<0.05$) reduced in group MDG having a value of 0.78mg/dl when compared to other groups. The group treated with the plant extract had increased uric acid concentration; other treated group was also reduced.

All the infected groups had elevated urea concentration. About seven-fold increase was observed in group MDB when compared to the normal control group. All the treated groups had increased mean urea values, ranging from 26.75 mg/dl to 51.55 mg/dl and these were significantly different ($p<0.05$) compared to the normal control group (NC).

**Heamatological parameters**

Table 5 shows the effects of treatment on the heamatological parameters. It was observed that on day two of treatment, all groups infected with P.berghei had a significantly decreased PCV values (ranging from 16.60% to 25.67%) compared to the normal control group with a mean PCV value of 45.40%. The malaria-infected control group (MDC) had the least PCV value among all the groups. The treated groups MDQ, MDB and MDP had their mean PCV values increased with MDQ having the highest percentage increase of about 46.75%.

The Hb level of the of the infected control group MDC was significantly ($p<0.05$) reduced. MDQ had the highest Hb which was comparable to NC. The groups treated with 5 mg/kg chloroquine (MDQ and MDB) had their RBC levels increased compared to the other treated groups. The MCH of all the groups was not significantly ($p>0.05$) changed from NC. The MCHC of all the different groups was not significantly different ($p>0.05$) except for group MDC which increased when compared to NC ($p<0.05$). Likewise the MCV of all the groups except MDC was not significantly different from the NC.

**Oxidative stress**

Figure 1 shows the mean plot of the total peroxidation potential (TPP) and oxidative stress index (OSI) of the different groups in the course of the experiment. MDC had the highest level of TPP and OSI while NC had the least values for both parameters. The group treated with the plant extract MDP had TPP lower than the groups treated with both standard drug (MDQ, MDG and MDB). Figure 2 shows the different levels of the mean total antioxidant potential (TAP) of the different groups during the course of the experiment. MDQ had a lowered TAP while the group treated with both standard drugs (MDB) had TAP which was more than othe treated groups.

**Histological studies**

**The Pancreas**

Result of the histological studies on the pancreas of treated and untreated malaria-infected and diabetic-induced mice are presented in plates 1-4. Photomicrograph of a section of the pancreas of normal control mice showed normal arrangement of the islets of langerhans of various sizes scattered throughout the exocrine tissue (Plate 1). The pancreatic acinic and duct system appeared normal. Photomicrograph also reveals normal arrangement of the glandular acinni. The result showed no observable histological defect in the pancreas of the control mice.

Histological observations of the pancreas of the malaria-induced diabetic control mice (MDC) showed focal areas of marked degeneration of the islet of langerhans and the presence of intestitial oedema (Plate 2), this was also observed in infected group treated with chloroquine (MDQ) as seen in plate 4, in which the intestitial oedema was severe. There was no visual changes in the pancreas of the co-infected group treated with the plant extract as can be seen in plate 3 in which the degenerative changes was markedly reduced.

**Kidney**

Histopathological defects observed in the photomicrographs of kidney sections of treated and untreated mice are presented in Plates 5 to 8. Histological studies on the kidney of normal control mice revealed no visible lesion as the renal corpuscles appeared normal in dense rounded structures (Plate 5). Photomicrograph from kidney of mice in the positive control group (MDC) showed dilation of tubules as seen in plate 6. Atropy of the glomerulus and dilation of the Bowman capsule was observed in the histology section of the kidney of the malaria-infected and diabetic-induced mice treated with the plant extract (MDP). Plate 8 showed severe dilation of the tubules in the malaria-infected and diabetic-induced mice treated with both standard drugs (MDB).

TABLE 5 Effects of treatment on the Heamatological parameters.

| Group | PCV-Day 2 | PCV-Day 4 | PCV-Day 6 | Hb | RBC | MCH | MCHC | MCV |
|---|---|---|---|---|---|---|---|---|
| NC | 45.40±1.88$^{d}$ | 45.55±2.04$^{e}$ | 51.25±0.63$^{e}$ | 19.12±1.17$^{de}$ | 9.15±0.51$^{e}$ | 20.97±1.15$^{a}$ | 0.37±0.02$^{ab}$ | 5.68±0.33$^{ab}$ |
| MDC | 16.60±3.75$^{ab}$ | 12.17±1.73$^{a}$ | 10.25±0.85$^{a}$ | 5.72±0.96$^{a}$ | 2.77±0.37$^{a}$ | 20.48±1.00$^{a}$ | 0.45±0.05$^{b}$ | 4.66±0.34$^{a}$ |
| MDP | 23.75±2.81$^{abc}$ | 23.77±3.41$^{bc}$ | 30.75±5.72$^{c}$ | 12.45±2.56$^{bc}$ | 5.35±1.42$^{bc}$ | 27.50±6.18$^{a}$ | 0.39±0.03$^{ab}$ | 6.40±0.97$^{ab}$ |
| MDQ | 25.67±8.02$^{abc}$ | 31.20±3.93$^{d}$ | 46.75±0.25$^{e}$ | 18.67±0.75$^{de}$ | 7.42±0.23$^{de}$ | 25.16±0.77$^{a}$ | 0.39±0.02$^{ab}$ | 6.36±0.19$^{ab}$ |
| MDG | 24.45±2.08$^{abc}$ | 19.32±0.78$^{ab}$ | 20.00±0.91$^{b}$ | 7.72±0.39$^{a}$ | 3.80±0.35$^{ab}$ | 20.68±1.48$^{a}$ | 0.37±0.01$^{ab}$ | 5.52±0.43$^{ab}$ |
| MDB | 18.35±3.93$^{ab}$ | 22.52±3.65$^{bc}$ | 33.25±1.79$^{d}$ | 15.75±0.73$^{cd}$ | 7.15±0.39$^{de}$ | 22.41±2.39$^{a}$ | 0.39±0.01$^{ab}$ | 5.61±0.58$^{ab}$ |

Values are mean±S.E.M. n=4; Values within a column having different supercripts are significantly different at p<0.05

NC- Normal control, MDC- Malaria diabetic control, MDP- Malaria diabetic treated 100mg/kg body weight of methanolic extraction of M.charantia, MDQ- Malaria diabetic co- infected treated with 5mg/kg body weight of Chloroquine phosphate, MDG- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide, MDB- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide and 5mg/kg body weight of Chloroquine phosphate

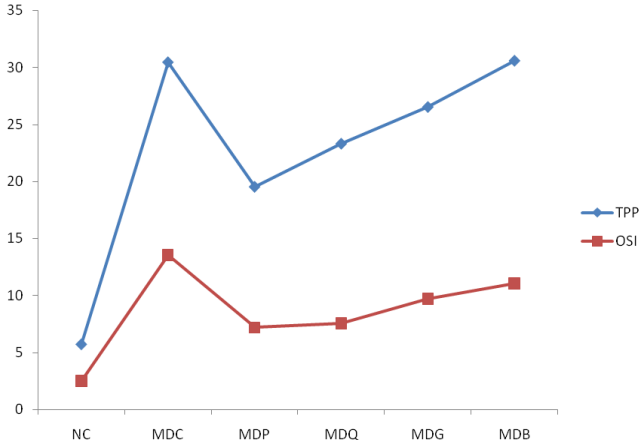


FIGURE 1 Effect of treatment on the total plasma peroxide (TPP) in µmol$H_2O_2$/L and oxidative stress index (%). NC- Normal control, MDC- Malaria diabetic control, MDP- Malaria diabetic treated 100mg/kg body weight of methanolic extraction of M.charantia, MDQ- Malaria diabetic co- infected treated with 5mg/kg body weight of Chloroquine phosphate, MDG- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide, MDB- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide and 5mg/kg body weight of Chloroquine phosphate

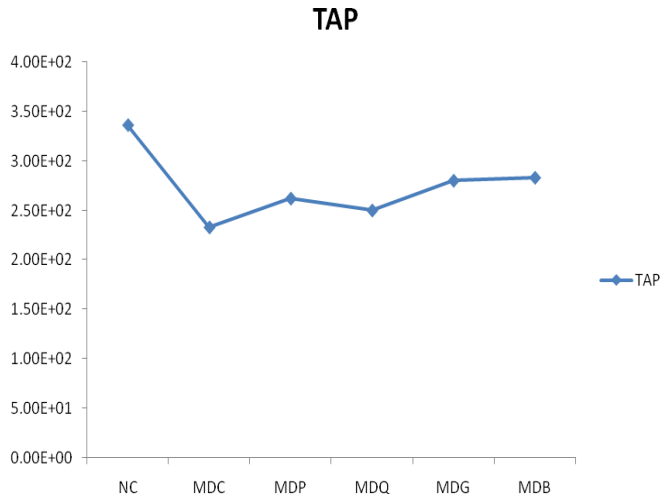


FIGURE 2 Effect of treatment on the Total antioxidant potential (TAP) in µmol.Trolox eqiuv/L. NC- Normal control, MDC- Malaria diabetic control, MDP- Malaria diabetic treated 100mg/kg body weight of methanolic extraction of M.charantia, MDQ- Malaria diabetic co- infected treated with 5mg/kg body weight of Chloroquine phosphate, MDG- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide, MDB- Malaria diabetic co- infected treated with 10mg/kg body weight of glibenclamide and 5mg/kg body weight of Chloroquine phosphate

## DISCUSSION

Prevalence of type 2 diabetes has been reported to be on increase worldwide (1998; Ghosh et al., 2004; Biyani et al., 2003). Although hypoglycemic agents, dietary fiber and low glycemic index foods are widely available, the public continues to maintain a substantial interest in dietary adjuncts that possess hypoglycemic properties. Over 1000 herbal products have been used by various cultures to lower blood glucose and treat diabetes. Among them, Momordiaca charantia (family Cucurbitaceae), commonly known as ku gua, karela, bitttergourd or bitter melon) is a popular herbal resource ( Marles and Farnsworth, 1995; Ethan et al., 2003).

Malaria caused by P. falciparum is a common and life-threatening disease responsible for over one million deaths annually (WHO, 2008). Presently, malaria is still the most dangerous parasitic infectious disease causing millions of death annually (Inga et al., 2002). In many countries where malaria is endemic the traditional medical methods hold a strong part in the public health care system. For safety reasons phytochemical, pharmacological and biochemical investigations on medicinal plants traditionally used as antimalarials are urgently needed (Inga et al., 2002).

Although *M. charantia* has been known for its hypoglycemic effect (Giron et al., 1991; Abascal and Yarnell 2005), its antiplasmodial effects had been reported by Munoz et al. (2000), and Inga et al. (2002), while Singh et al. (2006) demonstrated its larvicidal activity. *M.charantia* has been used separately in the treatment of diabetes and malaria infection; there has not been any literature report to the best of our knowledge that reported its use in treating co-occurrence of both diabetes and malaria. In this study, we investigated some biochemical and histological changes that could be associated with treatment of malaria and diabetes mellitus in mice using extract of *Mormodiaca charantia*.

The geometric increase in blood glucose concentrations observed in all mice after a single intraperitoneal injection of alloxan monohydrate confirmed the induction of insulin-dependent diabetes mellitus (IDDM) in the mice. Earlier works by Nimenibo-Uadia (2003) and Ghosh et al. (2004) revealed multiple increases in blood glucose concentration after a single injection of alloxan monohydrate. This increase has been attributed to the toxic effect of alloxan on the beta cells of the pancreas in which alloxan and the product of its reduction; dialuric acid established a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide and reactive oxygen species with a simultaneously massive increase in cytosolic calcium concentration that ultimately causes rapid destrution of the beta cells. This destruction results in the inability of the pancreas to synthesize and secrete adequate amount of insulin necessary for the metabolism of carbohydrate. All treated mice responded positively to treatment with the plant extract and the standard antidiabetic drug, glibenclamide. During the course of treatment, marked reduction in blood glucose concentration was recorded for all treated animals. Blood glucose level in the positive control group was reduced after the experiment (about 22%), probably due to the parasite. Reports have shown that Plasmodium parasite causes hypoglycemia in experimental mice (Elased et al., 1995; Mohapatra, 2001; Shalev et al., 1991; Taylor et al., 1992) and resulted in reduced voluntary food intake, with consequent weight loss. The group treated with the plant extract, MDP had their glucose levels reduced more than the group treated with the antidiabetic standard drug, glibenclamide. This better performance recorded by M. charantia may be due to a hypoglycemic polypeptide (polypeptide-P) of molecular weight of approximately 11,000 (166 amino acid residues) and a glycoalkaloid (vicine), which have been characterized from the fruits, seeds and leaves of the plant (Raman and Lau, 1996).

Malaria infection was established in the mice by a single intraperitoneal injection of 0.2ml of 1 X 107 parasitized red blood cells from donor mice of the same strain. Parasitemia was established in the mice when their tail blood was viewed under the microscope five days after induction. All treated groups had their parasitemia level decreased significantly ($p<0.05$). Group treated with chloroquine had their parasitemia decreased well above 50% while the treated with the extract had decrease in parasitemia with about 50%. It was observed that the parasitemia in the positive control group increased all through the course of the experiment. Parasitemia of the groups treated with chloroquine started decreasing after the first day of treatment while the plant extract started reducing the parasitemia between the third and fourth day of treatment. Although, there has not been a mechanism of action to the best of our knowledge, however, it has been reported that the fraction of M. charantia that had antimalarial effect contained flavones, alkaloids and phenolic that could be responsible for the observed antimalarial activity.

Five days after induction with P. Berghei, parasitemia was established in the animals after which they were made diabetic by a single intraperitoneal injection of alloxan monohydrate, the mean weight recorded for all animal groups was observed to reduce (data not shown). This could be as a result of low food and water intake that was observed in the infected groups. Other reasons for these observed low body weight may be due to the destructive effect of the injected alloxan monohydrate. Kliber et al. (1996) reported specific necrosis of the pancreatic islets which was also observed in the photomicrograph of the pancreas in this study. Histological examination showed necrosis of the pancreatic acini in all groups that were made hyperglyceamic using alloxan monohydrate. Also, malaria causes loss of apetite and this could also account for the weight loss in the different groups. The normal control group (NC) had a consistent increase in weight while the reverse was the case for the positive control group MDC.

Kidneys maintain optimum chemical composition of the body fluids by acidification of urine and removal of metabolite wastes as urea, uric acid, creatinine and ions (Biyani et al., 2003). During renal disease, the concentrations of these metabolites increase in blood. Increase in urea level has been observed in acute and chronic renal disease (Biyani et al., 2003). There was high level of urea in the malaria-infected and diabetic-induced groups in this study. Geoffrey (2006) reported that creatinine level can be either normal or high during renal diseases. Results showed that the group treated with plant extract had an increased creatinine value of 1.23 mg/dl. Photomicrogram of the kidney tissue of the malaria-infected

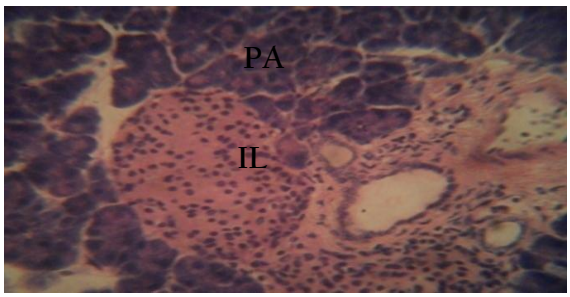


PLATE 1 Pancreas section showing normal appearance of the pancreatic acini (PA) and islet of langerhans(IL) in the pancreas of the normal control group (x 300) E & H.

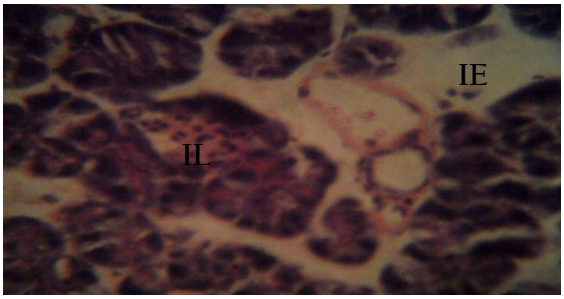


PLATE 2 Pancreas section showing marked degeneration of the pancreatic islets (IL) and interstitial oedema (IE) in the pancreas of the coinfected control group (x 300) E & H.

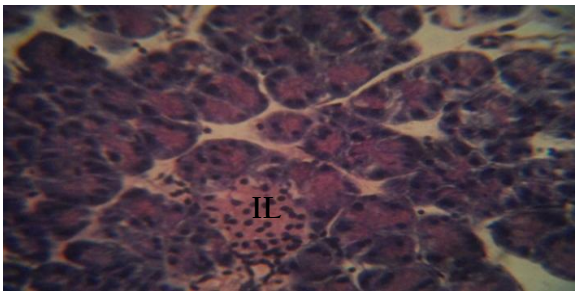


PLATE 3 Pancreas section of a mild degeneration of the islet of langerhan (IL) in the pancreas of the coinfected group treated with 100mg/kg of the methanolic extract of M.charantia (x 300) H & E.

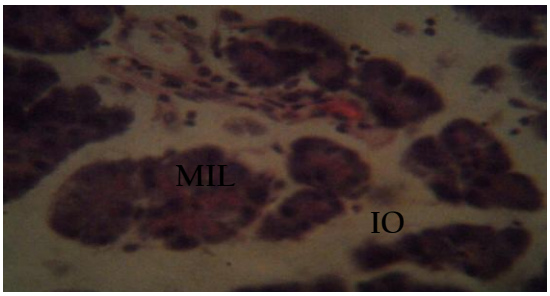


PLATE 4 Pancreas section of severe interstitial oedema (IO) and marked degeneration of the islet of langerhan (MIL) in the pancreas of coinfected group treated with 5mg/kg of chloroquine (x 300) E & H.

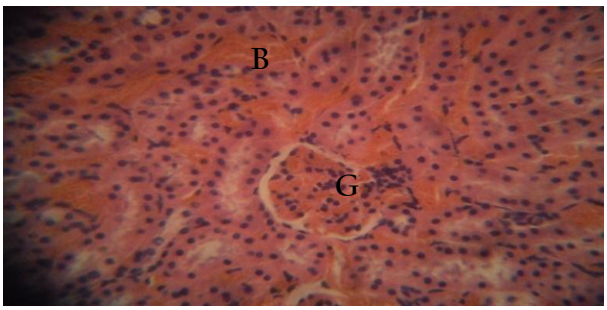


PLATE 5 Section of the normal control group showing normal appearance of the glomerulus (G), tissues and blood vessels (B) of the kidney (x 300) E & H.

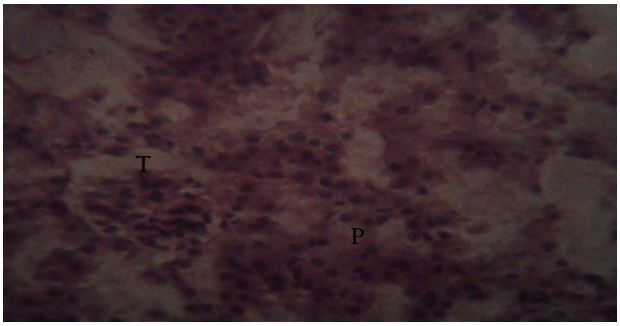


PLATE 6 Section of the kidney of the positive co-infected group showing dilation of the tubules (T) and luminar containig protein cast (P) (x 300) E & H.

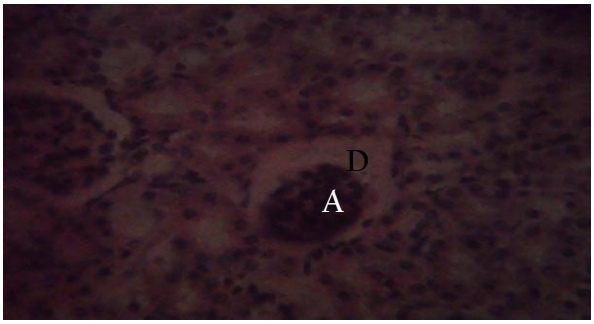


PLATE 7 Section of the infected group treated with the plant extract kidney showing mild atropy (A) of the glomeruli and dilation (D) of the Bowman space (x 300) H & E

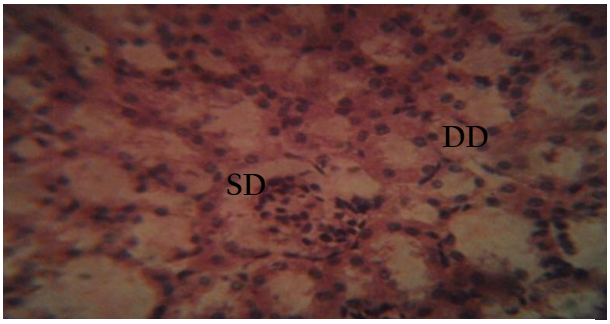


PLATE 8 Section of the co-infected group treated with both standard drugs showing severe dilation (SD) of the tubules in the glomeruli. (x 300) H & E.

and diabetic-induced mice revealed dilation of the bowman capsule. This could be linked to increased body fluids and metabolites observed.

Packed cell volume (PCV) which is a measure of the relative mass of cells present in a sample of blood was observed to increase in all the groups that were treated as observed on the day that the animals were sacrificed. This observation of anemic state has been reported in Plasmodium infected mice that were untreated (Iyawe and Onigbinde, 2009; Adam et al., 2005; Akpotuzor et al., 2007). Anaemia, a condition characterized by decrease in total cell mass of the blood, it is a state in which the blood index values of total haemoglobin is ‹12g/dl (Uthman, 1994). Result obtained from this study showed that all the groups infected with malaria were anaemic, although there was increase in the Hb level with treatment especially with the groups treated with the plant extract; this therefore suggests the haemopoetic properties of the extract used. Most diabetic patients suffer from oxidative stress probably as a result of increased pro-oxidant and lowered antioxidant concentration. In our study, MDC had the highest total peroxidation potential and oxidative stress index. This could be as a result of the increased level of pro oxidants produced in the pancreas as reported by Bansal et al. (1980).

## CONCLUSION

Result obtained from the study revealed the antiplasmodial and hypoglycemic potentials of *Mormodiaca charantia* for the treatment of malaria and diabetes mellitus. Results of physiological and biochemical analysis observed in this study have also suggest that treatment with the plant extract might not induce negative side effects on organ funtions at the tested dosages.

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