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Anti-malaria and hypoglycaemic activities of Diosgenin on alloxan-induced, diabetic Wistar rats

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Abstract

The rising threat of *Plasmodium falciparum* resistance to Monotherapies has prompted the world health organization (WHO) 2006 guidelines to recommend the use of different anti-malarias. In this study, the anti-malaria and hypoglycaemic activities of Diosgenin, a potent, yet poorly reported saponin was investigated on *P. falciparum* inoculated and Alloxan-Induced, Diabetic Wistar Rats. Fort two (42) adult male wistar rats of between 100g and 150g were procured, acclimatized (for two weeks), and grouped into seven of six (6) rats per group. While Group 1 (Normal control) received normal rat *chow* and water *ad libitum*, groups 2 – 4 received no treatment (untreated), 10 mg/kg body weight of anti-diabetic Metformin and 25 mg/kg body weight of diosgenin respectively after inducing diabetes mellitus (DM) with alloxan monohydrate; whereas, groups 5-7 (all malaria infected) were untreated (negative malaria control), 25 mg/kg body weight of diosgenin and 56 mg/kg body weight of anti-malaria coartem respectively. Following treatment period, blood samples were obtained and assayed for fasting blood sugar, packed cell volume (PCV) and total white blood cell count (TWBCC). From the result, *P. falciparum* exposed rats showed lowered PCV values than control with observed improvements in coartem (significant at $p < 0.05$) and diosgenin (insignificant) treatment groups. Also, diabetic, diosgenin treated rats showed an insignificant reduction in blood sugar levels compared to control, even though this change was apparently improved compared to diabetic, untreated group. Again, TWBCC caused notable decrease in diosgenin treated, though this decrease signified a huge recovery compared to untreated rats. Corroborative studies on diosgenin with other systems is recommended.

Keywords: Diosgenin; Coartem; Diabetes mellitus; Malaria

1. Introduction

Diosgenin [(3β, 25R)-spirost-5-en-3-ol], also known as sapogenin, is a hydrophilic sugar moiety associated with aglycone hydrophobic steroid present as glycosides (saponins) in fenugreek and yam (Dioscorea spp) [1]. It is a medication used pharmacologically to generate one of the world's most costly fertility medications and steroid hormones [2]. Balancing hormones, treating menstrual disorders, increasing fertility, increasing sexual libido, treating erectile dysfunction, increasing ovulation, increasing testosterone levels in men, increasing breast and buttock size in women, promoting hair growth, good for the skin, etc. have been documented [3]. For menopausal women, it is one of

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the best foods that balances their hormonal levels and decreases menopausal symptoms such as hot flashes, dryness of the vagina, unnecessary sweetening, mood changes, etc.

Diosgenin has been studied for its anti-infectious activity against fungi, bacteria, protozoa, and viruses over the past few years. Human pathogenic bacteria, C. Antifungal activity against *Candida albicans*. The antimicrobial activity of this steroid against all species tested was poor in Glabrata, *C. tropicalis* [4, 5]. In addition, diosgenin has a low to zero impact against the fungi *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma harzianum*, and *Fusarium oxysporum*; showing major inhibition areas when studied with multiple Gram-positive pathogens (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative pathogens (*Escherichia coli* and *Salmonella typhi*) [6]. Also, at molecular and cellular levels, its anti-amebial activity against *Naegleria fowleri* trophozoites has been explored. Interestingly, activity of the anti-surface membrane and Nf cysteine protease of fowleri trophozoites has been suggested.

More so, the therapeutic toxicity of this steroid to mammalian cells has been shown to be lower than that of the commonly used drug amphotericin B for the treatment of N. Fowlers' Diseases [7]. Furthermore, in some viral diseases, it has been shown to be an exciting molecule in certain cases. It can also be beneficial for HIV patients with dementia because of its antioxidant activity [8]. In *in vitro* studies, this steroid also displays antiviral activity against Hepatitis C Virus (HCV). Since plasma cholesterol can be reduced and HCV requires cholesterol to replicate effectively, this impact can be associated with viral replication inhibition [9].

There are also reports that support diosgenin's potential in diabetes management. Most reports report that diosgenin increases the dysfunction of oxidative stress and lipid metabolism. In addition, diosgenin-rich fenugreek enhances hepatic steatosis and hyperlipidemia in obese diabetic mice by suppressing mRNA lipogenic gene expression [10]. Additionally, the effects of diosgenin on blood glucose and intestinal amylase and ATPase's in streptozotocin-induced diabetic rats have been studied. In the proximal region of the small intestinal mucosa of diabetic rats treated with diosgenin, the function of alpha-amylase improved considerably.

In fasting blood glucose, decreased Na^{+}-k^{+}-ATPase activity was also observed in the proximal region of Ca^{2+} ATPase activity relative to diabetic control activity [11]. As the substance responsible for the inhibitory action of fenugreek, diosgenin prevents Triglyceride (TG) accumulation and lipogenic gene expression in HepG2 cells, leading to the therapeutic effects of fenugreek on lipid metabolism [12]. The effects of diosgenin on enzyme levels have recently been studied. In diabetic rats fed diosgenin, plasma glucose and glucose-6-phosphatase levels decreased significantly relative to diabetic control levels.

The liver function of ATP-citrate lyase, pyruvate kinase and glucose-6-phosphate dehydrogenase in diabetic rats has been substantially reduced compared to ordinary control rats [13]. 3T3-L1 pre-adipocyte lipid accumulation research has shown that diosgenin (levels ranging from 0.1 to 10 $molL^{-1}$) can promote the expression and differentiation of adipocytes, which can help minimize circulating lipids in the blood and contribute to hypolipidemic activity in type 2 diabetes rats [14]. The ability to produce anti-diabetic effects has been shown to reduce hyperglycemia and insulin resistance and mitigate metabolic dysregulation of the plasma and tissue lipid profile [15].

1.1. Aim of Study

This study investigated the anti-microbial and hypoglycaemic effects of diosgenin and coartem on alloxan-induced, diabetic Wistar rats. Specifically, the study;

- Investigated the hypoglycemic effects of diosgenin on alloxan-induced, diabetic wistar rats.
- Determined the anti-malaria effects of diosgenin on *Plasmodium falciparum* inoculated, coartem treated wistar rats.
- Examined the effects of diosgenin on the packed cell volume and white blood cell count of malaria infected wistar rats.

2. Material and methods

2.1. Animals

A total of forty two (42) male wistar rats of between 100-150g were procured from the animal unit of the college of medicine, Ambrose Alli University, Ekpoma, Edo State. Animals were then housed in wooden cages with full access to portable water and standard feed ad libitum, following which they were acclimatized for two weeks in compliance with guidelines from the National Institute of Health on the care and handling of laboratory animals.

Table 1 Study Design

Groups	Rats Condition	Treatments	Dose (Mg/Kg)
Group 1	Normal control	Standard feed and water	---
Group 2	Alloxan Diabetic (Negative control)	None	---
Group 3	Alloxan Induced (Diabetic)	Metformin	10mg / kg
Group 4	Alloxan Induced (Diabetic)	Diosgenin	25 mg/kg
Group 5	*P. falciparum* Infected	None	----
Group 6	*P. falciparum* Infected	Diosgenin	25 mg / kg
Group 7	*P. falciparum* Infected	Coartem	56 mg/kg

Above table shows the grouping of rats (Wistar) in the study. Here, a total of forty two (42) rats were grouped into seven (7) groups of six (6) rats each, then induced with diabetes mellitus (DM) and malaria (*P. falciparum*), then treated with varying regimens of coartem (anti-malaria), Metformin (anti-diabetes) and Diosgenin as listed.

2.1.1. Extraction of Diosgenin

First, about 1g of dried and ground fenugreek leaves/fruits was obtained, suspended in 25 ml HCl (3 M) and 25 ml n-hexane, stirred for 2 hours at 90-96℃ with a magnetic bar on a hot plate and extracted for diosgenin according to the method of Wang *et al*., (2007) [9]. Obtained mixture was then cooled and removed three times, each time with 25 ml of n-hexane. Next, the combined organic process was washed with 25 ml of KOH solution (1%) three times and then with 25 ml of distilled water three times, while using a rotary evaporator to evaporate the organic phase to dryness at 40℃.

The dry matter obtained was dissolved in 1 ml of acetonitrile and then analyzed by the HPLC method. On a Knauer (Berlin, Germany) HPLC system equipped with a Wellchrom K-1001 pump, a Wellchrom K-2600 UV-Visible detector and a Nucleosil RP C18 column (125 mm length, 4 mm inner diameter and 5 µm particle size, Knauer, Berlin, Germany), HPLC analyses were performed. At a flow rate of 1.0 ml min-1 at 35℃, a mixture of acetonitrile: water (90:10 v/v) was used as the solvent. Twenty sample microliters (in acetonitrile) have been injected and their chromatograms at 214 nm have been registered. All the study was done at least four times.

2.1.2. Confirmation of Diosgenin

By matching retention time (Rt = 12.27 min) with that obtained for pure (standard) diosgenin, Diosgenin was confirmed, and also by co-elution with pure diosgenin. The concentration of diosgenin in the samples was determined using their peak areas and the calibration equation obtained at different known concentrations for the normal (pure) diosgenin.

2.2. Preparation of Animals for Inoculation with *Plasmodium berghei*

From the laboratory animal building of the college of medicine, Ambrose Alli University Ekpoma, Edo State, two *Plasmodium berghei* infected (donor) rats were purchased from the same facility and were used shortly after purchase to inoculate some other rats (on the seventh day of acclimatization). The experimental animals were then acclimatized for 7 days and maintained at a temperature of 28℃ in a well-ventilated room and fed mash and water *ad libitum* to top feed growers for the entire duration. Good hygiene was maintained by constantly cleaning of the cage, replacement of beddings and disinfecting the floor where the cages were placed [16].

Parasite Inoculation: A modified method similar to Ryley and Peters was employed. Standard inoculums of 1×10^5 *Plasmodium berghei*-infected erythrocytes were injected intra-peritoneally into the experimental animals. Seventy-two hours later, the animals were administered with varying doses of diosgenin and monitored for changes.

2.3. Preparation and Administration of Coartem

The antimalaria drug Coartem (Artemether and Lumefantrine) was obtained from local pharmacy in Ekpoma, Edo State. The tablet containing 80/480 mg/kg of both active ingredient was meshed into powder and homogenized in 150ml of

distilled water (H_20). The homogenized mixture was then allowed to stand for 24 hours after a series of periodic stirring. The mixture was collected in a clean container and preserved in a refrigerator at minimum cool temperature. About 56 mg/kg (0.25ml) of it was given to animals (morning and evening) orally between 8:00am and 4:00pm for 3 days with the aid of an orogastric canula.

Induction of Diabetes: To induce experimental diabetes in rats, alloxan monohydrate was used using the method defined by Kalbag et al (2011). Animals were fasted for 24 hours, accompanied by a single dose injection of 150 mg/kg body weight of intraperitoneal alloxan monohydrate. For 3 days, the alloxanized mice were kept with free access to feed and water to develop hyperglycaemia. A Touch Glucometer was used to assess baseline fasting blood glucose levels (Lifescan, USA). For the study, rats with levels of glucose above 200 mg/dl were recruited.

2.4. Hematological Analysis

2.4.1. Determination of Packed Cell Volume (PCV)

Red blood was obtained using capillary tube via the ocular blood collection and then sealed using plasticin. The tubes were then centrifuged (spinned) using hematocrit centrifuge for 5 minutes. After centrifuging, the percentage of parked cell volume was then read using hematocrit reader (PCV table reader).

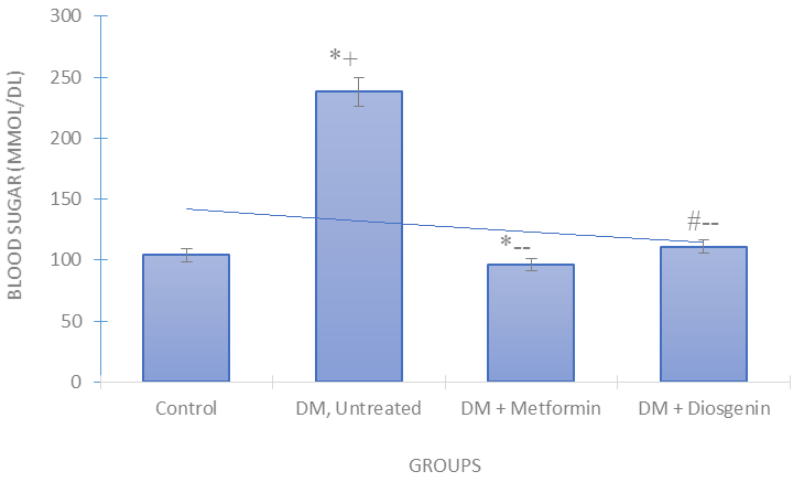
2.4.2. Determination of White Blood Count (WBC)

Drops (2) of the whole blood was obtained from the EDTA container using pasture pipette and dispensed into plain container. 0.3ml of the diluting fluid (Turk`s solution) was added and mixed gently. It was allowed to stay for 3minutes and the Naubaur chamber was flushed with 2-3 drops of the diluted blood, and after was loaded and allowed to settle for 5minutes undisturbed. After 5mins, it was focused with ×40 0bjective lens of the microscope. White blood cell where counted from each of the Naubaur chamber.

2.5. Statistical Analysis

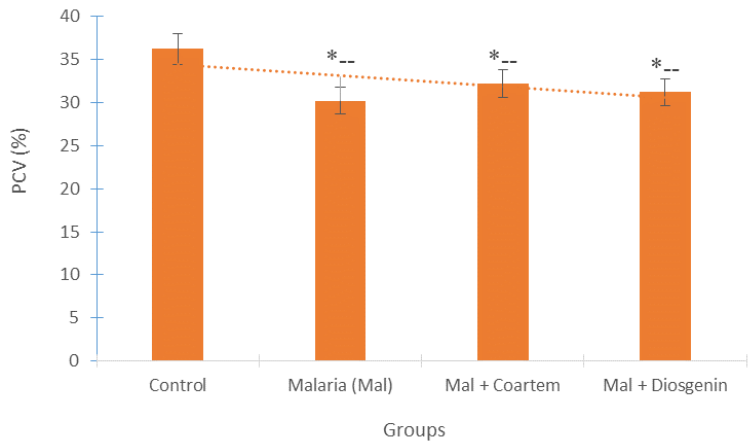
Obtained results were represented as mean standard deviation. Statistical analysis was done using the one-way analysis of variance (ANOVA) and post-hoc (tukey) test. Statistics was carried out with a graph pad prism software (version 8.0). A p-level less than 0.05 was considered as statistically significant.

3. Results and discussion



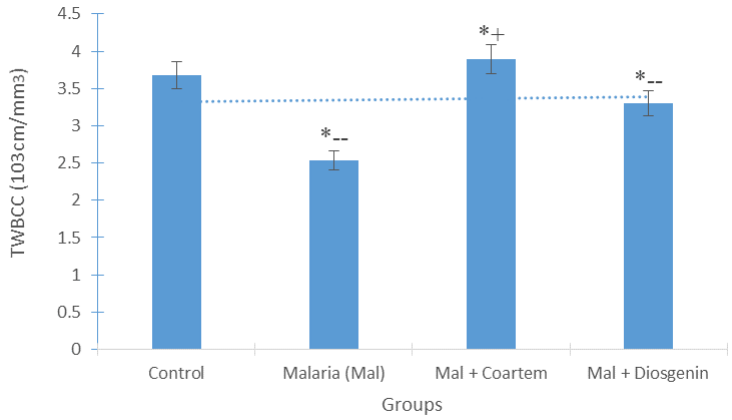
*+ = statistically significant increase ($p < 0.05$) compared with control, *-- = significant decrease
#-- = insignificant decrease ($p > 0.05$)

Figure 1 Comparison changes in blood sugar levels of diosgenin and metformin treated diabetic rats



*-- = significant decrease as compared with control group

Figure 2 Comparison changes in Packed Cell Volume (PCV) of malaria infected, coartem and diosgenin treated rats



*+ = statistically significant increase ($p < 0.05$) compared with control, *-- = significant decrease

Figure 3 Comparison Changes in Total White Blood Cell Count (TWBCC) of Malaria Infected, Coartem and Diosgenin Treated Rat.

This study investigated the anti-malaria and hypoglycemic effects of Diosgenin and Coartem on wistar rats with the view to explaining their hypoglycemic actions.

Results from current study (Figures 2) shows that compared with rats of the control group, mean packed cell volume (PCV) was significantly decreased in malaria infected, negative control mice (x = 30.24) who were left untreated than non-inoculated (control) animals (x = 36.25). This implies that on the average, inoculation of rats with *P. falciparum* lowered PCV values of rats that were exposed to it. This observation is consistent with previous findings of Ofem *et al.* (2013) who reported malaria infected human subjects to have significantly lower PCV levels than non-malaria sufferers, which ultimately results in low PCV [17].

For malaria infected rats that were administered with Coartem, current study observed that treatment with coartem significantly lowered the PCV levels compared to control, even though this decreased signified a recovery and improvement over *P. falciparum* untreated rats (negative control); contradicting strongly with previous reports of Dheyad, (2016), who observed a statistically significant difference with dexamethasone treated animals, reporting an increase (as such) in the PCV. This change was possibly achieved through retarding erythrophagocytosis and increasing erythropoietin production as such [18]. Howbeit, administration of antimalaria restored average PCV values towards normal. This is also observed to be consistent with previous studies from Sowunmi *et al.,* 2010, who reported that co-administration of coartem has greater effect in increasing PCV towards normalcy; an effect which may be attributed to the antioxidant activity of Vitamin-E and the A/L as observed by Onyesom *et al.* (2012) [19].

Again from this study, there was a significant reduction in the growth of the diabetic untreated rats. However, the diabetic treated rats with diosgenin showed an insignificant reduction in blood sugar levels compared to control, even though this change was apparently improved compared to diabetic, untreated group. This observation was inconsistent with previous reports. A possible reason for this may be that most other researches experimented with extracts of diosgenin not studied here, thus accounting for the differences in findings as such.

It should be stressed that that RBC counts were significantly reduced in malaria infection is consistently in agreement with findings from this present study (Figure 2). However, administration of Diosgenin caused a restoration in PCV towards normal, with more stable observable recovery compared to malaria infected, untreated group (Negative control). This proves the antimalarial efficacy of diosgenin as previously reported by Khan et al., (2015) [6]. This also corroborates with the findings of previous studies that A/L increases PCV values with subsequent increase in RBC count [20]. This malaria recovery effect of Diosgenin on PCV levels may be beneficial in antimalaria treatment.

As part of its research objectives, current study also investigated the changes in total white blood cell count (TWBCC) variables in diabetic and ACT treated rats (figures 1 and 2). Theoretically, even though reports on the effects of Diosgenin on TWBCC remains scanty, White blood cells (WBCs) are the known to be the centre of target mostly by malaria infection. Ani *et al.,* (2016) had reported a significantly higher value of total WBC (leucocyte) count in malaria positive individuals as against non-malaria infected subjects [21]. Their reports contradict findings of this study and those of Smita and Harish (2013) and Igbeneghu and Odaibo (2013) [22, 23], by showing a statistically significant decrease in average values of total WBC count of malaria positive, Diosgenin treated rats than those untreated (negative control). Here, total WBC counts of notably decreased in Diosgenin treated, though this decrease signified a huge recovery compared to untreated rats (Figures 3) were also is seen to be significantly increase in this study, compared to normal control group. This decreased TWBC count had been reported by Dheyad, (2016), who administered zinc to a group of spague dawley rats in his study. In this study however, the total WBC (leucocyte) counts were not significantly altered following administration of Coartem as reported by Ofem *et al.,* (2013) [17]. However, earlier report by Adeleye *et al.* (2012) shows that Coartem increased total WBC counts, which they attributed to immunological response induced by the drug at variance with the observation made by Ofem *et al.,* (2013)

4. Conclusion

Diosgenin's potential utilization in the management of diabetes mellitus has been shown in this study; coupled with its usefulness in improving the packed cell volume (PCV) and total white blood cell count (TWBCC) levels of *Plasmodium falciparum* inoculated rats. To this point, affirming most reports that diosgenin improves the dysfunction of carbohydrate metabolism and related defiant processes. In addition, diosgenin-rich fenugreek may help enhance blood glucose and intestinal amylase and ATPase's in alloxan-induced diabetic rats, while potentially improving PCV, TWBCC in other ailments that may have emanated due to reduced levels of these parameters

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that they do not have conflict of interest.

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