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IN VIVO EVALUATION OF *BRYOPHYLLUM PINNATUM* ANTIMALARIAL ACTIVITY, ACUTE ORAL TOXICITY AND IMPACT ON PANCREATIC INSULIN AND GLUCAGON EXPRESSIONS

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ABSTRACT

This study investigated the impact of Bryophyllum pinnatum on mice infected with malaria, assessing antioxidant activity, histopathological changes, insulin and glucagon expressions by the pancreas, and toxicological alterations. Thirty mice were randomly assigned to six groups (n = 5/group). Groups 1–3 received 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of the ethanol extract of Bryophyllum pinnatum (EEBp), respectively after infection. Group 4 (Positive Control) mice were not infected or treated (NINT). Group 5 (Negative Control) animals were infected but not treated (INT). Group 6 (Standard Control) mice were infected and treated with Lonart®DS at 20 mg/kg body weight. The treatment lasted for four days. The assays for antimalarial, antioxidant and toxicological activities, along with phytochemical profiling, pancreatic histopathology and immunohistochemical staining were conducted using standard procedures and documented methods. The phytochemical screening of EEBp revealed alkaloids, flavonoids, saponins, anthraquinones, phenols, terpenoids, tannins and triterpenoids. No toxicity was observed at 5,000 mg/kg. Antimalarial activity showed significant efficacy, with a mean survival time (MST) of 25.0 ± 0.8 days for the 400 mg/kg EEBp dose (MST for standard treatment = 29.3 ± 0.7) when tracked for 30 days. This dose also protected against oxidative damage in pancreatic tissue (histological score of 1/21), indicating significant treatment response. EEBp modulated insulin and glucagon expressions at doses of 200 mg/kg and 400 mg/kg, restoring levels to normal in infected mice. In conclusion, Bryophyllum pinnatum demonstrates potency in malaria treatment in experimental mice. However, further investigation is necessary to identify the active compounds for study as leads.

Keywords: *Bryophyllum pinnatum*, Malaria, Toxicity, Antioxidants, Insulin, Glucagon, Mice

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INTRODUCTION

Malaria, a persistent and pressing public health challenge, exerts a disproportionate burden on the African region. In 2022, out of the approximately 249 million malaria cases reported globally, the WHO African region bore the staggering weight of 94% of these cases (World Health Organization, 2023). Strikingly, this burden is not evenly distributed, as only four countries accounted for half of the world's malaria-related deaths. Nigeria takes the lead in this grim tally, contributing approximately 27% of these fatalities, surpassing Democratic Republic of the Congo (12%), Uganda (5%) and Mozambique (4%) (World Health Organization, 2023).

Over the years, the battle against malaria has primarily centered on the development of antimalarial drugs. This emphasis stems from the formidable challenge posed by antimalarial resistance, predominantly by the species *Plasmodium falciparum*. Emerging investigations have begun to explore the untapped potential of natural remedies. This shift in focus recognises the need for innovative approaches to combat the persistent threat of malaria. Among these innovative approaches is the study of the plant, *Bryophyllum pinnatum.*

Bryophyllum pinnatum, popularly known as air plant or miracle leaf (Ogidi *et al.* 2019) is a fast-growing succulent perennial herb native to African countries such as Madagascar, Nigeria and Cameroon, and Asian countries such as China (Tatsimo *et al.* 2012; Dogara 2022). Numerous medicinal properties of *Bryophyllum pinnatum* have been reported, including anthelmintic, immunosuppressive, hepatoprotective, antinociceptive, anti-inflammatory, anti-diabetic, nephroprotective, antioxidant, antimicrobial, analgesic, anticonvulsant, neuropharmacological and antipyretic activities (Muhammad and Samoylenko 2007). Secondary metabolites with therapeutic value, such as alkaloids, flavonoids, tannins, glycosides and phenolic compounds, can be obtained from various parts of the plant, including leaves, stems, and roots, which ultimately contribute to the plant's medicinal properties (Ujah and Onyishi 2023).

Severe malaria is linked to a range of clinical manifestations affecting the neurological, renal, haematological, cardiovascular, respiratory and metabolic systems which include cerebral malaria, acute kidney injury, anemias, hypertension and shock, pulmonary oedema and hypoglycaemia, respectively (Glaharn *et al.* 2018; Trampuz *et al.* 2003). Studies have shown that metabolic and homeostatic disorders, with a particular focus on pancreatic dysfunction related to insulin and glucagon, are prevalent in malaria-related complications (Mavondo *et al.* 2019).

Several studies have linked alterations in pancreatic histology to infectious diseases (Chen *et al.* 2001; Davis *et al.* 2002; Daher *et al.* 2003; De Souza *et al.* 2016). The knowledge of histopathological changes in pancreatic tissues during malaria infection is limited. To address these knowledge gaps, this study aimed to investigate both antimalarial activity and pancreatic expressions of insulin and glucagon by malarial infected mice.

MATERIALS AND METHODS

Plant Collection and Identification

The plant (*Bryophyllum pinnatum*) was harvested fresh from their natural habitat in Abraka, Ethiope East Local Government Area, Delta State, Nigeria, and its taxonomical identification was done using the LeafSnap application at the point of collection.

Preparation of extract

The fresh leaves of *Bryophyllum pinnatum* were plucked, washed properly with running water and air-dried at laboratory room temperature (22°C–33°C). The dried leaves were then, pulverised to fine powder using a laboratory blender (Binatone, Model BLG-450, MK2). Thereafter, 200 g of dried powder was soaked in 500 mL of ethanol for 72 h and then, filtered. The filtrate was percolated and the extraction solvent (ethanol) was evaporated under vacuum. The residue was air-dried and stored in the fridge (4°C) for use in the treatment of *P. berghei* induced malarial infection in experimental mice.

Animal procurement

Thirty (30) healthy, six months old, male BALB/c albino mice, weighing between 22 g–30 g was purchased from the Laboratory Animal Centre (LAC), Faculty of Basic Medical Sciences (FBMS), Delta State University (DELSU), Abraka, Nigeria, for the study. The animals were maintained under standard housing condition at LAC where they were tested for malaria parasite prior to inoculation to ensure that they were not already infected. They were fed with chow and water *ad libitum* and kept in well ventilated plastic cages to acclimatise for a week before commencement of the experiment.

Animal care and handling complied with the guidelines established by the Research and Bioethics Committee of FBMS, DELSU, Abraka; and approval for this research was given by the Research and Bioethics Committee of FBMS, DELSU, Abraka with the approval number RBC/FBMC/DELSU/24/301.

Experimental animal grouping, inoculation and treatment

The mice were separated and caged into six groups; five mice for each group to avoid a tight condition for the animals according to the method by Olorukooba *et al.* 2022 but with modifications. Then, they were infected by obtaining parasitized (*Plasmodium berghi* NK 65) blood by pricking the tail of infected mice (donour) obtained from NIMR, Yaba, Lagos State, Nigeria. The inoculum contained $1 \times 10^{5-7}$ parasite. Then, 100 µL of the infected blood was diluted in 900 µL of normal saline and 25 mice were inoculated intraperitonally, due to ease of administration and rapid confirmation of Parasitaemia after 72 hours of inoculation.

Groups 1, 2 and 3 Mice: They were infected and respectively treated with 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* (EEBp).

Group 4 Mice (Positive Control): They were not infected and were also not treated (NINT).

Group 5 Mice (Negative Control): They were infected, but not treated (INT).

Group 6 Mice (Standard Control): They were infected and treated with 20 mg/kg body weight of Lonart®DS (the standard ACT drug used for the treatment of malaria containing artemether and lumefantrine).

Euthanisation of animals and collection of blood and tissue samples

On Day 0, before inoculation, blood sample was collected and then on Day 3, Day 6, Day 9 and Day 12. After the 4day oral treatments of infected mice with EEBp and standard drug, the mice were euthanised on the 12th day after an overnight fast by cervical decapitation under ketamine anaesthasia, and blood/pancreatic tissue samples were collected. Blood was obtained with the aid of a capillary tube using the ocular puncture method and the samples collected were placed in plain, sterile tubes for biochemical assays. Laparotomy was carried out to expose the internal organs and the pancreas was excised and processed for both biochemical, histological and immunohistochemical examinations (Loha *et al.* 2019).

Qualitative Phytochemicals Profiling

Bryophyllum pinnatum was analysed for the presence of phytochemicals, including tannins, phlobatannins, saponins, steroids and terpenoids, according to the method of Ejikeme *et al.* (2014). Then, flavonoids, alkaloids and glycosides, according to the method of Sofowara (1993); anthraquinones, phenols, and triterpenoids according to the method of Sharma *et al.* (2015), Archana *et al.* (2012) and Abubakar *et al*. (2017), respectively.

Pancreatic Antioxidant Assay

The antioxidant levels (reduced glutathione and glutathione peroxidase, catalase, superoxide dismutase, malondialdehyde and nitric oxide) in pancreatic homogenate were determined spectrophotometrically according to previous methods. The reduced glutathione (GSH) level in the kidney was assayed using the method of Ellman (1959), superoxide dismutase (SOD) following Kakkar *et al.* (1984), catalase by Johansson and Borg (1988), and glutathione peroxidase as per Tappel (1978). Malondialdehyde (MDA) levels were measured according to Gutteridge and Wilkins (1982), and nitric oxide activity was determined using the method by Hunter *et al*. (1963).

Histopathological Analysis

The pancreas section was fixed in 10% neutral buffered formalin overnight at room temperature. Histopathology was performed by the method of Loha *et al*. (2019). The pancreatic tissues were interpreted based on seven histological criteria namely: presence of parasitised RBCs, oedema, haemorrhage, inflammatory infiltration, acinar necrosis, fat necrosis and fibrosis, under x200 magnification. The changes were graded on a scale of 0–3. All criteria were assessed in the lobule, interlobular and interglandular, except for fat necrosis, where the occurrence was at the peripancreatic tissue level. The grading criteria were based on previously described studies (Schmidt *et al*. 1992; Gülçubuk *et al.* 2005).

Immunohistochemical Study for Expressions of Insulin and Glucagon in Pancreatic Tissue

Insulin and glucagon expressions, along with the assessment of immunohistochemical staining, were carried out following the procedures outlined by Glaharn *et al*. (2018), with the only deviation being the magnification employed during the examination of pancreatic sections (x200).

Oral Acute Toxicity

The acute oral toxicity study was conducted according to the Organization of Economic Cooperation and Development (OCED) (2008) guidelines 420 for testing chemicals. Male BALB/c mice aged 6–8 weeks (n = 12), were fasted for 16 hours prior to the administration of a 5,000 mg/kg dose of ethanol leaf extract of *Bryophyllum pinnatum* dissolved in 10% Tween 20. The rats were observed hourly for the first 3 hours, then once daily for 14 days for any signs of acute toxicity.

Statistical Analysis

The data was recorded and analysed with SPSS software version 22.0. Values were expressed as mean ± SEM. The differences in parameters were tested using one-way ANOVA. The level of statistical significance (p value) used was less than or equal to 0.05 ($p \leq 0.05$).

RESULTS

Antimalarial Activity of *Bryophyllum pinnatum* Ethanol Extract

The percentage of parasite suppression in each group was calculated to determine the antimalarial effect and is summarised in Table 1. The table shows that the Standard Control Group (Lonart®DS) and the varying doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of EEBp induced significant suppression in parasitaemia; however, this suppression was not consistently dose-dependent. Notably, the 100 mg/kg dose of EEBp demonstrated the highest parasitaemia suppression by Day 12 (89.13%), suggesting it may be more effective for long-term suppression despite being a lower dose. In Figure 1, all groups treated with the doses of EEBp and the standard drug, Lonart®DS, exhibited reduced parasite levels in blood, reflecting the pattern of parasite suppression. The mean survival time (MST) was highest for the standard drug treated group, followed by those treated with 400 mg/kg, 200 mg/kg and then 100 mg/kg of EEBp when tracked for 30 days, indicating a trend in treatment efficacy, but with the recognition that the 100 mg/kg dose shows noteworthy effectiveness in suppressing parasitaemia over time.

Table 1: Parasite suppression in blood induced by treatment of infected mice with EEBp

Treatments	Suppression (%)			Mean Survival Time (MST)
EEBp (mg/kg	**Day 6**	**Day 9**	**Day 12**	
100	82.72 ± 0.19[a]	87.51 ± 0.11[a]	89.13 ± 0.62[a]	23.3 ± 1.5
200	84.53 ± 0.01[a]	87.23 ± 0.06[a]	88.38 ± 0.09[a/b]	23.7 ± 0.5
400	80.27 ± 1.24[b]	85.09 ± 0.12[b]	86.57 ± 0.06[b]	25.0 ± 0.8
20 (Lonard®DS)	83.67 ± 0.15[a]	84.23 ± 0.16[c]	88.87 ±1.31[a/b]	29.3 ± 0.7

Notes: Percentage of parasitemia suppression (mean ± SEM, n = 5 mice/group) induced by different doses of ethanol extract of *Bryophyllum pinnatum* (EEBp) and Lonart®DS (20 mg/kg: Artemether 80mg + Lumefantrine 480 mg tablet) on malaria-infected mice. Statistical comparisons used one-way ANOVA; values not sharing a common superscript are significantly different ($p \leq 0.05$). Mean survival times (MST, days) are also presented to indicate efficacy. Day 0: Inoculation with Plasmodium berghei; Day 3: Confirmation of parasitaemia and commencement of the 4-day treatment; Day 6: End of treatment; Day 9: Third day of post treatment; Day 12: Sixth day of post treatment.

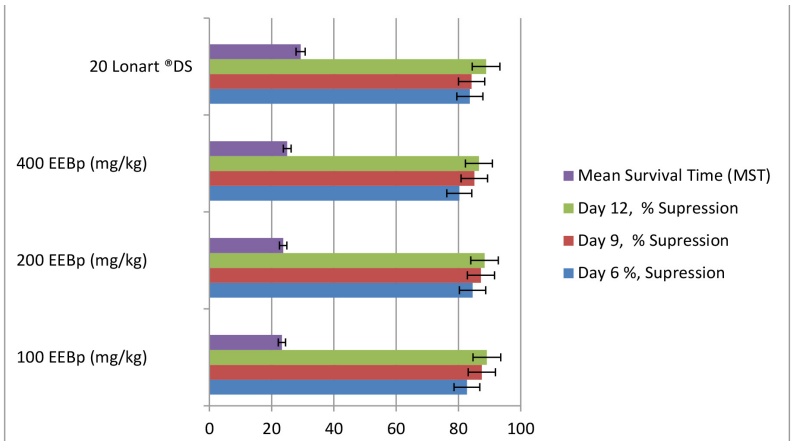

Figure 1: Bar graph showing the parasite suppression and MST in blood induced by treatment of *P. berghei* infected mice treated with with EEBp.

Notes: Error bars represent the standard error of the mean (SEM) for n = 5 mice/group with data recorded at key intervals during and post treatment; Day 0: Inoculation with Plasmodium berghei; Day 3: Confirmation of parasitaemia and commencement of the 4-day treatment; Day 6: End of treatment; Day 9: Third day of post treatment; Day 12: Sixth day of post treatment. Lonart®DS: Standard drug (Artemether 80 mg + Lumefantrine 480 mg tablet) EEBp: Ethanol extract of *Bryophyllum pinnatum*.

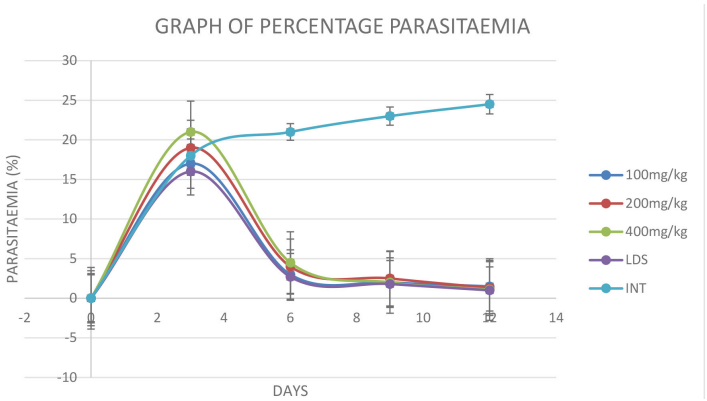

Figure 2: Changes in parasitaemia induced by the treatment of malarial (*P. berghei*) infected mice with ethanol extract of *Bryophyllum pinnatum*. LDS = Lonart®DS; INT = Infected not treated

Notes: Graph represents progression of parasitaemia levels in malaria-infected mice (for n = 5 mice/group) across treatment and post treatment. Error bars represent the standard error of the mean (SEM) for n = 5 mice/group with data recorded at key intervals during and post treatment; Day 0: Inoculation with Plasmodium berghei; Day 3: Confirmation of parasitaemia and commencement of the 4-day treatment; Day 6: End of treatment; Day 9: Third day of post treatment; Day 12: Sixth day of post treatment. Lonart®DS: Standard drug (Artemether 80 mg + Lumefantrine 480 mg tablet).

Phytochemical Screening of Plant Extract

Phytochemical screening of *Bryophyllum pinnatum* ethanol leaf extract revealed the presence of alkaloids, flavonoids, saponins, anthraquinones, phenols, terpenoids, tannins and triterpenoids (Table 2).

Table 2: Qualitative phytochemical screening of plant's ethanol leaf extract.

Phytochemical compounds	***Bryophyllum pinnatum***
Alkaloid	+
Flavonoid	+
Saponin	+
Anthraquinone	+
Phenol	+
Terpenoids	+
Tannin	+
Steroids	–
Triterpenoids	+
Glycoside	–
Phlobatannin	–

Note: + = Present; – = Not detected.

Pancreatic Function Markers in Blood

Table 3 summarises changes in pancreatic function markers in the blood of malaria-infected mice treated with ethanol leaf extract of *Bryophyllum pinnatum*. Outcome of treatment for the different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) were compared with Lonart (20 mg/kg) treatment and then with the infected not treated (INT) and not infected not treated (NINT) groups. The 400 mg/kg dose significantly increased the C-peptide level and decreased the glucose level when compared with the other treated groups. This dose also restored the pancreatic α-amylase activity. These data which compared well with the normal control and standard treated groups suggest significant improvement in pancreatic function. These results, obtained on the twelfth-day post-treatment, highlight the potential of *Bryophyllum pinnatum* in modulating pancreatic function in malaria-infected mice.

The results presented in Table 4 illustrate the impact of the treatment of malaria-infected mice with the ethanol leaf extract of *Bryophyllum pinnatum* on pancreatic oxidative markers. Significant variations were observed in multiple oxidative markers. The infected untreated group (INT) showed markedly elevated levels, indicating increased oxidative stress associated with malaria infection. At doses of 100 mg/kg and 200 mg/kg of *Bryophyllum pinnatum*, notable effects on pancreatic oxidative markers were observed. While both doses demonstrated improvements in certain markers compared with the INT, such as MDA, GSH, GPx, SOD and CAT, the impact was less pronounced than with the higher dose (400 mg/kg). These findings suggest a dose-dependent response, with the 400 mg/kg dose exhibiting more substantial enhancements in oxidative status.

Table 3: Changes in pancreatic function markers in blood of malaria infected mice treated with ethanol leaf extract of *Bryophyllum pinnatum*.

Dose (mg/kg)		**EEBp**			**LDS**	
Markers	**100**	**200**	**400**	**20**	**INT**	**NINT**
C-peptide (ng/ml)	0.85 ± 0.05^{a}	0.90 ± 0.00^{a}	1.55 ± 0.05^{a}	1.70 ± 0.20^{a}	6.25 ± 1.25^{b}	1.55 ± 0.35^{a}
Glucose (mg/dl)	91.1 ± 1.10^{a}	88.65 ± 0.55^{a}	$62.75 \pm 32.25^{a/b}$	$53.2 \pm 22.00^{a/b}$	38.10 ± 1.9^{b}	$57.55 \pm 26.95^{a/b}$
α-amylase (U/L)	$26.85 \pm 22.35^{a/b}$	53.6 ± 2.60^{a}	44.00 ± 1.60^{a}	40.3 ± 10.90^{a}	10.50 ± 1.50^{b}	56.45 ± 1.45^{a}

Notes: Changes in pancreatic function markers expressed as Mean ± SEM, n = 5 mice/group. observed on the twelfth day post-treatment of malaria-infected mice. Significant differences ($p \leq 0.05$) between groups were determined using one-way ANOVA. Values not sharing a common superscript differ significantly ($p \leq 0.05$). INT: Infected not treated; NINT: Not infected not treated; EEBp: Ethanol extract of *Bryophyllum pinnatum*; LDS: Lonart®DS; (Standard drug: Artemether 80 mg + Lumefantrine 480 mg tablets).

Table 4: Changes in pancreatic oxidative markers induced by the treatment of malaria infected mice with ethanol leaf extract of *Bryophyllum pinnatum.*

Doses (mg/kg)	**EEBp**			**LDS**		
Markers	**100**	**200**	**400**	**20**	**INT**	**NINT**
MDA (µM)	17.55 ± 0.05a	18.25 ± 7.35a	27.90 ± 1.50a	20.10 ± 4.90a	66.75 ± 9.65b	16.35 ± 5.15a
GSH (µM)	10.30 ± 0.10a	10.60 ± 0.60a	11.35 ± 1.15a	10.8 ± 0.80a	0.85 ± 0.35b	11.8 ± 0.6a
GPx (mU/mL)	6.35 ± 1.05a	5.2 ± 0a	5.60 ± 0.60a	4.35 ± 0.55a	0.70 ± 0.20b	3.65 ± 0.25a
SOD (U/mL)	0.88 ± 0.11a	0.78 ± 0.07a	0.73 ± 0.07a	0.72 ± 0.11a	0.05 ± 0.01b	0.68 ± 0.07a
CAT (U/mL)	9.55 ± 0.05a	9.45 ± 0.05a	11.8 ± 0.4a	10.4 ± 0.20a	1.55 ± 0.45b	10.30 ± 0.20a
NO (µmol/L)	22.4 ± 0a	22.9 ± 2.7a	35.15 ± 5.05b	11.55 ± 1.35a	3.55 ± 1.55c	16.65 ± 3.45d
TAC (µM)	0.05 ± 0a	0.06 ± 0a	0.05 ± 0a	0.05 ± 0a	0.02 ± 0.01b	0.05 ± 0a

Notes: Pancreatic oxidative stress markers (mean ± SEM, n = 5 mice/group) measured on the 12th day post-treatment in malaria-infected mice. Significant differences ($p \leq 0.05$) between groups were determined using one-way ANOVA. MDA = malondialdehyde, GSH = glutathione, GPx = glutathione peroxidase, SOD = super oxide dismutase, CAT = catalase, NO = nitric oxide, TAC = total antioxidant capacity, INT = infected not treated, NINT = not infected not treated, EEBp = ethanol extract of *Bryophyllum pinnatum*, LDS = Lonart®DS; (Standard drug: Artemether 80 mg + Lumefantrine 480 mg tablet).

Histopathological Changes in Pancreatic Tissue of Malaria Infected Mice

The histopathological examination of pancreatic tissues from infected mice (INT) revealed notable pathological features, including parasites in red blood cell vessels, oedema, haemorrhage, inflammatory infiltration, acinar necrosis, fat necrosis and fibrosis (Table 5). The grading scores for infected mice treated with the standard drug (20 mg/kg Lonart®DS) and 200 mg/kg of *Bryophyllum pinnatum* were equivalent, differing only in the occurrence of specific histological criteria. In comparison with the Positive Control (NINT), the plant extract at 400 mg/kg (*B. pinnatum*) displayed a lower grading score of 1/21, indicating a dose-dependent treatment response. This observation underscores the potential significance of dosage in influencing the therapeutic impact of *Bryophyllum pinnatum* at mitigating histopathological alterations associated with malarial infection.

Table 5: Histopathological scoring of pancreatic tissue in malaria infected.

				EEBp (mg/kg)		
Features	**NINT**	**LDS**	**INT**	**100**	**200**	**400**
pRBCs	0	0	3	0	0	0
Oedema	0	0	3	1	0	0
Haemorrhage	0	1	2	1	0	1
Inflammatory infiltration	0	1	3	0	0	0
Acinar necrosis	0	0	2	1	1	0
Fat necrosis	0	0	2	0	0	0
Fibrosis	0	0	3	0	1	0
Total Score		**2/21**	**18/21**	**3/21**	**2/21**	**1/21**

Note: INT = infected not treated; NINT = not infected not treated; EEBp = ethanol extract of *Bryophyllum pinnatum*; LDS = Lonart®DS; (Standard drug: Artemether 80 mg + Lumefantrine 480 mg tablet).

Immunohistochemical Staining: Expressions of Insulin and Glucagon

Cells expressing insulin and glucagon were identified by a brown coloration in the cytoplasm within the islets of Langerhans. Typically, the central region of the pancreatic islets exhibited insulin expression, while glucagon expression was predominantly situated at the periphery of the islets (Figure 3A–3B). Notably, in the malaria-infected group and those treated with 100 mg/kg of *Bryophyllum pinnatum* (Figure 3B and 3D, respectively), insulin expression prominently increased compared with the NINT. Conversely, Figure 3F demonstrated reduced insulin expression, indicative of increased glucose in mice treated with 400 mg/kg of *B. pinnatum* when compared with the normal mice and standard drug treated mice. Intriguingly, Figure 3C and 3E exhibited insulin expression akin to the control group (Figure 3A), suggesting that Lonart administration to malaria-infected mice effectively restored insulin and glucose levels to normal. Furthermore, a 200 mg/kg dosage of *Bryophyllum pinnatum* demonstrated efficacy in reinstating insulin levels in infected mice.

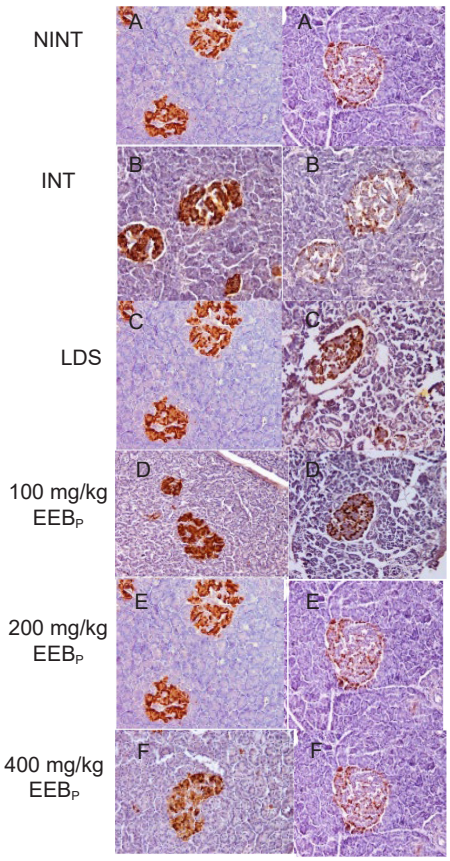

Figure 3: Immunohistoche mical analysis of pancreatic tissue stained for insulin (central region of islets) and glucagon (periphery of islets) in malaria-infected mice treated with ethanol extract of *Bryophyllum pinnatum* (EEBp) and Lonart®DS. Staining intensity (brown coloration) reflects hormone expression. A = NINT, B = INT, C = LDS [Lonart®DS; (Standard drug: Artemether 80 mg + Lumefantrine 480 mg tablet)], D = 100 mg/kg EEBp, E = 200 mg/kg EEBp, F = 400 mg/kg EEBp.

For glucagon expression, Figure 3B had a weaker staining compared with that of Figure 3A. Figure 3C and 3D had moderate effect on the level of glucagon in infected mice. This indicates that the antimalarial, Lonart, as well as the 100 mg/kg of the plant, *Bryophyllum pinnatum,* may have a mitigating effect on the disruption caused by the malaria infection, possibly helping to regulate glucagon expression within the pancreas. Conversely, the uniform glucagon staining in Figure 3E and 3F, similar to the NINT, suggests that the administration of 200 mg/kg and 400 mg/kg of *Bryophyllum pinnatum* potentially ameliorated the metabolic disturbances induced by malaria infection. This observation implies a positive impact on the regulation of glucagon expression, indicating a potential therapeutic effect of *Bryophyllum pinnatum* in managing the metabolic aspects affected by the infections.

Acute Toxicity

No fatalities related to the treatment were observed over the 3 h tracked time and the 14-day test period. Put together, observations indicated no discernible changes in general behaviour, and there were no toxicity signs noticed (Table 6). The LD50 of EEBp is, therefore, greater than 5,000 mg/kg body weight.

Table 6: Acute toxicity of ethanol leaf extract of *Bryophyllum pinnatum.*

Observation	**Control**	***Bryophyllum pinnatum***			
		1 h	2 h	3 h	14 days
General observation					
Consciousness	N	N	N	N	N
Grooming	N	N	N	N	N
Touch response	N	N	N	N	N
Sleeping duration	N	N	N	N	N
Movement	N	N	N	N	N
Gripping strength	N	N	N	N	N
Righting reflex	N	N	N	N	N
Food consumption	N	N	N	N	N
Water intake	N	N	N	N	N
Pinna reflex	N	N	N	N	N
Sound response	N	N	N	N	N
Signs of toxicity					

(continued on next page)

Table 6: (*continued*)

Observation	Control	*Bryophyllum pinnatum*			
		1 h	2 h	3 h	14 days
Tremors	A	A	A	A	A
Diarrhoea	A	A	A	A	A
Hyperactivity	A	A	A	A	A
Corneal reflex	N	N	N	N	N
Salivation	N	N	N	N	N
Skin colour	N	N	N	N	N
Lethargy	A	A	A	A	A
Convulsion	A	A	A	A	A
Faecal appearance	N	N	P	P	N
Mortality	0	0	0	0	0

Note: N = normal; A = absent; P = pasty.

DISCUSSION

The escalating resistance of the malaria parasite to existing antimalarial drugs has underscored the urgency in exploring alternative therapeutic avenues. Plant-derived drugs, with their diverse phytochemical compounds, have garnered attention as potential therapeutics for malaria treatment.

Severe malaria, often caused by *Plasmodium falciparum*, has been linked to various multi organ dysfunction (De Souza *et al*. 2016), with the pancreas emerging as a site of considerable interest. Histopathological studies have revealed distinctive alterations in pancreatic tissues during severe malaria, including oedema in interlobular and interglandular spaces, acinar necrosis and inflammatory reactions (Glaharn *et al*. 2018). These changes, although, frequently associated with hypoglycaemia in malaria patients, share similarities with histopathological findings in other infectious diseases like babesia, leptospirosis and dengue (Möhr *et al*. 2000; Daher *et al*. 2003; Shamim 2010). In our study, pancreatic tissues from infected mice (INT) exhibited various pathological features. Treatment with 400 mg/kg *B. pinnatum* showed a lower histopathological grading score (1/21) compared with the NINT, suggesting a dose-dependent response in mitigating the observed alterations. These findings are in agreement with studies reported by Glaharn *et al*. (2018) which showed the presence of red cells, oedema, acinar necrosis and presence of lymphocytes in severe malaria patients. Chen *et al.* (2001) also reported similar result.

Malaria disrupts glucose balance through factors like parasite metabolism, fever and immune system irregularities (Davis *et al*. 2002). This disruption occurs primarily with the glucose hormones, insulin and glucagon, initially leading to low glucose levels (hypoglycaemia), without intervention, it can progress to high levels (hyperglycaemia). The effectiveness of hormones and immune responses is vital, and when compromised, it

contributes to severe malaria-induced glucose imbalance (Mavondo *et al.* 2019). There is a scarcity of studies documenting the expression of insulin and glucagon in pancreatic tissues during severe *P. falciparum* malaria.

This present study revealed distinct effects on insulin and glucagon expression in mice infected with malaria and treated with *Bryophyllum pinnatum* and antimalarial drug, Lonart. Notably, 100 mg/kg of *B. pinnatum* and Lonart showed a significant increase in insulin expression, while 400 mg/kg of *B. pinnatum* indicated reduced insulin levels, possibly linked to hyperglycaemia. Interestingly, Lonart administration effectively restored insulin levels in mice with hypoglycaemia. Additionally, 200 mg/kg of *B. pinnatum* demonstrated efficacy in reinstating insulin levels. For glucagon expression, Lonart and 100 mg/kg of *B. pinnatum* showed potential mitigating effects, while 200 mg/kg and 400 mg/kg of *B. pinnatum* demonstrated regulation similar to the control. These findings are in agreement with the study conducted by Bosco *et al*. (2010) and Glaharn *et al*. (2018).

The study evaluated antimalarial effects by calculating parasite suppression percentages. The 400 mg/kg EEBp, displayed the lowest post-treatment parasite count, while 100 mg/kg EEBp displayed the highest post-treatment parasite count over time. Notably, all EEBp-treated groups and the standard drug demonstrated significant reductions in parasitaemia, supporting the antimalarial properties of EEBp. This result is in agreement with the study conducted by Singh *et al.* (2015) who reported that the ethanol leaf extract of the plant, *Bryophyllum pinnatum* had good antiplasmodial activity. The therapeutic prowess of medicinal plants can be owed to the presence of bioactive constituents which are referred to as phytochemicals. The qualitative analysis of the ethanol leaf extract of the plant, *Bryophyllum pinnatum* showed the presence of alkaloids, flavonoids, saponins, anthraquinones, phenols, terpenoids, tannins and triterpenoids. This finding corroborates with the study conducted by Ogidi *et al.* (2019).

Phytochemicals' protective role is linked to their antioxidant activity, countering the overproduction of oxidants (reactive oxygen species and reactive nitrogen species) implicated in the pathogenesis of numerous chronic diseases (Zhang *et al.* 2015). This study investigated the impact of the ethanol leaf extract of *Bryophyllum pinnatum* (EEBp) on pancreatic oxidative markers in malaria-infected mice, comparing it with Lonart (standard drug), INT and NINT groups. Notably, at 400 mg/kg of *B. pinnatum*, there was a significant increase in malondialdehyde (MDA) and nitric oxide (NO), indicative of lipid peroxidation, and a notable elevation in total antioxidant capacity (TAC), suggesting a potential compensatory response. This dosage also exhibited enhanced activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD), underscoring the extract's antioxidant potential. Contrastingly, the INT group demonstrated heightened oxidative stress, as evidenced by elevated MDA levels and reduced TAC. These findings collectively imply that *Bryophyllum pinnatum*, particularly at 400 mg/kg, may confer protective effects against oxidative damage induced by malaria infection, highlighting its potential as a therapeutic agent. In a parallel study, Bassey *et al*. (2021) employed the aqueous extract of *Bryophyllum pinnatum*, in contrast to our use of the ethanol leaf extract. Remarkably, their findings echoed our study, both showing decreased malondialdehyde (MDA) levels. Notably, at the 400 mg/kg dose of the ethanol leaf extract in our study, the observed decrease in MDA underscores the plant's robust antioxidant activity, suggesting its effectiveness regardless of the solvent used for extraction. In malaria-infected mice, *Bryophyllum pinnatum* ethanol leaf extract exhibited dose-dependent modulation of pancreatic function, particularly at 400 mg/kg, reflected in increased C-peptide levels and decreased glucose levels. Lonart (20 mg/kg) also displayed positive effects on these markers. Conversely, the INT group showed impaired pancreatic function, emphasising the impact of infection. These findings underscore the potential therapeutic role of *Bryophyllum pinnatum* in mitigating malaria-induced pancreatic

dysfunction. However, owing to the dearth of resources of the plant of choice, further investigations are necessary to substantiate its efficacy in addressing pancreatic function.

While medicinal plants are increasingly utilised as alternative treatment sources, their efficacy must be balanced with safety considerations. Therefore, conducting systematic assessments of potential toxic effects from plant extracts is crucial for establishing safe therapeutic dosages (Neergheen-Bhujun 2013). The toxicity of herbal compounds has been documented in numerous studies (Vaghasiya *et al.* 2011; Christapher *et al*. 2017). The acute toxicity study revealed no mortality with a 5,000 mg/kg ethanol extract dose of *Bryophyllum pinnatum* No changes were observed in various parameters, including grooming, movement, gripping strength, food intake, water consumption, sleep duration and hyperactivity. Normal conditions were noted in salivation, diarrhoea, gait, righting reflex, pinna reflex, sound and touch response, corneal reflex, skin color and consciousness. Tremors, letharg, and persistent diarrhoea were absent throughout the study period.

CONCLUSION

In conclusion, this study delved into the antimalarial, pancreatic antioxidant function phytochemical activity, acute toxicity, pancreatic expression of insulin and glucagon and histopathological changes in pancreatic tissues of the ethanol leaf extract of *Bryophyllum pinnatum*. Despite limited information, the plant exhibited promising efficacy across these domains. Higher doses of the plant extract were deemed effective and safe. However, the expression of glucagon at lower doses indicates that the plant exhibits biological activity even at reduced concentrations. To ensure its safety for therapeutic use, further investigation and assessment are imperative, as these doses may achieve therapeutic effects with fewer side effects and could be more practical for long-term use than higher doses. While the initial findings are encouraging, continued observations are essential to validate and strengthen the credibility of its efficacy.

Limitations of the Study

While both high and low doses were evaluated, there may be an insufficient exploration of intermediate doses. This could mean the optimal therapeutic dose that balances efficacy and safety might not have been identified. The study observed glucagon expression at lower doses, which could indicate potential metabolic side effects. However, this was not fully explored or explained in terms of its long-term effects on glucose regulation and overall metabolism, especially in malarial treatment.

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