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Research Article

Evaluation Of the Antimalarial and Hepatoprotective Effects of *Jatropha Curcas* Leaf Extract in *Plasmodium Berghei*-Infected Mice

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Abstract

The use of medicinal plants has been on the increase especially in the treatment of malaria. These medicinal plants are rich natural sources of ingredients that can be used in drug development and synthesis. The bioactive compounds that occur as secondary metabolites in plants include alkaloids, steroids, terpenes, flavonoids, saponins, glycosides and tannins. This study was carried out to investigate the antimalarial and hepatoprotective activities of ethanol extract of *Jatropha curcas* leaves in *Plasmodium berghei*-infected mice. The effect of the extract on parasitaemia and liver function was investigated in malaria-infected mice using standard biochemical methods. The phytochemicals were also studied. *Jatropha curcas* fresh leaves were gotten from Ibagwa community in Enugu state, Nigeria. The sample was characterized and authenticated by Mr. Nwafor. F, a Botanist at the University of Nigeria Nsukka, Enugu state. A total of thirty mice grouped into six of five mice each were used for the study. Group 1(negative control) uninfected group, Group 2 consisted of infected and untreated mice, Group 3 (positive control) received artesunate (80mg/kg) Groups 4-6 were treated with 200, 400, and 600 mg/kg of extract. Results were expressed as mean ± SD of six animals in each group. Statistical significance ($p<0.05$) was determined by one-way analysis of variance (ANOVA) and post hoc Least Significant Difference (LSD) test using Statistical Package for Social Sciences (SPSS) version 23.0. Results obtained from this study showed that the quantitative phytochemical analysis of the plant's leaf extract revealed the presence of; phenols (7426.15 ± 306.220mg/100 g), alkaloids (593.92±17.029mg/100 g), terpenoids (393.30 ± 7.400mg/100 g) and flavonoids (962.18 ± 15.086mg/100 g). Tannins (48.03 ± 3.023mg/100 g) and steroids (20.04 ± 2.010mg/100g) were relatively present in moderate concentrations while glycosides (3.00 ± 0.30mg/100 g) in small concentration. The percentage parasitaemia decreased significantly ($P< 0.05$) in the treated groups when compared to the untreated group. *P. berghei* infection in mice caused significant elevation ($P < 0.05$) in the activities of ALT, AST, and ALP which were restored after treatment with the extract. The findings from this study showed that the crude extract of the *Jatropha curcas* contains several bioactive compounds of medical importance and it can significantly reduce the parasitaemia level in mice and restored the integrity of the liver in *Plasmodium berghei*- infected mice. It can be concluded that *Jatropha curcas* has antimalaria activity and has no toxic effects on liver functionality.

Keywords: *Jatropha Curcas*, Liver, Parasitaemia, Antimalaria, *Plasmodium Berghei*

Introduction

Malaria is a tropical disease that is caused by a vector borne route which is the female anopheles' mosquitoes. In about 87 countries and territories, there is a recorded rate of infection with an estimated 241 million clinical episodes occurring and 627,000 deaths in 2021 (WHO malaria report 2022). It is still endemic in the African Regions with an estimate of 95% of deaths in 2020, particularly among those living in lower-income countries (WHO malaria report 2022). Human malaria is caused by intra-erythrocytic Plasmodium parasites. The bite of an infected female mosquito of the Anopheles species spreads these parasites [1,2] and Major consequences from malaria include cerebral malaria, acute renal failure, severe anaemia, and hypoglycaemia in infected individuals [3-5]. Many reports have revealed that the use of medicinal plants has been on the increase especially in the treatment of malaria [6-8] and these medicinal plants are rich natural sources of ingredients that can be used in drug development and synthesis. The bioactive compounds that occur as secondary metabolites in plants include alkaloids, steroids, terpenes, flavonoids, saponins, glycosides and tannins *Jatropha curcas* (Castor oil plant) is a semi-evergreen shrub which belongs to the family Euphorbiaceous, is a widespread forest plant that is distributed around the globe and is very much common in central American and in the tropical and Subtropical regions of the world. The shrub or tree grows up to 6 m, with spreading branches and stubby twigs, with a milky or yellowish rufescent exudate. The leaves of this plant are deciduous and alternates but is apically crowded, ovate, acute to acuminate and basally cordate. The flowers of this plant are several and sometimes many in greenish cymes, yellowish and bell-shaped. *Jatropha curcas* is increasingly being exploited as a potential source of new drug leads (*Mgbahunke et al., 2018*, *Imam et al., [09]* and *Akande et al., [10]*)., biofuel (*Neupane et al., [11]*). They are favoured for cooking with goat meat and said to counteract the peculiar smell. The stem and twigs are used in Nigeria and in Northern Cameroon as chew-sticks, producing a kind of foam. The watery sap is put onto fresh cuts and sores at the corner of the mouth *[12]*. The young leaves are eaten in Java. Some say that animals will not touch the leaves; others they will, but this must be rare as the leaves are reported to contain hydrocyanic acid. An infusion, hot or cold, of the leaves is also taken internally for fever. They are mashed up by the Tenda people for poultices, and a macerate is taken as an emetic (*Mgbahunke et al., 2018*).

Phytochemical analyses of Jatropha curcas unveil promising pharmacological compounds like tannins, flavonoids, and terpenoids as reported also by *Akande et al.,* [13] and *Rahu et al., (2021) [14].* There is however paucity of information on the antimalarial and hepatoprotective effects of *Jatropha curcas* leaf extract in. Therefore, this research aimed investigating the efficacy and safety of *Jatropha curcas* leaf extract in in *Plasmodium berghei*-Infected mice with particular interest in the antimalarial and hepatoprotective effects of the plant's leaves.

Materials and Method

Collection of Plant Materials

Jatropha curcas fresh leaves were gotten from Ibagwa community in Enugu state, Nigeria. The samples were characterized and authenticated by Mr. Nwafor. F., a Botanist at the University of Nigeria Nsukka, Enugu State, Nigeria.

Extraction of *Jatropha Curcas* Leaves Extract

A total of 3kg of fresh leaves of *Jatropha curcas* were collected and washed to remove dirt. The plant material was cut into pieces and shade-dried for 1 week. The dried leaves were pulverized into powdered form using a mechanical grinder. A known weight of the pulverized leave (1kg) was macerated in 3.5 Litres of 80% ethanol using a maceration flask. The suspension was left for 3 days with occasional agitation after which it was filtered into a flat-bottomed flask using a muslin cloth. Further filtration was achieved with the use of Whatman No 1 filter paper to remove fine residues. The filtrate was concentrated using a rotary evaporator to obtain the crude ethanol extract. The concentrated extract was stored in a labelled sterile beaker until it was needed for experimental use.

Quantitative and Qualitative Phytochemical Analysis of *Jatropha Curcas* Leaves Extract

The quantitative phytochemical analysis of the ethanol extract was carried out in order to ascertain the quantities of some plant's metabolites. The determinations were done utilizing standard conventional protocols described by Trease and Evans [15] and *Yakubu et al.,* [16]. The extract was subjected to qualitative phytochemical analysis utilizing standard conventional protocols described by *Harborne (1973).*

Experimental Animals

Thirty (30) Albino mice (15 - 30g) were used for this study. The animals were purchased from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were maintained under standard environmental conditions of temperature, relative humidity and light (12 hours of light and 12 hours of darkness) and fed on standard rat feed and water.

Acute toxicity study of *Jatropha Curcas* Leaves Extract

The acute toxicity study of the *Jatropha curcas* leaf extract was carried out by *Lorke's method (1983)*. The experimental animals were divided into groups 5 and administered 10mg/kg, 100mg/kg, 1000mg/kg, 2500mg/kg, and 5000mg/kg doses of the extract. Over a period 48 hrs., the animals' responses, including mortality rates, were recorded. The median lethal dose (LD50) was determined by interpolating the dose-response curve constructed from the data. This curve depicts the relationship between the administered doses and the animals' responses, enabling the estimation of the dose at which 50% of the animals succumb.

Experimental Design

A total of thirty mice grouped into six of five mice each were used for the study. Group 1 consisted of uninfected group, Group 2 consisted of infected and untreated mice (negative control), Group 3 (standard control) received artesunate (80mg/kg) Groups 4-6 were treated with 200, 400, and 600 mg/kg of ethanol leaf extract of Jatropha curcas respectively.

Collection of Blood Samples

Blood samples were collected from donor mice infected with *Plasmodium berghei* ANKA 65 resistance strain which was obtain from faculty of Veterinary Medicine, University of Nigeria, Nsukka Enugu State, Nigeria. 2ml of phosphate buffer at a pH of 7.2 was combined with 12 drops, then a standard inoculum of 1×107 of parasitized erythrocytes from a donor mouse in volumes of 0.2 mL was used to infect the experimental animals intra-peritoneally. The percentage parasitaemia was determined by microscopically examining the thin blood smear stained with Leishman stain using the method described by *Kalra, et al.* (2006) days after parasite inoculation has been carried out.

Determination of Liver Function Parameters

Determination of Serum Aspartate Aminotransferase (AST) Activity: Serum Aspartate Aminotransferase (AST) activity was assayed according to the method described by *Reitman and Frankel (1957)*. The assay principle is based on the reversible transfer of an amino group from aspartate to α-ketoglutarate, resulting in the formation of oxaloacetate and glutamate. The concentration of glutamate produced is determined spectrophotometrically at 546 nm. The assay reagents included a phosphate buffer (pH 7.4), aspartate, α-ketoglutarate, and Pyridoxal Phosphate (PLP) as a coenzyme. Serum samples were mixed with the reaction mixture and incubated at a controlled temperature. To initiate the reaction, 0.1 ml of serum was added to 2.9 ml of the reaction mixture containing phosphate buffer, aspartate, α-ketoglutarate, and PLP. The reaction was allowed to proceed for 30 minutes time at 37 o C temperature. After incubation, the reaction was terminated by the addition of a stopping reagent. Subsequently, the absorbance of the reaction mixture was measured spectrophotometrically at 546 nm at regular intervals. A standard curve was prepared using known concentrations of a standard AST enzyme solution. The activity of AST in the serum samples was determined by comparing the absorbance readings to the standard curve.

Determination of Serum Alanine Aminotransferase (ALT) Activity: Serum Alanine Aminotransferase (ALT) activity was assayed using a standard enzymatic method. The assay relies on the reversible transfer of an amino group between alanine and α-ketoglutarate, generating pyruvate and glutamate, with pyruvate production measured spectrophotometrically at 340 nm. Serum samples were mixed with a reaction mixture containing phosphate buffer, alanine, α-ketoglutarate, and NADH as a coenzyme, then incubated. The decrease in absorbance over time indicates ALT activity. A standard curve using known ALT concentrations was employed for quantification. Controls, including a blank and samples with known ALT concentrations, ensured assay reliability. This method allows precise measurement of ALT activity, offering insights into liver function and potential tissue damage.

Assay for Serum Alkaline Phosphatase (ALP) Activity: Serum Alkaline Phosphatase (ALP) activity was assayed following the

method outlined by *Aebi (1983*). The assay relies on the ultraviolet absorption of hydrogen peroxide (H2O2) at 240 nm. Upon catalytic decomposition of (H2O2) by ALP in the sample, the absorption decreases over time, allowing monitoring of ALP activity. The assay reagents included a phosphate buffer (pH 7.0), 0.2 M hydrogen peroxide, 5% potassium dichromate, and 5% glacial acetic acid. In the procedure, 2.5 ml of phosphate buffer was pipetted into test tubes, followed by 2.0 ml of H2O2 and 0.5 ml of serum. After gentle mixing, 2.0 ml of dichromate acetic acid reagent was added, and the absorbance was measured at 240 nm at 1-minute intervals against a corresponding reagent blank.

Determination of Total Bilirubin

The total bilirubin assay method involves preparing assay reagents including a phosphate buffer (pH 7.0), 0.2 M hydrogen peroxide, and a dichromate acetic acid reagent. Serum samples are then prepared. Test tubes are filled with 2.5 ml of phosphate buffer, followed by the addition of 2.0 ml of hydrogen peroxide and 0.5 ml of serum to each tube. After gentle mixing, 2.0 ml of dichromate acetic acid reagent is added to each tube. The absorbance of the samples is measured at 240 nm at 1-minute intervals against a corresponding reagent blank. This method allows for the determination of total bilirubin concentration in the serum samples.

Statistical Analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance ($p<0.05$) was determined by one-way analysis of variance (ANOVA) and post hoc Least Significant Difference (LSD) test using Statistical Package for Social Sciences (SPSS) version 23.0.

Results

Quantitative and Qualitative Phytochemical Analysis of *Jatropha curcas* Leaves Extract

The results from this investigation revealed that the dried crude sample (1000g) of *Jatropha curcas* leaves yielded 29.20 g (2.92 %). The phytochemicals present in the extract were found to be relatively high. The detected phytochemicals were: phenols (7426.15 ± 306.220mg/100 g), alkaloids (593.92±17.029mg/100 g), terpenoids (393.30 ± 7.400mg/100 g) and flavonoids (962.18 ± 15.086mg/100 g). Tannins (48.03 ± 3.023mg/100 g) and steroids (20.04 ± 2.010mg/100g) were relatively present in moderate concentrations while glycosides (3.00 ± 0.30mg/100 g) were present in small concentration, this is summarized in (Table 1) below.

Table 1: Phytochemical constituents of the extract.

Phytochemicals	Qualitative Analysis (Bioavailability)	Quantitative Analysis (mg/100g)
Alkaloids	++	593.92 ±17.029
Flavonoids	+++	962.18 ± 15.086
Glycosides	+	3.00 ± 0.300
Steroids	+	20.04 ± 2.010
Tannins	+	48.03 ± 3.023
Terpenoids	++	393.30 ± 7.400
Phenols	+++	7429.15 ± 306.220

Note*: + means slightly present, ++ moderately present, +++ means highly present, Results are expressed as mean ± standard deviation, n=3.

Acute Toxicity of *Jatropha curcas* Leaves Extract

The acute toxicity assessment of the Jatropha curcas extract revealed no instances of mortality or adverse responses among the test subjects across a dosage spectrum spanning from 10 to 5000 mg/kg of body weight. This outcome underscores the leaf extract's apparent safety profile, indicating its non-toxic nature.

Effect of *Jatropha curcas* Leaves Extract on Parasitaemia in *Plasmodium berghei*-Infected Mice

Table 2: Effect of the extract on parasitemia in *Plasmodium berghei*-infected mice.

Treatment Group	Parasitemia (%)	
	Before Treatment	After Treatment
Group 1(uninfected)	0.00±0.00[a]	0.00±0.00[a]
Group 2 (untreated)	78.46±10.46 [b]	90.00±12.00[d]
Group 3(artesunate 80 mg/kg)	65.415±17.38[b]	26.24±12.82[c]
Group 4 (extract 200 mg/kg)	74.00±9.00[c]	35.00±6.12[c]

Group 5 (extract 400 mg/kg)	68.56±15.27^{c}	43.00±0.03^{b}
Group 6 (extract 800 mg/kg)	57.33±11.39^{c}	34.00±12.02^{b}

Note*: *Results are expressed as mean ± standard deviation, n = 3. Mean values with different letters as superscripts down the columns are considered significant at $p < 0.05$.

Table 2 shows the percentage parasitaemia of the groups before and after treatments. The percentage parasitaemia after treatments with the graded doses of Jatropha curcas extract was found to be significantly ($p<0.05$) lower when compared to the untreated group (group 2) also the percentage parasitaemia of the untreated group (group 2) was significantly ($p<0.05$) higher when compared to the normal control (Table 2).

Effect *Jatropha curcas* leaves Extract on Liver Function Parameters in *Plasmodium berghei*-Infected Mice

Table 3 presents findings on the levels of total bilirubin concentration and the activities of AST, ALT, and ALP in the experiment. Results indicate a statistically significant increase ($p < 0.05$) in these parameters in the negative control compared to the normal control. However, administration of graded doses of *Jatropha curcas* extract resulted in significant reductions ($p < 0.05$) in total bilirubin concentration, AST, ALT, and ALP activities when compared to the negative control. Notably, there were no statistically significant differences ($p > 0.05$) observed in total bilirubin concentration, AST, ALT, and ALP activities between the *Jatropha curcas* treated groups and the standard control when compared with each other (Table 3).

Table 3: Effect of the extract on the liver function parameters in *Plasmodium berghei*-infected mice.

Treatment groups	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	Total Bilirubin (mg/dl)
Group 1	20.10±0.52^{a}	26.67±2.03^{a}	33.20±0.25^{b}	0.892±0.03^{ac}
Group 2	40.26±1.51^{c}	38.00±1.40^{c}	57.67±3.60b^{b}	1.292±0.30^{b}
Group 3	31.00±8.12^{b}	24.00±2.42^{b}	23.67±2.50^{a}	0.890±0.02^{a}
Group 4	29.07±3.26^{b}	23.23±3.02^{b}	22.17±4.64^{a}	0.839±0.30^{a}
Group 5	25.10±0.25^{b}	22.44±1.54^{b}	19.36±1.38^{a}	0.877±0.07^{ac}
Group 6	28.27±2.73^{ab}	23.10±1.33^{ab}	13.76±2.20^{b}	0.850±0.23^{c}

Note*: *N: B; Alanine Transaminase (ALT); Aspartate Transaminase (AST) and Alkaline Phosphate (ALP)Results are expressed as mean ± standard deviation, n = 3. Mean values with different letters as superscripts down the columns are considered significant at $p < 0.05$.

Discussion

In response to the disease burden, medicinal plants have gained traction for their rich repository of bioactive compounds (*Gutierrez-Grijalva, et al., 2018*). Many reports have revealed that the use of medicinal plants has been on the increase especially in the treatment of malaria [17,18]. These medicinal plants are rich natural sources of ingredients that can be used in drug development and synthesis. The bioactive compounds that occur as secondary metabolites in plants include alkaloids, steroids, terpenes, flavonoids, saponins, glycosides and tannins. The leaf extract of *Jatropha curcas* is at the core of this experiment. Phytochemical analyses of *Jatropha curcas* unveil promising pharmacological compounds like tannins, flavonoids, and terpenoids as reported also by *Akande et al.,*[19]and *Rahu et al., (2021).*

Further exploration into *Jatropha curcas's* efficacy against malaria reveals its potential as an antimalarial agent. The phytochemicals present in the extract were found to be relatively high. The detected phytochemicals were: phenols (7426.15 ± 306.220mg/100 g), alkaloids (593.92±17.029mg/100 g), terpenoids (393.30 ± 7.400mg/100 g) and flavonoids (962.18 ± 15.086mg/100 g). Tannins (48.03 ± 3.023mg/100 g) and steroids (20.04 ± 2.010mg/100g) were relatively present in moderate concentrations while glycosides (3.00 ± 0.30mg/100 g) were present in small concentration. Alkaloids and flavonoids present in *Jatropha curcas*, especially, show promise as precursors for synthesizing antimalarial drugs like artemisinin [20-22]. Additionally, flavonoids and phenolic compounds demonstrate antioxidant properties, countering oxidative damage induced by malaria infection [23-25].

The percentage parasitemia after treatments with the graded doses of *Jatropha curcas* extract was found to be significantly ($p<0.05$) lower when compared to the untreated group (group 2) also the percentage parasitemia of the untreated group (group 2) was significantly ($p<0.05$) higher when compared to the normal control. In the experiment, the level of parasitemia in groups 3, 4, 5 and 6 decreased significantly ($p<0.05$) after treatment with *Jatropha curcas* leave extract when compared to the negative control (group 2) i.e., the untreated group, highlighting the efficacy of the extract in reducing parasitemia levels. Furthermore, *Jatropha curcas* impact on liver function parameters suggests its hepatoprotective potential, attributed to flavonoid content [26,27]. Liver function tests, including ALT, AST, ALP and total bilirubin, provide insights into the extract's hepatoprotective effects [28-35].The activities

of ALT, AST, and ALP were also assayed properly and the *P. berghei* infection in the mice caused significant elevation ($P < 0.05$) in the activities of ALT, AST, and ALP but after the administration *Jatropha curcas* in the treated groups there was a significant decrease ($P < 0.05$) in the activities of ALT, AST, and ALP when compared to the negative control i.e. the untreated group. The potentials of this plant are enormous hence further research could still be carried out for the optimum utilization of these potentials in solving health related challenges.

Conclusion

The findings from this study showed that the crude extract of the *Jatropha curcas* contains several bioactive compounds of medical importance and it can significantly reduce the parasitemia level in mice and restored the integrity of the liver in *Plasmodium berghei*- infected mice.

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Conflicts of Interest

The authors declared that there are no conflicts of interest.

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