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ORIGINAL ARTICLE

Modulation of thrombocytes, thrombopoietin, thrombopoietin receptors and ribonucleic acid expression with Jatropha tanjorensis using albino Wistar rats

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Abstract

Background: *Jatropha tanjorensis* leaf extract possesses some bioactive compounds with potential medicinal properties. Its role in thrombopoiesis and the associated molecular pathways could offer useful insights into its therapeutic potential in conditions related to platelet disorders. This study is aimed at investigating the modulatory effects of this leave extract on platelet functions, thrombopoietin (TPO), cyclic myeloproliferative leukaemia virus oncogene(c-MPL) and messenger ribonucleic acid (RNA) in the modulation of thrombopoiesis using male albino Wistar rats.

Materials and Methods: A total of twenty (20) male adult albino Wistar rats weighing between 150g-250g were selected into four (4) groups of five rats each. The groups were control groups(A), treated group B(1000mg/kg leave extract, treated group C(2000mg/kg leave extract, and treated group D(4000mg/kg leave extract. Platelet counts and platelet indices were done using a three-part Sysmes Haematology autoanalyzer. RNA extraction and semi-qualitative polymerase chain reaction (PCR) were used to isolate from rat bone marrow; complementary DNA was synthesized and subsequently amplified. Gel electrophoresis was used to determine the bands of the genes.

Results: Platelet counts were significantly higher in the treated groups compared to the control group ($p<0.05$). RNA expression of TPO was significantly lower in the control group compared to the test group ($p<0.001$). Treated groups B, C and D showed significantly higher expression of TPO when compared to control($p<0.001$). Meanwhile, the mRNA expression of c-MPL showed no significant effect when compared to all the groups, even with varying concentrations of the plant extracts.

Conclusion: *Jatropha tanjorensis* leaf extracts significantly increase platelet counts and TPO expression but have no significant effect on c-MPL gene expression. These results suggest that the leaf extract could influence thrombopoiesis by modulating the expression of the key regulating factor.

Keywords: Platelets, *Jatropha tanjorensis*, Thrombopoietin, Ribonucleic acid.

Introduction

Plants function as a vital source of a wide range of biofunctional compounds, and some of these biologically functional compounds are very important to humans (1) Due to this importance, humans relied on some of these plants and their products for their therapeutic effect on various forms of diseases and maintaining a healthy life over the years (2) One such useful plant is the green leafy medicinal plant called *Jatropha tanjorensis* (3) The name *Jatropha* coined from the Greek words *jatrós* which stands for doctor and *trophé* which means food implies usefulness in the field of medicine (4). *Jatropha tanjorensis, commonly called "hospital too far" " Catholic vegetable or "Iyana Ipaja", is one of the plants belonging to the Euphorbiaceae family and is a common weed of about 1.8m in* height and grown mainly in the tropical woods of West Africa (5).

Each part of the plant, comprising the seeds, leaves, and bark, has been found useful in traditional medicine and even in animal medicine (6). It has a lifespan of several years as a perennial herb and serves as a middle point regarding phenotypic features between *Jatropha curcas* and *Jatropha gossypifolia* (7). The plant leaves possessing 3-5 lobed palmates bears glandular hairs and can develop under different climatic conditions (8). It grows well on different soil types and even on barren land; it is, however, resourceful due to its ability to revitalize perfectly, grow and reproduce effectively for lots of years (9). *Jatropha tanjorensis* is originally from Central America and has spread across many tropical and sub-tropical areas of the World like Africa, India and North America. (10) The leaf is often ingested as a vegetable in some areas in the Southern part of Nigeria like Edo State and has been used routinely as cure for diabetes in this area of Nigeria (11)

Although having a primary use for fencing, the leaves of *Jatropha tanjorensis* are very useful in the therapy of anaemia (as it helps to improve blood quality by boosting haemoglobin levels and red cell count), diabetes as well as cardiovascular diseases, which all occur as a result of free radical synthesis (12). The leaves of the plant were used as food in Nigeria and consumed in form of soups and also in form of tonic as it was believed that the leaves of the plant help to expand blood volume (13). Previous studies on *Jatropha tanjorensis* have been carried out as regards haematopoiesis, hypolipidaemia activity, hypoglycaemic and antidiabetic action and also its action against microbes (14).

Phytochemical examination of *Jatropha tanjorensis* attributed the healing action of this plant to its phytochemical components, which include alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (15). Igbinaduwa and colleagues also conducted another research showing the role this plant has on Human Immunodeficiency Virus-1, which was revealed to be a powerful anti-HIV agent (16). Previous studies have shown that *Jatropha tanjorensis* is highly rich in antioxidants like phosphorus, selenium, zinc and vitamins C and E (17). A study carried out in 2012 showed that the leaf of this plant exhibits blood renewal capabilities and contains some biological components vital for the swift haemopoietic process in the bone marrow (18). The synthesis of thrombocytes from the multipotential haemopoietic stem cells is a highly regulated process, and so far, thrombopoietin (TPO) has been studied to be the main regulator (19). Platelets are released into blood circulation through the megakaryocytes, which develop from a very organized haemopoietic stem cell commitment process, proliferation of the cells and differentiation consecutively (20)

Animal and human models have been used in previous studies to explain the function of thrombopoietin (TPO) in the process of megakaryocytopoiesis and thrombopoiesis (2). In mice lacking thrombopoietin (TPO) and thrombopoietin receptor (C-MPL), platelet production was reduced drastically (22,23).

Studies carried out by administering the *Jatropha tanjorensis* powder to rabbits led to a significant development in the haematological indices, which directly reflects the role of the bone marrow (24). The plant is highly rich in iron, which helps increase the iron concentration made available for the process of erythropoiesis (25).

Materials and Methods

This study was conducted at the University of Benin, Edo State, Nigeria. A total of twenty (20) male albino Wistar rats were used in this study. The rats were housed in the Department of Anatomy, University of Benin, after their purchase and were exposed to dark and light cycles to allow for acclimatization for a two-week period with access to feed and water *ad libitum*

Identification of *Jatropha Tanjorensis* Leave Extract

The leaves were harvested from the University environment and transported to the Department of Plant Biology and Biotechnology, Faculty of Life Science, for proper identification and labelling. The leaves were identified, and voucher number UBH-G532 was given.

Crude Extraction

Aqueous extract of the leaves were obtained by weighing 300g and placed in three 1000ml beakers. Water was added and allowed to soak for 3 days. After 3 days, the extract was filtered, and the solution was placed in a freeze-dryer to obtain a dry compound paste.

Study Design

Animals in group A were fed with grower mash and water only.

Animals in group B were given grower mash and water, administered with 1000mg/kg body weight of *Jatropha tanjorensis* crude extract.

Animals in group C were given grower mash and water, administered with 2000mg/kg extract of *Jatropha tanjorensis*.

Animals in group D were given grower mash and water, administered with 4000mg/kg crude extract of *Jatropha tanjorensis* . The experimental period lasted for five (5) weeks and body weight of animals were taken weekly and recorded.

Sacrifice of the Animals

At the end of the experimental period which lasted for five (5) weeks, the animals were grossly observed for general physical characteristics, and were weighed using a weighing balance. A midline incision was made through the ventral wall of the rats under mild anesthesia using chloroform.

Sample Collection

Five millilitre of blood samples were collected by terminal blood collection into sample bottles containing EDTA and plain sample bottles, respectively by terminal blood collection. All samples collected in plain sample bottles were spun at 4000 rpm for 10 minutes; the serum was collected into correspondingly labeled plain sample bottles and stored at -20^{o}C till assay. The EDTA samples were used for complete blood count, and the bone marrow of the rats was also harvested and stored in 0.3ml of TriZol reagent.

Laboratory Analysis

Full Blood Count

All full blood count samples were analyzed using the automated three-part ERMA Haematology Autoanalyzer PCE-210N (Diamond Diagnostic; Holliston, USA). Prior to the analysis, all samples were placed on a

mixer and well mixed by gentle inversion. The automated analysis was done according to the manufacturer's operational guidelines. All the samples were analyzed within 30 minutes of collection.

Procedure for Full Blood Count

The whole blood was properly mixed and inserted into the probe. Then, 20 µL of the blood was aspirated into the instrument. The analysis was immediately done, and the results were displayed on the screen after about 1-2 minutes, which were printed by the printer.

RNA extraction and Semi-Quantitative PCR

RNA was isolated from the rat bone marrow with TRIzol Reagent (ThermoFisher Scientific) and converted to cDNA using ProtoScript First Strand cDNA Synthesis Kit (NEB). PCR amplification of TPO mRNA and c-MPL mRNA was done using OneTaq® **2X Master Mix (NEB).**

Procedure for RNA extraction

After sacrificing the animals, the bone marrow harvested was imbedded in 0.3mL of TRIzol (Invitrogen Life Technologies, Inc., Carlsbad, CA) reagent inside an Eppendorf tube for proper tissue preservation. The tissues were homogenized using a plastic pestle. RNA lyase buffer was then added to the homogenate to break down the tissue cell membranes further, after which it was spun at 10000 rpm for 10 minutes. The RNA supernatant was carefully removed and placed in a separate Eppendorf tube. The RNA precipitating buffer was centrifuged at 10000 rpm for 30 minutes. The supernatant was carefully removed, leaving the RNA precipitate at the bottom of the tube. RNA wash buffer was added and centrifuged again at 10000 rpm for 5 minutes. This step was repeated three times to remove excess solutions and previously added buffers. Nuclease-free water was added to break down the RNA in a pellet form and the phosphodiester bond of the RNA. The solution also contained a nuclease inhibitor, which removed other DNA or RNA contaminants from the medium. The RNA was quantified using a UV spectrophotometer at 260nm.

Complimentary DNA (cDNA) Synthesis

All components of the cDNA kit were added according to the RNA following the manufacturer's instruction; these components included random primer, oligonucleotide, primer or deoxynucleotides, and reverse transcriptase buffer. After adding all components to the RNA, the mixture was then incubated at 42^0C in a thermocycler for 1 hour. Then, the temperature was increased to 75^0C to denature the reverse transcriptase. Thereafter, all the RNA was converted to cDNA. For Real-time Quantitative Polymerase Chain Reaction (RQ-PCR) on rat samples, the expression levels of TPO and c-MPL genes were normalized to the levels of the GADPH housekeeping gene.

Gene Amplification

An equal volume of forward and reverse primer, PCR mix (master mix), Taq polymerase, and magnesium was added. The mixture was placed in a thermocycler for amplification and programmed for 30 cycles.

Gel Electrophoresis

After the PCR process, the DNA gel loading dye was added to the mixture. The agarose gel was prepared by dissolving 1% of the gel in Tris Borate EDTA buffer. The gel was allowed to solidify, and the sample was loaded. The gel was connected for electrophoresis. A snapshot was taken thereafter, and the image was transferred to ImageJ. The intensity of the bands from agarose gel electrophoresis was quantified densitometrically using ImageJ software.

Statistical analysis

Data obtained from this research was presented and analyzed using Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc. USA), while analysis of variance (ANOVA) was used to compare means and results were expressed in the standard error of the mean, bar chart and line graph as it may occur. A value of $p<0.05$ was accepted as significant.

Results

Table 1 shows the effect of *Jatropha tanjorensis* on platelet parameters in Albino Wistar rats. Platelet counts were significantly higher in test rats than in controls($p=0.0203$). The highest platelet count was observed in rats administered with the highest concentration of 4000mg/kg leave extract. Mean platelet volume (MPV), platelet distribution width (PDW) and platelet crit (PCT) were not altered significantly in test rats compared to control rats ($p=0.3656$, $p=0.025$ and $p=0.4404$, respectively).

Total white blood cells were significantly reduced in test rats compared to control rats($p=0.0003$), Lymphocytes were significantly higher in test rats compared to control rats($p=0.0067$), while Neutrophils and mixed monocytes (MID) were significantly lower in test rats compared to control rats($p<0.0001$ and $p<0.0001$ respectively as shown in table 2.

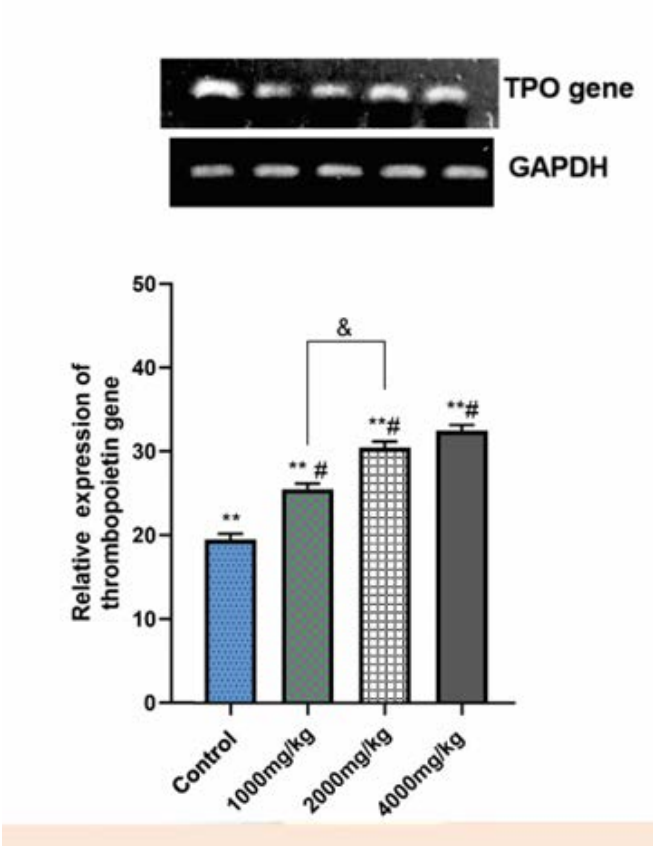
Fig 1 shows the expression of the TPO gene as represented by gel electrophoresis. The mRNA expression of the TPO gene was significantly higher ($p<0.05$) in test rats compared with controls, and the higher the concentration of leave extract, the higher the expression of the TPO gene. Figure 2 shows the relative expression of c-MPL in Albino Wistar rats. There was no significant expression of c-MPL in test rats compared with control rats.

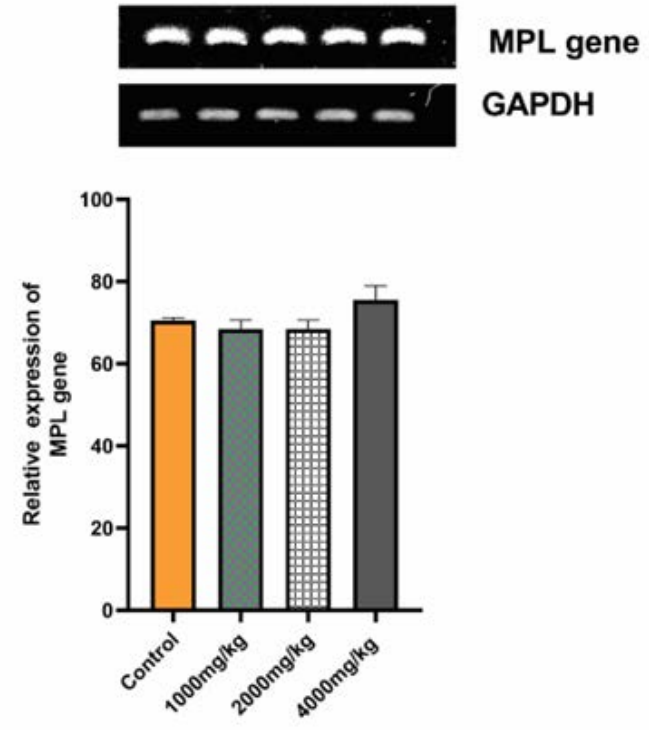
Table 1. Effect of *Jatropha tanjorensis* on Platelet parameters in male Albino Wistar rat.

Parameters	Platelet Count ($X10^3\mu L$)	MPV (μM^3)	PDW (%)	PCT (%)
Control	691.7±59.73	8.4±0.5148	10.02±0.914	0.698±0.09129
1000mg/kg	741.4±30.45	8.75±0.4924	10.75±0.6665	0.4925±0.02496
2000mg/kg	789.2±42.25	8.42±0.299	11.16±0.7935	0.692±0.05877
4000mg/kg	20853±20062	7.76±0.2112	9.52±0.4386	0.566±0.09862
F Value	3.517	1.158	3.333	1.007
P value	0.0023	0.3656	0.025	0.4404

Table 2: Effect of *Jatropha tanjorensis* on White Blood Cell parameters in male Albino Wistar rat.

Parameters	Total WBC (10^3/µl)	Lymphocyte Count (%)	MID (%)	Granulocyte (%)
Control	14.37±2.57	75.83±6.75	19.3±2.05	8.2±2.801
1000mg/kg	6.65±0.74	90.85±2.19	5.77±0.8664	3.37±1.411
2000mg/kg	6.6±0.79	93.82±0.70	4.97±0.3772	1.82±0.1715
4000mg/kg	6.92±0.61	92.2±1.00	6±0.3782	2.2±0.4572
F Value	8.164	4.561	31.56	27.24
P value	0.0003	0.0067	<0.0001	<0.0001





Discussion

This study was carried out to determine the effect of *Jatropha tanjorensis* on platelets,expression of TPO and c-MPL genes in mediating the thrombopoietic process. Thrombopoiesis is a process that encompasses the development of megakaryocytes into maturing platelets that support haemostasis. It was observed that the leaf extract stimulated a significant increase in platelet count with increasing concentration of plant extract as the platelet count was highly raised in the group administered with the highest dose(4000mg/kg) of plant extract. This could be attributed to an increase in the number of megakaryocytes and their progenitors in the marrow and spleen.

In stimulating the megakaryocyte's progenitor cells, platelet numbers will invariably increase. A study carried out in 2012 showed that the leaves exhibit blood renewal capabilities and contain some biological components vital for the swift haemopoietic processes in the bone marrow (26). The mean platelet volume, which assesses the average size of platelets, did not change significantly with the various administration of plant extract in the test rats compared to the control rats. This could be attributed to the fact that this leaf extract does not directly act on already-formed platelets but acts more on the progenitor megakaryocytes. This is at variance with another study that observed an increase in mean platelet volume (27). This may be attributed to the varying amounts of extract that were administered.

Platelet distribution width, which is a numerical measure of the variability in size, was not significantly affected. This may be because the extract of J tanjorensis does not act on already formed platelets, but its progenitors increase the number of progenitor megakaryocytes without altering the shape and size of the platelets. This agrees with the study by Igbinaduwa *et al. (14), who* also observed no significant change in this parameter. Plateletcrit (PCT), which measures total platelet mass as a percentage of the volume occupied in circulation and effectively detect platelet number abnormalities, did not significantly change in our study's various doses of plant extract. This is in line with the fact that mean platelet volume, which was not affected by the plant extract, is largely responsible for the plateletcrit not being altered as well.

White blood cells, which play a key role in immune responses, were significantly lower in the groups treated with various extracts of the leaves when compared to the controls. This may be because *J tanjorensis* leave extract may not favour the maturation of white cells nor been destructive to white cells. Lymphocytes, which are directly responsible for antibody production, direct cell-mediated killing of viruses and tumour cells, and the regulation of immune response, were significantly higher in all the treated groups than in the control group. This is largely because the plant extract readily encourages lymphocyte maturation of progenitor cells, causing them to mature at a higher rate, leading to high lymphocyte count. On the other hand, neutrophils, which are largely responsible for destroying bacteria, were significantly lower in the treated groups than in the control groups. Extract of *J tanjorensis* may not contain chemical properties that may encourage the maturation of neutrophils progenitor cells in the bone marrow.

Thrombopoietin (TPO) is the circulating regulator of platelet production, which plays an important role in the proliferation and maturation of megakaryocytes. This is done by triggering the proliferation of marrow megakaryocytic progenitor cells upon binding with c-MPL receptors expressed on platelets and megakaryocytes, thus acting as receptors for TPO. The signal activation leads to the transcription of genes necessary for the production and maturation of platelets due to the proliferation of megakaryocytes.

In our study, the mRNA expression of TPO gene was significantly expressed in the group treated with 1000mg/kg of *Jatropha tanjorensis* leave extracts compared to control groups($p<0.05$). There was also a significantly higher expression of the TPO gene as crude extract concentration increased from 2000mg/kg to 4000mg/kg. This may be attributed to the fact that *Jatropha tanjorensis* leave extract acts directly on thrombopoietin, aiding its active production and hence can act on megakaryocytic progenitor cells, causing the proliferation of platelet numbers.

The c-MPL gene plays a vital role in megakaryocytic and platelet development. This is done by acting as receptors for TPO. In our study, there were no significant differences in the expression of c-MPL in the treated groups when compared to the control groups. This may invariably imply that the extract of *Jatropha tanjorensis* acts directly on thrombopoietin and does not alter the expression of c-MPL. Administration of the leave extract has been shown by other researchers to improve haematological indices as well as improving bone marrow function (28)

Conclusion

This research findings demonstrate that *Jatropha tanjorensis* leave extracts is associated with an increase in platelet numbers and a significant decrease in white blood cells. Also, there was significant TPO expression, which was highly dependent on the concentration of leave extract. The leaf extract did not significantly affect the expression of the c-MPL gene. These results suggest that *Jatropha tanjorensis* could influence thrombopoiesis by modulating the expression of the key regulatory factor thrombopoietin, providing insights into potential therapeutic significance for thrombopoietic related conditions.

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