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ORIGINAL RESEARCH

Ameliorative Effect of *Faidherbia albida* Against *2.4*-Dinitrophenylhydrazine Induced Hyperbilirubinemia in Wistar Albino Rats

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Background: *Faidherbia albida,* popularly known as gawo in Hausa, is traditionally used to treat jaundice in Zuru emirate of Kebbi State. Herein, the ameliorative effect of *F. albida* against 2.4-dinitrophenylhydrazine-induced hyperbilirubinemia in Wistar albino rats was investigated.

Methods: Thirty healthy rats were administered 75 mg of 2.4-dinitrophenylhydrazine to induce hyperbilirubinemia. Thereafter, groups 1–3 received 500, 750, and 1000 mg/kg body weight of the methanol stem-bark extract, and 15 mg/kg of phenobarbitone (standard drug) was administered to group 4. Groups 5 and 6 served as the untreated and normal controls, respectively. The phytochemical composition was evaluated using standard methods, and acute oral toxicity was evaluated using standard OECD 2008 guidelines.

Results: Phytochemical analysis revealed the presence of alkaloids, phenols, and a substantial amount of tannins. A significant ($P<0.05$) reduction of direct bilirubin, total bilirubin, and total protein levels for all the doses of the extract and standard drug compared to untreated groups was observed. Similarly, there were significant reductions in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels of the group treated with the standard drug and all extract-treated groups compared to elevated levels observed in untreated controls. However, a significant ($P<0.05$) increase in serum albumin (ALB) levels, red blood cells, hemoglobin, and pack cell volume was observed in all extract-treated compared to the untreated control in contrast to a significant decrease in MCH levels in treated groups compared to the untreated group.

Conclusion: *F. albida* ameliorated the hyperbilirubinemia induced by 2.4-dinitrophenylhydrazine in Wistar albino rats, thus providing some support for its use in traditional medicine to treat jaundice.

Keywords: *Faidherbia albida*, jaundice, liver enzymes, hyperbilirubinemia, 2, 4-*dinitrophenylhydrazine*, red blood cells

Introduction

Jaundice, a disorder where the skin, mucous membranes, and whites of the eyes turn yellow, is brought on by an excess of bilirubin in the blood as a result of an overproduction of or failure to metabolize and eliminate bilirubin.[1] The excess bilirubin that accumulates in the skin is produced from the breakdown product of heme called biliverdin.[2] It is a manifestation that underlies disorders of bilirubin metabolism, biliary obstruction, or hepatocellular dysfunction. Jaundice is usually observed in neonates and is not common among adults, and although neonatal jaundice is not necessarily fatal, the accumulation of bilirubin can cause permanent brain damage.[3] The unbound unconjugated bilirubin can cross the blood-brain barrier, thus causing toxicity to the central nervous system.[4] Although the yellowish color caused by the accumulation of unconjugated bilirubin is visible to the eye, it is the onset and determination of hyperbilirubinemia in the laboratory that leads to the diagnosis of neonatal jaundice.[5] A total serum bilirubin level of

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2.7–4.0mg/dl is the lowest range at which neonatal jaundice can be visually detected.[5] The laboratory test for hyperbilirubinemia usually includes an evaluation of bilirubin (conjugated and unconjugated) and liver enzymes including alanine aminotransferase, aspartate aminotransferase, total protein, and alkaline phosphatase.

Indeed, jaundice is among the most widespread disease conditions in many parts of the world. It is more prevalent in underdeveloped and developing countries. For instance, a recent study reported that 24.46% of 417 newborn babies encountered neonatal jaundice in southern Ethiopia.[6] A similar incidence was previously reported in Nigeria with male neonates accounting for 56.8% of babies admitted with Jaundice whereas, 14% of the neonates developed complications as a result of jaundice that resulted in death (69.4%).[7] The global prevalence of severe neonatal jaundice ranged from 8.31% to 31.49% with the highest incidence recorded in Africa.[8] Indeed, the African region accounted for the highest incidence of exchange blood transfusion and an estimated 7.52% jaundice-related deaths.[8]

From ancient times, plants have served as the foundation of the folklore medical system, which has rapidly gained commercial significance.[9] The majority of the global population (80%) relies on medicinal plants and alternative medicine.[10] Plants have been used on the African continent for a very long time; in some African nations, up to 90% of the population relies on medicinal plants as a source of medications.[11] This can be linked to several factors, including the fact that conventional medication is expensive and has serious side effects, while traditional medical consultancies are far more affordable and accessible.[12] A recent ethnobotanical study identified 28 medicinal plants that are used to treat jaundice.[13] The stembark of *Faidherbia albida* (Delile) A. Chev is boiled and a cup full of the filtrate is orally administered twice a day for three days as treatment for jaundice.[13] It belongs to the Fabace has a wide spread across Africa and is also found in Israel. In addition, *F. albida* is also used to treat other diseases such as malaria, fever, toothaches, vomiting, diarrhea, and other digestive disorders.[14]

However, the potential effect of *F. albida* against jaundice has not been investigated. Therefore, the present study investigated the in vivo ameliorative effect of the methanol stem bark extract against 2.4-dinitrophenylhydrazine-induced hyperbilirubinemia in Wistar rats.

Materials and Methods

Collection of Plant and Preparation of Plant Extract

F. albida was collected from Zuru in Kebbi State, Northwestern Nigeria, the sample was transported in a polythene bag to the herbarium of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology Aliero. The specimen was identified, assigned voucher number (Ksusta/psb/h/319) by a taxonomist, and the specimen was deposited in the herbarium as previously described.[13] For preparation, the stem bark of *F. albida* was shed dried, and then pulverized using a blender. 150 g of the pulverized sample was soaked in absolute methanol for 48 h and was filtered using cheesecloth, the filtrate was then concentrated at 45°C using a vacuum rotary evaporator. The concentrate was further air dried at room temperature and the extract was kept in a sterile petri dish and was stored refrigerated until needed for use.

Qualitative and Quantitative Phytochemical Analysis

Quantitative and qualitative assays were carried out using standard methods.[15–17]

Qualitative Phytochemical Analysis

Test for Alkaloids (Dragendroff's Test)

A filtrate comprising the extract (0.5 g) and 5 mL of HCL (1%) was prepared by continuous stirring on a steam bath and thereafter, a few drops of Mayer's reagent and Dragendroff's reagents were each separately added to 1 mL of the filtrate. The preliminary evidence of the presence of alkaloids was based on the precipitation or turbidity of the filtrate mixture.

Test for Flavonoid (Alkaline Reagent Test)

The presence of flavonoids was determined by observing for yellowish coloration following the mixing of the extract (5 mL) and drops of aqueous NAOH.

Test for Tannins (Ferric Chloride Test)

The presence of tannins was determined by observing for a blue-black, green, or precipitate following the addition of a few drops of ferric chloride to a filtrate containing plant extract (0.5 mL) in 10 mL of distilled water.

Test for Phenolic Compound

A mixture containing the extract and ferric chloride solution in a ratio of 1:1 (2 mL each) was shaken and placed under observation for the possible appearance of a dark green color as an indicator of the presence of phenolic compounds.

Quantitative Phytochemicals Analysis

Determination of Alkaloids

A total of five grams (5 g) of the powdered sample was dissolved in 100 mL of 20% acetic acid in ethanol and incubated for 4 h. Thereafter, the filtrate was concentrated and concentrated ammonia hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration using filter paper and weighed.

Calculation

$$\%\ \text{Alkaloids}\ (\text{g}\%) = \frac{\text{Alkaloids residue}}{\text{Weight of Sample}} \text{x}100$$

Where

W_1 = Weight of empty filter paper

W_2 = Weight of the sample

W_3 = Weight of filter paper + Alkaloids

Flavonoids

The technique used that has been previously described was adopted.[18] The reaction mixture consists of 2 ml of two (2%) percent $AlCl_3$ dissolved in ethanol, and mixed with 2 ml of varying concentrations of the standard (0.1 to 1 mg/mL) in methanol. One milligram/milliliter concentration of the extracts was also added to two (2) milliliters of 2% AlCl3 in methanol, and the spectrophotometric reading was obtained at 420 nanometres after a one-hour incubation at room temperature.

Determination of Total Tannins Content

This technique used by[19] was adopted. Tannic acid was diluted to a standard solution (5–100 ug/mL) and then one milliliter of sample/ standard was diluted with distilled water to 7.5 ml. One milliliter Folin-Denis reagent was added and two milliliter of 7.5% of sodium carbonate solution was added to both tubes respectively. Absorbance reading was taken at 700nm. The result was calculated as milligrams of tannic acid equivalent per gram (mg TAE/g).

Total Phenolic Content

This was determined using a previous method.[19] The different concentrations of gallic acid or extract (0.2–1mg/mL) were prepared and 0.5 mL was added into tubes with 6.5 mL of distilled H20, 0.5 mL tenfold diluted folin ciocalteu reagent, and 5 mL of 7% sodium carbonate. The mixture was incubated for 90 min at room temperature. The experiment was done in triplicate and the absorbance was read at 760 nm. The total phenolic content was expressed as Gallic acid equivalent (GAE).

Experimental Animals

Following the granting of ethical approval (KSUSTA/FLS/UREC/20-2) by the faculty of life sciences ethics committee at Kebbi State University of Science and Technology, Aliero. The animals were purchased and housed in ventilated cages according to previously described conditions.[20] Also, The guide of care and use of animals in research and teaching of

the animal ethics committee of Kebbi State University Aliero, Nigeria, in line with the National Institutes of Health guidance for the care and use of laboratory animals (NIH publications No. 8023, revised 1978) were followed in maintaining the rats.

In vivo Bilirubin Assay

The animals were placed into six different groups. For induction of hyperbilirubinemia, 75 mg/kg body weight of 2.4-dinitrophenylhydrazine was administered via intraperitoneal route to groups 1–5 for five (5) days according to the previous method.[21] Whereas, group six (6) served as the normal control (un-induced) that did not receive the 75 mg/kg body weight of 2.4-dinitrophenylhydrazine. Following confirmation with combi9 strip and the level to be 4 g/dl of hyperbilirubinemia, the animals were then administered with different concentrations of the extract for group 1 (500 mg/kg), group 2 (750mg/kg) and group 3 (1000 mg/kg), whereas group 4 received 15 mg/kg body weight of the phenobarbitone standard drug for 14 days. 24 h after the last administration, the animals were anesthetized in a CO_2 jar, and blood was collected through cardiac puncture for biochemical and hematological analysis.

Biochemical Analysis

Biochemical analysis of liver function parameters was determined as follows;

Determination of Aspartate Amino Transferase (AST)

This was done according to the technique developed by (Frankel, 1957; Schmidt and Schmidt, 1976).

The reaction mixture was composed of two (2) sets of tubes: the sample (in triplicates) and the reagent blank tube. 0.1 mL of the sample was added to the sample tubes, and 0.1mL of distilled water was added to the blank tube. 0.5 mL of RI (phosphate buffer, 100 mm/L, PH 7.4, L-aspartate, 100 mm/L, and α-ketoglutarate, mm/L) were added into all tubes, and they were then mixed and left to stand for 20 min at 25°C. Five millilitres of 0.4 molar sodium hydroxide was added into all tubes and thoroughly mixed; the spectrophotometric value of the sample against the blank was measured at 546 nm after 5 min.

Determination of Alanine Aminotransferase/glutamic pyruvic transferase (ALT),

The reaction mixture consists of two (2) sets of tubes samples (in triplicates) and reagent blank tube, 0.1 mL of the sample was added to sample and 0.1 mL of distilled water to the blank tube, 0.5 mL of RI (phosphate buffer 100 mmol/L $_P$H 7.4, L-aspartate 100 mmol/l, and α-ketoglutarate 2 mmol/l) were added to all tubes and was mixed thoroughly and incubated for exactly 30 min at 37°C, 0.5 mL R2 (2,4 dinitrophenylehydrazine 2 mmol/L) was added to all tubes while 0.1 mL of the sample was added to reagent blank tube and was then mixed and allowed to stand for 20 min at 25°C, 5 mL of 0.4 m sodium hydroxide was added to all tubes and was thoroughly mixed, the absorbance of sample against blank was measured at 546 nm after 5 min.

Alkaline phosphatase (ALP),[22]

The test consisted of four (4) sets of tubes in triplicate test, test blank, standard, and standard blank, to each tube 1 mL of phosphate buffer ph 7.4 was added followed by 1 mL phenyl phosphate to test and test blank tubes, and mixture was mixed and incubated at 37°C for 2 min, then 100 ul of serum was added to test, standard and standard blank tube and was mixed and incubated at 37°C for 15 min, this followed by the addition of 800 ul of 0.5 N NaoH, 100 ul of serum was added to test blank tube, this was followed by the addition of 1.2 mL, 1 mL,1 mL each of 0.5 m NaHCo3, amino antipyrine, and potassium ferricyanide to each tube, this was thoroughly mixed and absorbance recorded at 500 nm using a spectrophotometer. Serum alkaline phosphatase was calculated using = T-TB/S-B x 10 units/100mL).

Where T= absorbance of test, TB= absorbance of test blank, S= absorbance of sample,

B= absorbance of blank.

Total Protein determination,[23]

The experiment consisted of three (3) sets of tubes; a blank, a standard, and a sample tube. 1000 ul of reagent one was dispensed into each tube, followed by 20 ul of the standard into the standard tube and 20 ul of sample serum into the sample tube. The tubes were left to stand for ten (10) minutes at 37°C, and absorbance was read at 546 nm.

$$\text{Total protein concentration}(g/dl) = \frac{\text{absorbance of sample}}{\text{Absorbance of standard}} \text{x concentration of std}$$

Bilirubin Estimation

The experiment consists of two (2) sets of tubes in triplicate, test, and blank. To each tube, 200 ul of reagent one (1) was dispensed, then one drop of reagent two (2) to only the test tube, and then 1000 ul, 200 ul, and 1000 ul of reagent three (3), sample, and reagent four (4) were added serially to both the test and blank tubes, respectively. The mixture was left to stand for ten (10) minutes at 25°C, and absorbance read at 578 nanometres for the total concentration of bilirubin and 546 nanometres for the direct bilirubin.

Manual calculation

Total bilirubin

Mg/dl = 10.8 x absorbance

Direct bilirubin

Mg/dl= 14.4 x absorbance.

Total Albumin Determination[24]

The test consists of three sets of tubes; blank, standard, and sample tubes. To each tube, 1 mL of reagent was dispensed, then 5ul of known solution of albumin to the positive control tube and 5ul of serum to the sample tube. The test tube constituents were mixed thoroughly and left to stand for ten minutes at 25°C. The absorbance of the sample and standard were read against a blank at 630 nm, all experiments were performed in triplicate.

Calculations:

$$\text{Albumin concentration Isample}(g/dl) = \frac{\text{Abs of sample} - \text{Abs of blank}}{\text{Abs of standard} - \text{Abs of blank}} \text{x conc, of std}$$

$$\text{Conversion factor g/dl x144.9} = \text{umol/l}$$

Hematological Analysis

Following the previous method,[25] hematological parameters were analyzed using an automated hematological analyzer Sysmex XS800i (Sysmex Corporation, USA).

Acute Oral Toxicity Assay

Following the OECD 2001 guideline and after overnight fasting with access to water, five (5) animals were administered 5000 mg/kg of the extract. The animals were observed 8 h, 48 h, and 14 days after administration for any sign of toxicity such as weakness, loss of appetite, slow breath, paw licking, dropping of feces, loss of weight, and mortality.[26]

Data Analysis

The Data is presented as mean ± SEM. Analysis of variance (ANOVA) using the Duncan multiple comparison test was conducted using SPSS Version 20 to compare the statistical significance between untreated and various treated groups. The p-value ($P<0.05$) was considered statistically significant.

Results

Phytochemical Analysis of F. Albida

An analysis showed that the phytoconstituents include alkaloids, tannins, and phenols with substantial amounts of tannins (Table 1).

Acute Toxicity Profile of F. Albida

There was no evidence of toxicity or death recorded in the Wistar rats throughout the observation period following the administration of 5000 mg/kg of the stembark extract. This indicates that the LD_{50} is above 5000 mg/kg body weight.

Table 1 Qualitative and Quantitative Phytochemical Constituents of F. Albida Methanol Stem Bark Extract

Phytochemical	Qualitative	Quantitative
Alkaloids	+	0.09 ± 0.00 mg/mL
Flavonoids	–	
Tannins	+	8.28±0.13mg/mL
Saponins	–	
Phenols	+	0.30±0.01mg/mL

Notes: (+) Present, and (-) Not detected.

The Wistar rats administered with 5000 mg/kg of the extract did not show any sign of toxicity, and no death was recorded throughout the observation period indicating that the LD_{50} is above 5000 mg/kg body weight.

Effect of F. Albida Extract on Hyperbilirubinemia and Liver Functions

F. albida extract caused a significant (P<0.05) reduction in serum AST, ALT, and ALP of the group treated with standard drug (Phenobarbitone) and all extracts treated groups compared to untreated control, whereas, a significant increase in AST, ALT, and ALP was observed in untreated control compared to normal control. There was a significant (P<0.05) reduction in serum bilirubin direct and total (DB and TB) in all extracts treated, standard drug-treated, and normal control groups compared to the untreated control group. In addition, the level of serum total protein was significantly (P<0.05) reduced in all treated groups (extract and phenobarbitone) in comparison to the induced untreated hyperbilirubinemia group. In contrast, the serum level of albumin was significantly elevated in all extract-treated, standard drug, and normal control groups compared to untreated control (Table 2).

Effect of F. Albida Extract on Hematological Parameters

The methanol stem bark extract induced a significant (P<0.05) increase in red blood count (RBC) in extract-treated groups (500 and 750mg/kg) and standard drug-treated group compared to the untreated control group but was not significant (P<0.05) different compared to normal control. Similarly, a significant (P<0.05) increase in hemoglobin (HGB) was observed in groups treated with standard drug (Phenobarbitone), all extracts treated groups (500, 750, and 1000 mg/kg), and normal control compared to untreated control. The leave of pack cell volume (PCV) was significantly (P<0.05) increased only in the group treated with 500 mg/kg of the extract compared to both untreated and normal control groups. Whereas, a significant (P<0.05) decrease in MCH was observed in all extracts-treated groups, normal control, and standard drug-treated groups compared to the untreated control group (Table 3). Meanwhile, there was

Table 2 Anti-Hyperbilirubinemia Activity of F. Albida Methanol Stem Bark Extract on Albino Rats

Parameter	Normal Control Distilled H_2O 5mL/kg	Phenyl Hydrazine (75 mg/kg)	Phenobarbitone (15 mg/kg)	Extract (500 mg/kg)	Extract (750 mg/kg)	Extract (1000 mg/kg)
AST (U/L)	518.00 ± 1.08^{e}	564.00 ± 1.47^{f}	158.75 ± 0.58^{a}	428.00 ± 1.08^{d}	359.25 ± 0.48^{c}	189.25 ± 0.47^{b}
ALT (U/L)	568.50 ± 1.19^{e}	579.00 ± 1.68^{f}	249.00 ± 0.41^{a}	428.50 ± 0.65^{d}	389.00 ± 0.41^{c}	338.75 ± 0.48^{b}
ALP (U/L)	420.25 ± 0.85^{e}	474.25 ± 1.10^{f}	168.75 ± 0.49^{a}	248.50 ± 0.65^{d}	218.75 ± 0.48^{c}	178.75 ± 0.63^{b}
TB (G/l)	1.23 ± 0.06^{a}	4.13 ± 0.17^{d}	1.93 ± 0.18^{bc}	2.20 ± 0.09^{c}	1.85 ± 0.03^{b}	1.43 ± 0.25^{a}
DB (G/l)	0.65 ± 0.03^{a}	2.05 ± 0.03^{d}	0.90 ± 0.04^{bc}	1.03 ± 0.08^{c}	0.88 ± 0.05^{b}	0.55 ± 0.03^{a}
TP (G/l)	6.29 ± 0.09^{b}	12.40 ± 0.18^{c}	6.43 ± 0.11^{b}	1.50 ± 0.01^{a}	1.62 ± 0.02^{a}	1.65 ± 0.03^{a}
ALB (mg/dL)	16.25 ± 0.09^{c}	13.13 ± 0.18^{a}	14.12 ± 0.17^{b}	19.10 ± 0.51^{d}	22.78 ± 0.15^{f}	20.53 ± 0.40^{e}

Notes: Values are presented as mean ± SEM (n = 4) Values with different superscript letters (a,b,c,d,e,f) along the rows were statistically significantly different at P<0.05. **Abbreviations**: AST, Aspartate Amino Transferase; ALT, Alanine Amino Transferase; ALP, Alkaline Phosphatase; ALB, Albumin; TP, Total Protein; Bil, Bilirubin; ALB, Albumin.

Table 3 Effect of F. Albida Stem Bark Extract on Hematological Parameters of Phenylhydrazine Induced Hyperbilirubinemia in Albino Rats

Parameter	Normal Control Distilled H_2O 5mL/kg	Phenylhydrazine (75 mg/kg)	Phenobarbitone (15 mg/kg)	500 mg/kg	750mg/kg	1000 mg/kg
WBC (10^{-6}/UL)	7.91±0.32^{a}	5.02±0.24^{a}	8.63±3.60^{a}	5.18 ±0.09^{a}	5.53±0.39^{a}	4.39±0.11^{a}
RBC (10^{-6}/UL)	7.61±0.35^{b}	2.91±0.23^{a}	6.43±1.13^{bc}	7.41 ±0.19^{c}	7.66±0.44^{c}	4.77±1.06^{ab}
HGB (g/dl)	12.60±0.36^{b}	9.64±0.55^{a}	16.15±0.18^{c}	18.73 ±0.56^{d}	17.43±0.54^{cd}	13.63±0.67^{b}
MCV (%)	59.13±1.56^{a}	62.95±1.01^{ab}	68.88±2.25^{b}	83.35 ±3.99^{c}	68.23±0.35^{b}	68.48±0.90^{b}
MCH (%)	20.78±0.13^{a}	31.50±0.87^{c}	24.50±1.92^{b}	25.30±0.73^{b}	21.45±0.35^{a}	23.18±0.49^{ab}

Notes: Values are presented as mean ± SEM (n = 4). Values with different superscript letters (a,b,c) along the rows were statistically significantly different at P<0.05.
Abbreviations: WBC, white blood count; RBC, red blood count; HGB, hemoglobin; MCV, Mean corpuscular volume; MCH, mean corpuscular hemoglobin.

a non-significant (P>0.05) reduction in white blood count (WBC), of the group treated with both standard drug and extract-treated groups (500, 750, and 1000 mg/kg) when compared to both normal and untreated control.

Discussion

Jaundice is a disease characterized by yellowish skin discoloration and hyperbilirubinemia with a reported death of 2.6 million in 2016 with more than half of the deaths occurring in countries such as India, Pakistan, Ethiopia, Nigeria, etc.[27] Undoubtedly, plants have been used for decades to treat various diseases including jaundice amongst others attributable to the perceived safety and affordability. The usage of plants to treat cancer, viruses, and jaundice was recently reported.[21,28,29] In fact, 28 medicinal plants including *F albida* were recently identified and reportedly used to treat jaundice by traditional medicine practitioners in Zuru emirate Kingdom of Kebbi State, Nigeria.[13] Also known by the synonym *Acacia albida, F. albida* has been reportedly used as treatment for diarrhea, malaria, inflammation, vomiting, cold, fever, and cleansing of wounds.[30–32] Besides, pharmacological studies have also reported the bioactivities of *F. albida* including anti-inflammatory, antipyretic, antibacterial, anti-malaria, antidiarrheal, and anti-trypanosomiasis.[31,33–36]

Herein, the present study investigated the potential ameliorative effect of *F. albida* against 2.4-dinitrophenylhydrazine-induced hyperbilirubinemia. Firstly, phytochemical screening revealed the presence of alkaloids, with an appreciable number of phenols and a substantial number of tannins. The phenolic acids and tannins demonstrate anti-hyperbilirubinemia potentials.[37] Hence the anti-hyperbilirubinemia activity of *F albida* methanol stem back extract as observed in the current study might not only be due to the presence of these phytochemicals. The acute toxicity test revealed that the stem back extract is relatively non-toxic at acute doses since the LD_{50} is above 5000 mg/kg. Substances with LD_{50} values higher than 5000 mg/kg or 15000mg/kg are considered non-toxic or harmless, respectively.[38] The chemical agent phenyl hydrazine, when administered via the intra peritoneal route, induced jaundice by raising bilirubin levels. The considerable change in bilirubin and other liver function markers in the untreated control could be ascribed to phenylhydrazine's ability to cause hyperbilirubinemia and liver damage.[39] Indeed, as shown in Table 2, the administration of 2.4-dinitrophenylhydrazine induced significant hyperbilirubinemia in comparison to normal (un-induced) control. Interestingly, the administration of the stem bark extracts (500 mg/kg, 750 mg/kg, and 1000 mg/kg) and the standard control drug (15 mg/kg phenobarbitone) caused a reversal of hyperbilirubinemia with a significant reduction in total bilirubin and direct bilirubin levels almost comparable to the normal un-induced control. This is similar to the previous study that reported the ameliorative effect of *Agaricus brasiliensis* extract against phenylhydrazine-induced neonatal jaundice characterized by dose-dependent reduction of elevated bilirubin levels.[21] Liver enzymes including ALP, ALT, and AST are considered markers for liver toxicity. The levels of enzymes in the serum were significantly increased in the phenyl hydrazine-induced group although a dose-dependent reversal was observed upon treatment with all doses of the stembark extract as well as the standard drug. Similarly, elevated total protein levels were significantly reduced by the stembark extracts. Contrarily, phenylhydrazine caused a reduction in albumin levels that was significantly elevated by the stembark extracts. This suggests that the stembark extract reversed potential liver toxicity induced by phenyl hydrazine and restored the natural capacity of the liver. This is necessary as albumin is essential for the uptake of bilirubin into the liver for processing to prevent build-up in the body and the eventual development of yellowish skin.[40] Bilirubin is

produced as a result of the breakdown of the erythrocytes which contain hemoglobin and thus must be cleared from the system. The normal erythrocytes have smaller-sized reticulocytes and thus brisk reticulocytosis will have a higher MCV and MCH. In cold agglutinin disease and hyperbilirubinemia, the aggregation of red blood cells will falsely increase the MCV and MCH.[41] Finally, hemolysis can lead to folate deficiency and a rise in both MCV and MCH.[42] In the present study, *F. albida* methanol stem back extract showed strong anti-hemolytic potentials toward normalizing both hemoglobin and red blood concentration in hyperbilirubinemia-induced albino animals.

Conclusion

The methanol stem bark extract of *F. albida* ameliorated 2.4-dinitrophenylhydrazine-induced hyperbilirubinemia in Wistar rats via reversal of elevated bilirubin levels, liver enzymes, and elevation of albumin levels to facilitate uptake of bilirubin to the liver. Therefore, the present findings provide scientific evidence of *F. albida* in the treatment of jaundice and further support the utilization of this plant in traditional medicine. Future studies should further investigate the underlying mechanism mediating the reversal of hyperbilirubinemia and isolate the bioactive compound of interest.

Data Sharing Statement

The data analyzed in this work will be provided upon a reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors disclose that there are no competing interests in this work.

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