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Effect of Jatropha Gossypiifolia Leaves Extract in Wistar Albino Rats Against Gentamicin-Induced Biochemical Injury

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Abstract

Jatropha gossypiifolia L. (Euphorbiaceae), widely known as "bellyache bush," is a medicinal plant largely used throughout Africa and America. Several human and veterinary uses in traditional medicine are described for different parts and preparations based on this plant. The protective potentials of ethanol leaf extract of Bryophyllum pinnatum against gentamicin induced biochemical injury in Wistar albino rats were evaluated using serum biochemical parameters. Twenty-five male albino rats were divided randomly into five groups A to E. Groups C and D received 100, 200 and 300 mg/kg Jatropha gossypiifolia leaves extract respectively. Groups A and B were fed with chow (normal and positive controls) while Group E received 100 mg/kg of Vitamin C. Biochemical injury was induced in rats in groups B-E with gentamicin (i.p) at the dose of 80 mg/kg body weight on the 16th day of study. The rats were

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then fasted for 48 h and sacrificed by cervical dislocation. Serum was collected for biochemical analysis using standard methods and analytical biochemical kits. There was a significant increase in the serum levels of AST, ALT and ALP of the rats administered with gentamicin (Group B - positive control) compared to the Group A- normal control. However, treatment of rats with 100, 200 and 300 mg/kg body weight of ethanol leaf extract of Jatropha gossypiifolia L significantly decrease these biochemical parameters compared to Group B ($p < 0.05$). Also, there was a significant increase in the serum levels of conjugated bilirubin and total bilirubin in Group B compared to the normal control. Rat pre-treated with 200 and 300 mg/kg body weight of extract showed significant decrease in the conjugated and total bilirubin compared to the non-treated rats in Group B ($p <0.05$). Serum level of urea and creatinine significantly increased in Group B compared to normal control. Rats in groups C and D pre-treated with 200 and 300 mg/kg body weight of extract showed significant decrease in the levels of urea and creatinine compared to Group B ($p <0.05$). There was significant decrease in the serum levels of albumin and total protein of the rats administered with gentamicin compared to the normal control. Rats pre-treated with 200 and 300 mg/kg body weight of extract showed significant increase compared rats in non-treated Group B ($p <0.05$). The results obtained from this study shows that the ethanolic leaf extract of Jatropha gossypiifolia L has protective functions against gentamicin-induced hepatic and nephrotic damage in Wistar albino rats.

Keywords: Effect, Jatropha Gossypiifolia, Leaves, Wistar Albino, Rats, Gentamicin, Induced Biochemical, Injury

INTRODUCTION

The Euphorbiaceae family, which is considered one of the largest families of Angiosperms, covers about 7,800 species distributed in approximately 300 genera and 5 subfamilies worldwide. These species occur preferentially in tropical and subtropical environments [1, 2].

Among the main genera belonging to this family, there is Jatropha L., (figure 1) which belongs to the subfamily Crotonoideae, Jatropheae tribe and is represented by about 200 species. This genus is widely distributed in tropical and subtropical regions of Africa and the Americas [1]. The name "Jatropha" is derived from the Greek words "jatros," which means "doctor" and "trophe," meaning "food," which is associated with its medicinal uses [3]. The Jatropha genus is divided into two subgenera, Jatropha and curcas, from which the subgenus Jatropha has the widest distribution, with species found in Africa, India, South

America, West Indies, Central America, and the Caribbean [4]. Jatropha species are used in traditional medicine to cure various ailments in Africa, Asia, and Latin America or as ornamental plants and energy crops [3]. Several known species from genus Jatropha have been reported for their medicinal uses, chemical constituents, and biological activities such as Jatropha curcas, Jatropha elliptica, Jatropha gossypiifolia, and Jatropha mollissima, among others [3].

From these species, Jatropha gossypiifolia L. (Figure 1) is discussed here. It is a vegetal species widely known as "bellyache bush" and is a multipurpose medicinal plant largely used in folk medicine for the treatment of various diseases [3, 5, 6]. It is widely distributed in countries of tropical, subtropical, and dry tropical weather and tropical semiarid regions of Africa and the Americas [7].

In Brazil, it predominates in the Amazon, Caatinga, and Atlantic Forest and is distributed throughout the country in the North, Northeast, Midwest, South, and Southeast regions [8]. Several human and veterinary uses in traditional medicine are described for different parts (leaves, stems, roots, seeds, and latex) and preparations (infusion, decoction, and maceration, among others) based on this plant, by different routes (oral or topical). The most frequent reports concern its antihypertensive, anti-inflammatory, antiophidian, analgesic, antipyretic, antimicrobial, healing, antianemic, antidiabetic, and antihemorrhagic activities, among many other examples [3, 5, 7, 9]. Other uses are also related to this plant, such as biodiesel production, pesticide, insecticide, vermifuge, ornamentation, and even its use in religious rituals [3, 6, 10–13].

An important feature of J. gossypiifolia species is that, due to its important potential medicinal applications, it is included in the National List of Medicinal Plants of Interest, which is includes 71species of medicinal plants that have the potential to generate pharmaceutical products of interest to public health [14].

Regarding its phytochemical constitution, alkaloids, coumarins, flavonoids, lignoids, phenols, saponins, steroids, tannins, and terpenoids were already detected in different extracts from different parts of this plant [15]. Among the main activities already studied for this species (including various types of extracts from different parts of the plant), the antihypertensive, antimicrobial, anti-inflammatory, antioxidant, and antineoplasic activities mainly stand out, supporting some of its popular uses [3, 16].

Some toxicity studies have shown that despite the known toxicity of Jatropha species, J. gossypiifolia presented low toxicity in some in vitro and in vivo experiments. However, some studies have indicated that ethanolic extract from the leaves, in acute oral use, is safe for rats, but with chronic use, it could be toxic [17–19].

The leaf juice of the plant was used traditionally as antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumor, hypocholesterolemic, antioxidant, diuretic, antiulcer, antidiabetic, antiseptic, cough suppressant, antihistamine and anti-allergic (Okwu and Josiah, 2006).

The plant has also been used for the treatment of oedema of legs [20]. Leaf juice is used in the treatment of coughs, bronchial affections, blood dysentery, jaundice and gout (Ghani, 2003). In South-eastern Nigeria, the herb is used to facilitate the expulsion of the placenta after delivery and applied on the body of young children when they are sick [21].

Gentamicin is an aminoglycoside antibiotic widely used for the treatment of bacterial infections. Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals. Its use is known as one of the most common causes of acute renal failure and nephrotoxicity [22]. Vitamin C, selenium, vitamin E, taurine and the carotenoids (betacarotene, lutein and lycopene) were reported to decrease the gentamicin-induced reduction in the glomerular filtration rate and the severity of the tubular damage [23,24].

So, in view of the potential applications of this plant, this study aims to provide an up-to-date on the effect of Jatropha gossypiifolia leaves in wistar albino rats against gentamicin-induced biochemical injury.



Figure 1: Jatropha gossypiifolia plant showing the leaves and the fruits

Table 1: Phytochemical Composition of Jatropha Gossypiifolia; Methanol, Aqueous Extract, Stem Bark, Roots-Bark and Leaves

S/N	Phytochemicals	Jatropha gossypiifolia				
		Methanol	Aqueous	Stem bark	Roots bark	Leaves
1	Tannins	+	+	+	-	+
2	Alkaloids	+	+	-	-	+
3	Phenols	+	+	+	+	+
4	Flavonoids	+	+	+	+	+
5	Terpenoids	-	+	+	+	+
6	Glycosides	+	+	+	+	-
7	Anthraquinones	+	+	+	+	-
8	Saponin	+	+	+	+	+
9	Cyanoglycoside	+	+	+	+	-
10	Cardiac glycosides	+	+	+	+	+
11	Phlobatannin	+	+	+	+	+

MATERIALS AND METHODS

Animals: Twenty-five Wistar Albino male rats weighing 110 to 1320g were obtained from the Animal House of the HAUEMM Animal Farm, Federal Housing Estate Bajabure Gerie. Adamawa State. The animals were house in stainless cages and maintained under standard conditions for the period of the study. The animals were acclimatized for two weeks and maintained at the temperature of 25± 2ºC, 45 ± 5% relative humidity and 12 h light/dark cycle. The animals had access to water and food freely.

Chemicals

Absolute methanol was product of BDH Chemical Company 164 Ltd, Poole, England. Rat chow was purchased from Pfizer Nigeria Plc.

Collection of Plant Materials

The plant Jatropha Gossypiifolia was collected from HAUEMM Animal Farm, Federal Housing Estate Bajabure Gerie. Adamawa State

Extract Preparation

The leaves of Jatropha Gossypiifolia were collected and air dried under shade for two weeks. The dried leaves were ground into powder using an electric blender. 600 g of the powdered leave were macerated in 1800 ml of ethanol at room temperature for 24 h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was dried in a water bath at 37ºC, and the 50 g viscous concentrate was kept in air tight bottle at 4°C until use.

Experimental Design

Twenty-five (25) adult male Wistar albino rats (eight weeks old) were randomly divided into five (5) groups of five (5) animals per group, labelled A to E and were treated as follows:

1. Group A: served as the normal control (fed and water only).
2. Group B: served as the positive control.
3. Group C: received 100 mg/kg body weight of extract orally.
4. Group D: received 200 mg/kg body weight of extract orally.
5. Group E: received 300 mg/kg body weight Vitamin C orally (Standard antioxidant).

All treatments lasted for fifteen (15) days. Biochemical injury in animals in Group B to E were induced with gentamicin (i.p) at the dose of 80 mg/kg body weight on the 16th day of study, fasted for 48 h and then sacrificed through cervical dislocation.

Collection of Samples

Blood samples were collected in plain bottles through the cardiac puncture and were centrifuged at 2,800 rpm for 10 min. Serum obtained was used for biochemical analysis.

Biochemical Assay

Serum transaminase (ALT and AST) was determined by method of Reitman and Frankel (1957). ALP by the phenolphthalein monophosphate method (Babson, 1965). Total protein was determined by colorimetric method (Biuret method), as modified by Gornallet et al. (1994) method. Bilirubin was estimated by colorimetric method of Jendrassik and Grof (1938). Serum Urea was estimated by Natelson (1951) method, and serum creatinine by Jelliffe (1971).

Statistical Analysis

The results were expressed as mean ± SD. Data was analyzed by one-way analysis of variance (ANOVA). Sequential differences among means were calculated at the level of $P < 0.05$, using Turkey contrast analysis as needed.

RESULTS

Table 2. Effect of Jatropha Gossypiifolia on Liver Indices in Gentamicin-Induced Wistar Albino Rats.

Group	JATROPHA GOSSYPIIFOLIA ON LIVER INDICES							
	Treatment (Extract)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Albumin (g/dl)	Total Protein (g/dl)	Conj. Bilirubin (µmol/l	Total Bilirubin (µmol/l)
A	Normal Control	20.23±2.94a	18.00±1.58a	40.19 ± 2.10a	36.89 ± 1.00c	64.36± 1.36c	1.55±0.17a	5.76±0.64a
B	Positive Control	26.61±6.06c	30.20±4.76c	44.19 ± 1.55a	24.19 ± 1.55a	45.73± 2.65a	1.84±0.15b	9.22±1.42b
C	100mg/kg bwt	23.22±2.38b	22.40±4.04b	44.24± 5.43b	34.24± 5.43b	58.97± 3.71b	1.60±0.23a	6.37±1.24a
D	200mg/kg bwt	22.63±2.19b	23.00±4.30b	43.46± 6.23b	36.89 ± 1.00c	57.42± 4.72b	1.56±0.13a	6.39±0.73a
E	300mg/kg bwt	22.23±2.38b	22.80±4.15b	40.15± 6.50b	24.19 ± 1.55a	55.83± 1.83b	1.47±0.16a	6.25±0.42a

Data are mean ± SD (n = 5). Means values in the same column with different superscript letter(abc) are significantly different; $p < 0.05$

Table 3. Effect of Jatropha Gossypiifolia on Kidney Indices in Gentamicin-Induced Wistar Albino Rats.

Group	**JATROPHA GOSSYPIIFOLIA ON KIDNEY INDICES**		
	Treatment (Extract)	UREA (mmol/l)	CREATININE (mmol/l)
A	Normal Control	5.34 ± 0.51a	69.41± 3.47a
B	Positive Control	9.13± 0.37b	92.37± 6.71c
C	100mg/kg bwt	6.29±0.92a	76.27±7.46b
D	200mg/kg bwt	6.11± 1.12a	76.35± 6.12b
E	300mg/kg bwt	6.36±0.41a	73.27±5.57a

Data are mean ± SD (n = 5). Means values in the same column with different superscript letter(s) are significantly different; $p < 0.05$

The results of the effect of ethanol leaf extract of Jatropha Gossypiifolia on liver indices in wistar rats are presented in Table 1. There was significant increase in serum levels of AST, ALT, ALP of the rats administered with gentamicin (26.61±6.06c, 30.20±4.76c, 24.19 ± 1.55a), respectively when compared to the normal control group (20.23±2.94a, 18.00±1.58a, 40.19 ± 2.10a), ($p < 0.05$). However, pre-treatment of rats with 200 and 300 mg/kg body weight of Methanol leaf extract of Jatropha Gossypiifolia significantly decrease AST, ALT, ALP (22.63±2.19b, 23.00±4.30b, 30.46± 6.23b) and (22.23±2.38b, 22.80±4.15b, 33.15± 6.50b), respectively, compared to non-treated positive control group ($p < 0.05$).

However, there was a significant increase in the serum levels of conjugated bilirubin and total bilirubin in non-treated rat positive control group (1.84±0.15b and 9.22±1.42b), respectively, compared to the normal control (1.55±0.17a, and 5.76±0.64a). Rats in the groups treated with 200 and 300 mg/kg body weight of extract showed significant decrease in the conjugated and total bilirubin (1.56±0.13a and 6.39±0.73a) and (1.47±0.16a, and 6.25±0.42a), respectively, compared to the non-treated positive control group ($p < 0.05$).

The serum level of urea and creatinine significantly increased in gentamicin induced rats' group (9.13± 0.37b and 92.37± 6.71c), respectively, compared to normal control (5.34 ± 0.51a and 69.41± 3.47a), ($p < 0.05$) (Table 2). Rat in groups D and E treated with 200 and 300 mg/kg body of extract showed significant decrease in the levels of urea and creatinine (6.11± 1.12a and 76.35± 6.12b) and (6.36±0.41a and 73.27±5.57a), respectively, compared to the positive control group ($p < 0.05$).

There was a significant decrease in the serum levels of albumin and total protein of the rats administered with gentamicin (24.19 ± 1.55a and 45.73± 2.65a), respectively, compared to the normal control (36.89 ± 1.00c, and 64.36± 1.36c).

Rats treated with 200 and 300 mg/kg body weight of extract showed significant increase (36.89 ± 1.00c and 57.42± 4.72b) and (24.19 ± 1.55a and 55.83± 1.83b), respectively, compared to rats in non-treated group ($p < 0.05$).

DISCUSSION

Many researchers have directed their efforts towards the provision of empirical proof to back up the use of many tropical plants for trado-medical practices [26,27, 28]. This study was undertaken to explore the hepatoprotective and nephroprotective effect of Jatropha Gossypiifolia leaf extract in the hepatic and nephrotic damage caused by the administration of gentamicin. The plant was observed to have a potential with strong protein oxidation inhibitory potency. This could be attributed to the antioxidant activity of extracts from J. gossypiifolia which was evaluated by Kharat et al. [29].

In this work the high content of phenols, tannins, and flavonoids in the leaves prompted the authors to evaluate the antioxidant activity of the leaves. DPPH free radical, ferric thiocyanate, and nitric oxide scavenging methods were used to analyze the antioxidant activity in vitro of methanol, ethyl acetate, and aqueous extracts, demonstrating positive results. The authors attributed the free radical scavenging activity to the presence of flavonoids [30]. On the other hand, a study showed that different extracts (petrol ether, chloroform, ethyl acetate, and n-butanol) from whole plant of J. gossypiifolia had only partial antioxidant activity in DPPH scavenging, total antioxidant capacity, and lipid peroxidation tests. Among them, the Methanol and ethyl acetate extract was the most active, which correlates positively with its higher content of phenolic compounds in comparison with the other extracts [30]. Thus, the decreased levels of serum albumin and total proteins in non-treated rat group, is an indication of hepatotoxicity [31].

Albumin is essential for tissue growth and aids in preventing the leakage of fluids from blood vessels. It plays an important role in transporting both endogenous and exogenous substances, serving as protein reserves, as well as maintaining osmotic pressure [32]. However, there was significant increase in the levels of albumin and total protein in extract treated rat groups.

The present study corroborates suggestions made by Davies and Goldberg, [33] that the herb Jatropha Gossypiifolia leaf extract may have strong protein oxidation inhibitory potency and thus, may be a good source of medicines against diseases in which lipids and protein oxidation are involved, such as toxic hepatitis since protein degradation seems to occur by distinct mechanism [34]. The significant increase in serum level of urea and creatinine in gentamicin induced rats' group is also an indication of nephrotoxicity.

Therapeutic doses of gentamicin and other aminoglycoside antibiotics can produce nephrotoxicity in humans and animals and use of this class of antibiotics is known as one of the most common causes of acute renal failure possibly due to increased renal uptake of the antibiotic mainly by the proximal tubules. Nephrotoxicity is the most common side effects associated with the use of gentamicin [35]. Rats in groups treated with extract showed significant decrease in the levels of urea and creatinine. Kanika, [36] and Nwali et al. [37], reported some phytochemical constituents of Jatropha Gossypiifolia leaf extract to include; vitamin E, selenium, vitamin C, taurine and the carotenoids (beta-carotene, lutein and lycopene) which have the potentials to decrease the gentamicin-induced reduction in the glomerular filtration rate and the severity of the tubular damage [38, 39].

The aqueous extract of Jatropha Gossypiifolia leaves was earlier reported to possess potent nephroprotective activity in gentamicin induced nephrotoxicity in rats. The results obtained from this study show that the methanolic leaf extract of Jatropha Gossypiifolia leaf extract has protective function against gentamicin-induced Albino rats.

CONCLUSION

As demonstrated by this study, J. gossypiifolia presents an important potential for the generation of pharmacological and/or biotechnological products, based on popular uses and biological studies scientifically showing its properties. However, regarding specifically its medicinal properties, further studies are still necessary to assay important folk uses of the species and characterize the major compounds responsible for the bioactivity. Thus, the results obtained from this study show that the ethanolic leaf extract of Jatropha Gossypiifolia leaves has protective function against gentamicin-induced damage in rats.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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