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NEPHROPROTECTIVE EFFECTS OF N-HEXANE EXTRACT OF *JATROPHA GOSSYPIFOLIA* LEAVES IN ISONIAZID INDUCED KIDNEY DAMAGE IN WISTAR RATS

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ABSTRACT: *This study aimed to investigate the effect of n-hexane extract of Jatropha gossypifolia on isoniazid-induced kidney damage in Wistar rats. Serum levels of urea, creatinine, sodium, potassium, chloride, and bicarbonate, alongside kidney tissue antioxidant activities (superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA)), were assessed using standard methods. The study utilized a completely randomized block design (CRBD) with 25 rats (200 g–300 g) divided into five groups. Groups 1 (normal control) and 2 (positive control) received distilled water, while groups 3, 4, and 5 were pretreated with 200 mg/kg Jatropha gossypifolia extract, 400 mg/kg extract, and 200 mg/kg vitamin E, respectively. After daily pretreatment for 21 days, 50 mg/kg of isoniazid was administered to groups 2–5 to induce kidney damage. All treatments were administered orally. On day 21, the rats were sacrificed under chloroform anesthesia, and blood and kidney tissue samples were collected for analysis. Results indicated that isoniazid significantly ($p<0.05$) increased serum urea, creatinine, sodium, potassium, chloride, and MDA levels, while SOD, CAT, GSH, and bicarbonate activities were significantly ($p<0.05$) reduced compared to the normal control. However, Jatropha gossypifolia treatment (200mg/kg and 400mg/kg) significantly enhanced antioxidant activities and bicarbonate levels while reducing serum urea, creatinine, sodium, potassium, chloride, and MDA levels. These findings suggest that Jatropha gossypifolia contains antioxidant compounds that may protect against isoniazid-induced kidney damage.*

KEYWORDS: Jatropha gossypifolia, Serum, Isoniazid, Antioxidant, Nephroprotection.

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INTRODUCTION

Plants have been integral to human health and well-being since ancient times, serving as a rich source of medicinal compounds and therapeutic remedies. Across cultures and civilizations, traditional herbal medicine has played a vital role in treating various ailments, offering a holistic approach to healthcare that harnesses the healing properties of botanicals. In Nigeria, a country endowed with diverse flora, traditional medicine remains deeply rooted in the cultural fabric, providing accessible and affordable healthcare solutions to millions of people, especially in rural areas where modern medical facilities may be limited (Okigbo & Mmeka, 2016; Erukainure *et al.*, 2015). The World Health Organization estimates that up to 80% of the world's population relies on traditional medicine for primary healthcare needs, with plant-based remedies constituting a significant portion of these treatments. Plants contain a myriad of bioactive compounds, including alkaloids, flavonoids, terpenoids, and polyphenols, which exhibit diverse pharmacological properties such as anti-inflammatory, antimicrobial, antioxidant, and analgesic effects. These natural compounds serve as the basis for the development of modern pharmaceutical drugs and continue to inspire scientific research into novel therapeutic agents (Fabricant & Farnsworth, 2001; Azimi & Khodaie, 2017). In Nigeria, traditional herbal medicine, known as "agbo" or "dawa," has been practiced for centuries by indigenous healers and traditional medicine practitioners. The country's rich botanical diversity, comprising over 8,000 plant species, offers a treasure trove of medicinal resources that have been utilized for generations to treat a wide range of ailments, from malaria and respiratory infections to gastrointestinal disorders and skin conditions. Despite advances in modern medicine, traditional herbal remedies remain deeply ingrained in Nigerian healthcare practices, reflecting the enduring trust and efficacy attributed to plant-based treatments by the populace (Anosike & Obidoa, 2018; Sofowora *et al.*, 2008).

MATERIALS AND METHODS

Study area

The administration of treatment to the animal subjects was carried out in the department of Biochemistry, Niger Delta University. Similarly, the biochemical analysis took place at the Chemical Pathology Laboratory of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State. Okolobiri is a rural village situated in the Yenagoa Local Government Area of Bayelsa State. With a population of over 20,000, it is essentially a riverine town located at 4.88540N and 6.07840E geographical coordinates. Okolobiri is a fast-developing suburb and hosts several multinational oil companies and has been plagued by oil pollution for decades. The common vocations of natives and residents of Okolobiri are mainly farming, fishing, petty trading, company workers and civil service (Ikimi & Addy, 2024).

Collection and identification of plant sample

Fresh leaves of *Jatropha gossypifolia* were gotten from Ekiti State, Nigeria. The plant was identified by Professor Kola Ajibesin of the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State.

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Preparation of plant extract

The plant leaves were collected in large quantities and left under shade at room temperature for 2 weeks to dry. Afterwards, they were ground into powder. Thereafter, 247g of the powder was soaked in 1.5 liters of n-hexane and allowed to stand for 48 hours. The extract was collected, filtered and concentrated under increased pressure using a rotary evaporator at 60°C and 40 rpm.

Animal specimen and study population

Twenty-five healthy male wistar albino rats weighing between 200g and 300g were used for this study. The animals were procured from the animal house of the University of Port Harcourt. The rats were kept in standard animal cages and acclimatized in the animal house, in the Department of Pharmacology, Niger Delta University, for 14 days and allowed free access to pellet chicken feed and water. Mead's resource equation was utilized for the calculation of the sample size (Kirkwood & Robert, 2010). The equation is stated and the components defined. E = N – B – T, where N is the total number of individuals or units in the study (minus 1). B is the blocking component, representing environmental effects allowed for in the design (minus 1). T is the treatment component, corresponding to the number of treatment groups (including the control group) being used or the number of questions being asked (minus 1). E is the degrees of freedom of the error component and should be somewhere between 10 and 20. The study consisted of five groups (T = 4), with 5 animals per group, making 25 animals in total (N = 24), without any further stratification (B = 0); then E would equal 20, indicating that the sample size is very suitable for the research.

Ethical clearance

Ethical clearance was obtained from the animal research ethics committee of the Department of Biochemistry, Niger Delta University, Bayelsa State. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were strictly adhered to (Benjamin & Jean, 2016).

Experimental design

Completely randomized block design (CRBD) was used for this study. The animals were randomly grouped into 5 groups of 5 rats each in a standard rat cage and were treated as follows:

Group 1 (Normal control) was fed only pellet chicken feed and distilled water.

Group 2 (Positive control) was administered isoniazid (50 mg/kg) daily for 21 days.

Group 3 (Test group 1) was administered 200 mg/kg of plant extract + 50 mg/kg isoniazid for 21 days.

Group 4 (Test group 2) was administered 400 mg/kg of plant extract + 50 mg/kg isoniazid for 21 days.

Group 5 (Standard control) was administered Vitamin E (200 mg/kg) + 50 mg/kg isoniazid for 21 days.

All groups were fed with pellet chicken feed and distilled water *ad libitum.*

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Selection criteria

Rats used were apparently healthy and active as confirmed and approved by a veterinary doctor. Rats showing signs and symptoms of illness were excluded from the research. Also excluded were rats with any form of derangement. Contaminated blood samples were rejected.

Collection and preparation of blood and tissue samples

Blood was collected via cardiac puncture into plain bottles and allowed to stand for 30 minutes for coagulation to take place so as to obtain only the serum. Afterwards, the blood samples were centrifuged for 10 minutes at 2000 rpm and the supernatant was collected for the analysis. A portion of the kidney was also collected to prepare the homogenate for the antioxidant assays (Sule *et al.*, 2017; Ness, 1999).

Analysis of samples

Serum urea, creatinine, sodium, potassium and chloride were determined by spectrophotometric methods using Randox kits, as reported by Ikimi *et al.,* 2023 and Ikimi *et al.,* 2024. Serum bicarbonate was determined using an ion selective electrode (ISE) analyzer method (analyzer ISE 4000), as stated by Bolarin and Azinge (2010). SOD activity was determined by the method of Xin *et al.* (1991) as contained in the Randox commercial kit leaflet; the method of Habig *et al.* (1974) was followed in estimating the level of reduced glutathione (GSH); catalase activity was determined according to the method of Aebi (1983); and lipid peroxidation analyses were carried out by determining the concentration of MDA formed using the method of Varshney and Kale (1990), as modified and reported in Agoro *et al.* (2017) and Agoro *et al. (2019).*

Statistical analysis

Results were analyzed statistically using One-way analysis of variance (ANOVA) under the Tukey-Kramer Multiple Comparison Test and Statistical Package for Social Sciences (SPSS) version 20. Values are expressed as mean ± standard deviation (SD) and were considered statistically significant at $p < 0.05$.

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RESULTS

Table 1: Effect of isoniazid and *Jatropha gossypifolia* on the mean body weight (g) of wistar rats

Experimental group	Mean weight before treatment (g)	Mean weight after treatment (g)	% Mean weight change
Normal control	159.2±4.44	197.6±8.02	24.12[a]
Positive control	161.4±4.28	197±11.35	22.06[a]
Test Group 1	159.8±3.19	199.25±13.15	24.69[a]
Test Group 2	160.8±4.70	211±15.94	31.22[b]
Standard control	161.4±3.36	202.4±11.35	25.40[a]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying the same superscripts are not significantly different ($p < 0.05$).

Results (table 1) show that pretreatment with *Jatropha Gossypifolia* (200 mg/kg body weight and 400 mg/kg body weight) caused a ($p < 0.05$) increase in the rate of weight gain (24.69% and 31.22%) when compared with normal (24.12%) and positive control (22.06%).

Table 2: The protective role of *Jatropha gossypifolia* on isoniazid-induced albino wistar rats

Experimental group	Urea (mg/dl)	Creatinine (mg/dl)	Chloride (mmol/l)	Sodium (mEq/L)	Bicarbonateate (mEq/L)	Potassium (mmol/l)
Normal control	61.49±3.48a	0.80±0.30a	23.03±3.94[a]	69.68±3.48[a]	40.3±1.7[a]	1.49+0.07[a]
Positive control	99.82+5.65[b]	2.93+0.37[b]	60.11+5.57[b]	109.12+6.13[b]	22.99+1.3[b]	3.01+0.30[b]
Test Group 1:	78.06+7.96[c]	1.67+0.39[c]	46.83+2.18[c]	86.51+8.39[c]	30.85+1.8[c]	2.38+0.26[b]
Test Group 2:	73.0.8+2.30[d]	0.93+0.37[d]	38.75+2.82nd	79.20+3.49[d]	36.84+2.9[d]	1.92+0.20[c]
Standard control	72.96+3.47[d]	0.93+0.37[d]	32.52+2.86[e]	78.05+3.15[d]	39.02+1.7[d]	1.92+0.14[c]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying the same superscripts are not significantly different ($p < 0.05$).

Table 2 shows that the administration of isoniazid caused a significant increase ($p<0.05$) in serum concentrations of urea (99.82±5.65), creatinine (2.93±0.37), chloride (60.11±5.57), sodium (109.12±7.13) and potassium (2.38±0.26) and a decrease ($p<0.05$) in bicarbonate (22.99±1.38) relative to normal control rats. Treatment with *Jatropha gossypifolia* at doses of mg/kg body weight and mg/kg body weight showed a significant decrease ($p < 0.05$) in serum concentration of urea (78.06 ± 7.96 and 73.08 ± 2.30), creatinine (1.67 ± 0.29 and 0.93 ± 0.3), chloride (46.83 ± 2.18 and 38.75 ± 2.82), sodium (86.51 ± 8.39 and 79.20±2.49) and potassium (3.01 ± 0.30 and 2.38 ± 0.26) and an increase ($p < 0.05$) in bicarbonate (30.85 ± 1.80 and 36.84 ± 2.92) concentrations, respectively, with respect to the positive control group.

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Table 3: The antioxidant effects of *Jatropha gossypifolia* on isoniazid-induced wistar albino rats

Experimental group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	MDA (U/mg protein)
Normal Control	9.16±0.46[a]	7.37±0.31[a]	6.74±0.36[a]	1.84±0.17[a]
Positive Control	2.95±0.15[b]	2.63±0.17[b]	2.12±0.07[b]	6.98±0.50[b]
Test Group 1	4.89±0.21[c]	4.26±0.22[c]	3.84±0.08[c]	4.02±0.13[c]
Test Group 2	6.50±0.52[d]	4.98±0.10[c]	4.66±0.4[d]	2.9±0.14[d]
Standard Control	6.93±0.29[d]	5.42±0.22[d]	4.89±0.46[d]	2.67±0.16[d]

Data are expressed as the mean ± SD (n = 5). The means within the same column carrying the same superscripts are not significantly ($p < 0.05$) different.

Table 3 shows that isoniazid administration caused a significant decrease ($p<0.05$) in kidney SOD (2.95±0.15), CAT (2.63±0.17) and GSH (2.12±0.07) activities and a significant increase ($p<0.05$) in kidney MDA concentration (6.98±0.50) related to normal control rats. However, treatment with *Jatropha gossypifolia* at doses of mg/kg body weight and mg/kg body weight caused a significant ($p<0.05$), dose-dependent elevation of kidney activities of SOD (4.89±0.21 and 6.50±0.52), CAT (4.26±0.22 and 4.98±0.10) and GSH (3.84±0.08 and 4.66±0.40), and a significant decrease in the kidney MDA (4.02±0.13 and 2.9±0.14) concentrations relative to the positive control group.

DISCUSSION

The exploration of medicinal plants as fundamental components of healthcare practices across diverse cultures has garnered substantial attention, driven by their perceived therapeutic benefits (Iwu and Uwakwe, 2019; Mizanur-Rahaman *et al.,* 2023). Among the myriad botanical remedies, *Jatropha gossypifolia* stands out for its potential medicinal properties, sparking interest in its efficacy in addressing various ailments, ranging from cancer to diabetes (Boakye-Yiadom, 2021; Ojewole, 2004). This study delved into the intricate interplay between isoniazid and *Jatropha gossypifolia*, a botanical species native to equatorial regions, renowned for its purported antioxidant and hepatoprotective properties (Manoharan *et al.,* 2016; Biswas *et al.,* 2018).

Elevated levels of malondialdehyde (MDA), indicative of heightened lipid peroxidation, coupled with decreased activities of superoxide dismutase (SOD), catalase, and glutathione (GSH) enzymes, point towards compromised antioxidant defense mechanisms in isoniazid-exposed rats (Sharifi-Rad *et al.*, 2020; Asbaghi *et al.*, 2020). Yet, treatment with *Jatropha gossypifolia* extract and vitamin E showcases significant reversals of these trends, highlighting their antioxidative prowess and reinforcing the plant's potential as a natural therapeutic agent. The observed biochemical effects are postulated to stem from the phytochemical composition of *Jatropha gossypifolia*, which boasts an array of antioxidant compounds such as flavonoids, alkaloids, tannins, and cardiac glycosides (Zengin *et al.,* 2021; Babalola, *et al.*, 2014). These bioactive constituents are believed to synergistically contribute to the plant's therapeutic effects, offering protection against isoniazid-induced renal injury in a

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dose-dependent manner **(**Alabi, *et al.,* 2017: Ajayi *et al.,* 2015)

The findings of the study unveil intriguing insights into the potential of *Jatropha gossypifolia* extract in mitigating weight gain, shedding light on its possible role in weight management. This discovery adds a new dimension to the understanding of the plant's therapeutic effects, expanding its utility beyond conventional medicinal applications (Abatan *et al.,* 2017; Mohammed *et al.,* 2018). The study dissects the complex dynamics of isoniazid-induced renal injury, aiming to unravel the mechanistic underpinnings of *Jatropha gossypifolia*'s purported protective effects. This study represents a supplementary endeavor in pharmacological explorations, expanding the horizons of therapeutic interventions in tuberculosis management (Ashok and Nargund, 2016; Jegede and Ogunyinka, 2017). By elucidating the potential of *Jatropha gossypifolia* as a mitigating agent against isoniazid-induced renal damage, this research holds promise for transcending conventional treatment paradigms, heralding a new era of personalized and holistic approaches to combat the multifaceted challenges posed by tuberculosis and its pharmacotherapeutic arsenal (Abolaji and Folarin 2015; Manoharan *et al.,* 2016).

The kidneys, integral organs in the body's excretory system, play a pivotal role in eliminating waste products and toxins (Hoilat *et al.,* 2023; Folarin and Babalola, 2016). The study's findings underscore the deleterious effects of isoniazid exposure on renal function, as evidenced by the significant elevation in serum concentrations of urea, creatinine, chloride, sodium, and potassium, coupled with decreased bicarbonate levels (Oloyede and Ajao, 2018; Oyedemi and Yakubu, 2017). However, pre-administration of n-hexane extract of *Jatropha gossypifolia* exhibited promising outcomes, attenuating the adverse biochemical alterations induced by isoniazid and hinting at its potential nephroprotective properties. Moreover, the investigation delves into the antioxidative mechanisms underlying the observed effects (Ogundipe and Moody, 2019; Ogunyinka *et al.,* 2018). In essence, this study not only unveils the potential of *Jatropha gossypifolia* as a therapeutic agent in combating kidney injury but also underscores the intricate interplay between phytochemical constituents and biological mechanisms underlying its pharmacological effects (Agoreyo and Okafor, 2019; Uchendu *et al.,* 2016; Olorunsogo *et al.,* 2014).

However, further research is warranted to unravel the precise molecular pathways involved, paving the way for the development of novel therapeutic interventions harnessing the power of medicinal plants. Expanding on this study, future investigations could explore additional parameters such as histopathological changes in renal tissues, gene expression profiling of antioxidant enzymes, and long-term effects of *Jatropha gossypifolia* administration (Mohammed**,** *et al.,* 2019; Folarin *et al.,* 2016). Moreover, conducting clinical trials to validate these findings in human subjects would be crucial for translating preclinical evidence into clinical practice. Additionally, elucidating the safety profile and potential adverse effects of prolonged *Jatropha gossypifolia* usage would be essential for ensuring its suitability as a therapeutic agent. By addressing these aspects, researchers can further enhance our understanding of the therapeutic potential of medicinal plants and pave the way for their integration into mainstream healthcare practices (Manoharan *et al.,* 2016; Akande & Folarin, 2016). This finding of this study underscores the significance of continued research in advancing the field of phytopharmacology and expanding the repertoire of therapeutic options available for managing renal injury induced by pharmaceutical agents like isoniazid. This research contributes to the growing body of evidence supporting the efficacy of natural

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remedies in complementing conventional pharmacotherapeutic approaches, fostering a more integrative and holistic approach to healthcare.

CONCLUSION

The findings of this study revealed that the n-hexane extract of *Jatropha gossypifolia* contains antioxidant compounds with biochemical properties in rats with isoniazid-induced kidney damage, and these biochemical effects were dose-dependent. Further investigations are crucial to unravel the specific mechanisms through which *Jatropha gossypifolia* provides kidney protection.

Conflict of interests

The authors declare no conflict of interest.

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