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HEPATO PROTECTIVE AND ANTIOXIDANT FUNCTION OF INDIAN ALMOND (TERMINALIA CATAPPA) LEAF EXTRACT IN AUGMENTIN-INDUCED WISTAR RATS EXPERIMENTAL MODEL

BY

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Abstract

Exploration of the potential hepato protective benefit of the Indian almond in rats exposed to liver damage was the focus of this investigation. Parameters such as AST, ALT, ALP, T.P and albumin levels, as well as antioxidant activities of Superoxide dismutase (SOD), CAT, GSH, and MDA were evaluated. Thirty Wistar rats that weighted 150 to 200grams were divided into five groups of six rats each. The study was designed such that; ***Group 1*** *(Normal control),* ***Group 2*** *(Positive control) were both given (10ml/kg) dose of normal saline per body weight.* ***Group 3*** *and* ***Group 4*** *had 200 mg/kg and 400 mg/kg doses of the extract per body weight, respectively, while* ***Group 5*** *(standard) was orally administered 200 mg/kg of vitamin E from day 1 to day 14. On days 15 to 21,* ***Group 2, 3, 4,*** *and* ***5*** *were treated with 30mg/kg dose of augmentin. The rats were then sacrificed painlessly (under chloroform anesthesia) on day 22nd, and blood sample by cardiac puncture collected to test liver function. Liver tissues were obtained and homogenized for antioxidant analysis. The results showed that augmentin-intoxicated group of rats had significant ($p < 0.05$) decrease in SOD, CAT, GSH, T.P, and albumin levels compared to the normal control group. There was significant increase in ALT, AST, ALP, and MDA levels when compared to normal control group. However, pretreatment with Indian almond leaf extract (at doses of 200 mg/kg and 400 mg/kg for Groups 3 and 4, respectively) led to significant increases in SOD, CAT, GSH, T.P, and albumin levels. Also, there was significant ($p<0.05$) decrease in MDA, ALT, AST, and ALP levels compared to the positive control group. Meanwhile, administration of 200mg/kg dose of Vitamin E caused significant increase in SOD, CAT, GSH, T.P, and albumin levels, along with decrease in MDA, ALT, AST, and ALP levels compared to the positive control group. Pretreatment with extract in group 3 and 4, also caused significant ($p<0.05$) decrease in mean weight gain, when compared with the normal and positive control groups. From this study, it can be inferred that Indian almond leaf extract has antioxidant activities and can give protection to Wistar rats' liver that were subjected to augmentin-induced hepatic damage.*

Keywords: *liver, antioxidant enzymes, Indian almond, ALT, AST, ALP.*

INTRODUCTION

The liver has been described as the largest internal organ in the body, distinct for its dual blood supply, with more than 500 important functions attributed to it (Kalra *et al*., 2023; Colombia University, 2022). Few of the numerous functions of the liver include, metabolism of fats in the small intestine through bile production, carbohydrates storage in form of glycogen, storage of vitamins A, D, E, K, (Kalra *et al*., 2023). Some hormones and essential proteins are produced by the liver which is also involved in the immune system of the body (Kalra *et al*., 2023).

The liver can be exposed to a plethora of diseases including Cirrhosis (Trefts *et al*., 2017).with a devastating toll on the body due to interference with numerous functions that are

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associated with the liver health (Trefts *et al*., 2017). It is therefore imperative to regularly check the functional status of such vital organ as the liver, in order to maintain healthy life (Trefts *et al*., 2017). This could be enhanced by carrying out liver function tests (LFTs) that operate on the principle of assaying for some parameters such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, total protein and albumin in serum or plasma (Lala *et al*., 2023); Meanwhile, ALT and AST elevated levels in blood though unspecific, have been recognized markers in liver tests (Ribeiro *et al*., 2019; Giannini *et al*., 2005).

The quest of mankind to maintain and improve self-preservation has seen centuries of identifying and applying traditional herbal remedies or sometimes processing them into orthodox medication for health. There has been apparent, increasing acceptance regarding application of herbal remedies in recent decades. This has drawn attention of researchers and scientists to explore beneficial therapeutic properties of many plants with positive outcomes. For instance, some secondary metabolites that show great promise in pharmaceutical application have been found in plants, with many of them contributing to the development of approved drugs by relevant regulatory agencies (Chavan *et al*., 2019); and innovative beneficial therapeutics, that is heralding developmental changes in the health and pharmaceutical sectors, (Dzobo *et al*., 2022).

In line with this, the Indian almond, scientifically known as Terminalia catappa is one of the myriads of plants that several investigators have been studying in recent times, including a team of researchers led by Sule; that showed its association with antioxidant activities and some protective health benefits for some essential internal organs/tissues in experimental rat models (***unpublished***). It was therefore an objective in this study, to investigate whether Indian almond can actually give protective health benefits for rats subjected to hepato-toxic exposure in experimental model.

METHODS

An obtained sample *of Indian almond* from Niger Delta University was identified by Professor Kola Ajibeshin of the Department of Pharmacognosy, in the University located in Bayelsa State, Nigeria. Then, under room temperature, fresh leaves of the plant were made to dry for 14 days, grounded into powdery form. 2 litres of ethanol was added to 500g of the powder to have it diluted. The mixture was stirred at intervals within 48 hours it was allowed to stand. Afterwards, Whattman filter paper of 110 mm size was used to obtain the filtrate, which was evaporated at 40 ^{0}C by a rotary evaporator. The powdery residue was now re-diluted with distilled water.

Five Groups, **1 to 5** of wistar rats each containing 6 were used for the study. All the rats weighted between 150g – 200grams. They were housed under laboratory condition in standard plastic rat cages for 14 days, and given free access to grower's mash, water and fresh air with a regulated 12 hour light and 12 hour darkness cycle. In the initial 14 days, **Group 1** and **2** were given 10ml/kg of normal saline every day per body weight, representing normal and positive controls respectively. 200mg/kg of the extract was administered per body weight to **Group 3** (representing test group 1) while **Group 4** (representing test group 2) was given 400mg/kg body weight of the extract. 200 mg/kg Vitamin E was administered to **Group 5** (representing standard)**.** On the day 15 of the study, **Group 1, 2, 3** and **4** rats were all orally given 30mg/kg per body weight of augmentin, for seven days after which, all rats were sacrificed by day 22.Blood sample was obtained by cardiac puncture, placed in bottles, and allowed to settle for 30 minutes. After coagulation, centrifugation was done at 2000 RPM for 10 minutes. Then, the supernatant was obtained for biochemical analysis, while portion of liver tissue was collected to prepare homogenate for antioxidant studies. Parameters assessed were AST, ALT, ALP, T.P and albumin levels, for liver protection potential, as well as Superoxide dismutase (SOD), CAT, GSH, and MDA for antioxidant activities. Standard protocol outlined in biochemical kits (Randox product) was followed for all analyses.

Serum AST, ALT, ALP and serum albumin were assayed using kit manual instructions as in the reports of (Gowda *et al*., 2009; Vagvala & O'Connor, 2018).

Antioxidant activities were assessed; total protein level, superoxide dismutase (SOD) activity, reduced glutathione (GSH) level, Catalase activity were also determined as reported in (Beutler et al., 1963; Sinha A., 1971), and lipid peroxidation by measuring the thiobarbituric acid reactive substances.

Statistical analysis

Results were analyzed statistically using one-way analysis of variance (ANOVA)under Turkey Kramer Multiple Comparison Test. All values are expressed as mean ± Standard deviation and deemed significant at $p< 0.05$.

RESULTS

The results of the present study are summarized in tables 1, 2 and 3. Table 1 shows the mean body weight of augmentin induced wistar rats before and after pretreatment with *TerminaliaCatappa*. Table 2 shows mean serum concentrations of ALT, AST, ALP, Albumin and total protein while table 3 shows the mean liver concentrations of SOD, Catalase, GSH and MDA in wistar rats pretreated with *TerminaliaCatappa* for 14 days and induced with augmentin for 7 days.

Table 1 Effect of Augmentin and Indian almond on the Mean Body Weight (g) of Albino Wistar Rats

EXPERIMENTAL GROUP	Mean Weight before treatment(g)	Mean Weight after treatment (g)	% mean weight increase
Normal Control with normal saline	144.6±12.5	192.8±31.4	33.3[a]
Positive Control with 30mg/kg	151.2±38.3	201.5±29.1	33.2[a]

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augmentin			
Test Group 1with 200mg/kg extract and 30 mg/kg augmentin	141.5±26.2	182.3±18.2	28.9[b]
Test Group 2 with 400mg/kg extract and 30mg/kg augmentin	162.5±12.1	189.9±12.7	27.4[b]
Standard Control with 200mg/kg Vitamin E and 30mg/kg augmentin	149.8±12.7	224.1±32.2	49.6[c]

Data are expressed as the mean ± SD (n = 6). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Results in table 1 shows that pretreatment with Indian almond (200 mg/kg and 400 mg/kg body weight) caused a significant ($p < 0.05$) decrease in the rate of weight gain (28.9% and 27.4%) when compared with normal (33.3%) and positive (33.2) control.

Table 2: The protective role of Indian almond on augmentin induced albino wistar rats

EXPERIMENTAL GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (mg/dl)	Tot. Protein (g/dl)
Normal Controlwith normal saline	38.9±1.0[a]	84.2±12.5[a]	86.9±4.2[a]	3.5±1.3[a]	7.7±0.4[a]
Positive Control with 30mg/kg augmentin	122.1±6.1[b]	172.4±9.2[b]	143.1±9.1[b]	1.5±0.3[b]	4.3±0.6[b]
Test Group 1with 200mg/kg extract and 30 mg/kg augmentin	69.3±7.9[c]	126.7±15.0[c]	88.8±4.1[a]	2.3±0.2[c]	5.6±0.5[c]
Test Group 2 with 400mg/kg extract and 30mg/kg augmentin	66.5±5.5[c]	109.3±4.0[d]	84.2±9.2[a]	2.8±0.1[d]	6.1±0.4[d]
Standard Control with 200mg/kg Vitamin E and 30mg/kg augmentin	69.3±7.0[c]	120.4±7.0[e]	78.1±13.5[e]	2.8±0.2[d]	6.1±1.0[d]

Data are expressed as the mean ± SD (n = 6). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Table 2 shows that augmentin administration caused a significant ($p<0.05$) increase in serum ALT (122.1±6.1), AST (172.4±9.2) and ALP (143.1±9.1) activities of positive control and a significant ($p<0.05$) decrease in serum albumin (1.8±0.3) and total protein (4.3±0.6) concentration compared to normal control rats. However, treatment with Indian almond doses of 200 mg/kg b.wt and 400 mg/kg b.wt significantly ($p<0.05$) decreased serum ALT (69.3±7.9 and 66.5±5.5), AST (126.7±15.0 and 109.3±4.0), and ALP (88.8±4.1 and 84.2±9.2), and a significant increase in albumin (2.5±0.1 and 2.8±0.1) and total protein (5.6±0.5 and 6.1±0.4) concentration relative to the augmentin treated group in a dose dependent fashion.

Table 3 The antioxidant effect of Indian almond on augmentin induced wistar albino rats

EXPERIMENTAL GROUP	SOD (U/mg protein)	Catalase (U/mg protein)	GSH (U/mg protein)	MDA (U/mg protein)
Normal Control with normal saline	7.01±0.6[a]	5.23±0.3[a]	6.06±0.1[a]	6.30±0.6[a]
Positive Control with 30mg/kg augmentin	2.63±0.4[b]	2.87±0.3[b]	2.99±0.2[b]	13.65±0.5[b]
Test Group 1 with 200mg/kg extract and 30 mg/kg	5.80±0.5[c]	3.73±0.4[c]	4.15±0.3[c]	9.35±0.2[c]

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augmentin				
Test Group 2 with 400mg/kg extract and 30mg/kg augmentin	8.17±0.4^{d}	4.36±0.2^{c}	4.51±0.3^{c}	8.90±0.1^{c}
Standard Control with 200mg/kg Vitamin E and 30mg/kg augmentin	6.43±0.7^{e}	4.76±0.3^{c}	4.72±0.4^{c}	9.78±0.3^{c}

Data are expressed as the mean ± SD (n = 6). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Table 3 shows that augmentin administration caused a significant ($p<0.05$) decrease in liver SOD (2.63±0.5), Catalase (2.87 ± 0.3) and GSH (2.99 ± 0.2) activities of positive control and a significant ($p<0.05$) increase in liver MDA concentration (13.65±0.5) relative to normal control rats. However, treatment with Indian almond doses of 200 mg/kg b.wt and 400 mg/kg b.wt significantly ($p<0.05$), elevated SOD (5.80±0.5 and 8.17±0.4), Catalase (3.73±0.4 and 4.36±0.2) and GSH (4.15±0.3 and 4.51±0.3), and a decrease in the MDA (9.35±0.2 and 8.90±0.1) concentration, in a dose dependent fashion, relative to the augmentin treated group.

DISCUSSION

The liver in humans has been described as the largest solid organ in the body, distinct for its dual blood supply, and credited with well over five hundred functions in the body, (Kalra et al., 2023; Colombia University, 2022). Its delicate nature and inadvertent exposure to plethora of diseases, by virtue of the functionality, has put it in the spot light among organs requiring cautious handling and care.

This current study was undertaken to investigate protective benefits that medicinal plants or herbs may provide for the liver. Therapeutic plants have been utilized for a long time in traditional medicine practices. They synthesize a wide array of chemical compounds that have protective effects against oxidative damage. (Gershenzon and Ullah, 2022).

The current study aimed to investigate the impact of an ethanolic leaf extract of *Terminalia catappa* on liver injury induced by a high dose of augmentin in male Wistar rats. Results obtained showed that pretreatment with *Terminalia catappa* (200 mg/kg body weight and 400 mg/kg body weight) caused a significant ($p < 0.05$) decrease in the rate of weight gain when compared with normal and positive control. This suggests that the extract may possess weight control properties (Table 1).

There was significant ($p<0.05$) increase in serum ALT, AST, and ALP activities, and notable decrease in serum albumin as well as total protein levels in the positive control group compared to the normal control group, (see table 2). This observation implies a disruption in the architecture of hepatocytes, leading to leakage of AST, ALT, and ALP into the bloodstream and subsequent elevation (McGill, 2016). However, pretreatment with *Terminalia catappa* at doses of 200mg/kg and 400mg/kg per body weight significantly decreased serum AST, ALT, and ALP activities and increased serum albumin and total protein levels significantly. Treatment with vitamin E (at a dose of 200mg/kg body weight) also led to a significant decrease in serum ALT, AST, and ALP compared to the positive control group.

Furthermore, results from this investigation showed significant increase in serum MDA levels of the positive control (**Group 2**) compared to the normal control (**Group 1**), as seen in table 3. This is suggestive of increase lipid peroxidation, then tissue damage and failure of antioxidant defense mechanisms to prevent the excessive formation of free radicals. Moreover, a significant ($p<0.05$) decrease in SOD, CAT, and GSH enzyme activities in the positive control group compared to the normal control group, was observed, which is indicative of potential toxic effects of reactive oxygen species produced during augmentin intoxication. But, Test **Group 2 and 3**, treated with 200 mg/kg and 400 mg/kg body weight of *Terminalia catappa* leaf extract, respectively, exhibited significant dose-dependent reversal of MDA, SOD, and catalase activities compared to the positive control group (Table 3). From these, it can be inferred that the ethanol leaf extract of *Terminalia catappa* possess antioxidant properties. Meanwhile, in **Group 5**, animals pretreated with Vitamin E at a dose of 200mg/kg body weight showed increased SOD, catalase, and GSH levels compared to the positive control group, along with a significant decrease in MDA activity. Umoren *et al*., (2023), had reported that *Terminalia catappa* attenuated Phenylhydrazine-induced hepato-renal toxicity in male Wistar rats and up-regulated *invivo* antioxidants; and findings from this study are corroborative of that.

The observed capacity of the ethanol leaf extract of *Terminalia catappa* to protect the liver from augmentin-induced damage could be attributed to its rich phytochemical composition. The plant leaves contain a variety of beneficial compounds, including flavonoids, alkaloids, tannins, cardiac glycosides, anthraquinones, triterpenes, saponins, phenolic compounds, carbohydrates, and reducing sugars (Olutokun*et al*., 2018). These compounds may have contributed to the hepatoprotective and antioxidant effects observed. But more studies to fully comprehend the underlying mechanisms and

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to explore the potential applications of *Terminalia catappa* in promoting liver health is recommended. The protection of the liver by the extract was dose dependent with the higher dose (400 mg/kg body weight) showing more activity.

Conclusion

The results of this study gives premise to infer that the ethanol leaf extract of Indian almond contain antioxidant compounds that have hepatoprotective effects; which appear to be dose dependent. While further investigation remains a recommendation, to elucidate the specific mechanisms through which these actions may have occurred.

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