

DOI: <http://dx.doi.org/10.21123/bsj.2022.6107>

Effect of *Alstonia Boonei* Stem Bark Extracts on the Activity of Liver Maker Enzymes in Rats Induced by Ccl4

Victor E. Okpashi^{1*} 

Robert I. Uroko² 

Joy Ogana³ 

A. Agbafor² 

C. P. Nwuke² 

¹Department of Biochemistry, Cross River University of Technology, Calabar.

²Department of Biochemistry, Micheal Okpara University of Agriculture Umudike, Nigeria.

³Department of Applied Biochemistry, Nnamdi Azikwe University, Awka, Anambra State.

*Corresponding author: vic2reshu@gmail.com

E-mails address: uroko.robert@unn.edu.ng , oganajoy@unn.edu.ng , amarachi.agbafor@gmail.com , chinedu.nwuke@gmail.com

Received 28/2/2021, Accepted 27/7/2021, Published Online First 20/3/2022, Published 1/10/2022



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Abstract:

This study investigated the outcome of *Alstonia boonei* stem bark on liver enzymes after inducing the Wistar albino rats with carbon tetrachloride (CCl₄). This effect of plant extract was compared with silymarin – a drug commonly used for the treatment of chronic hepatocyte disorder. The plant sample was extracted with ethanol; acute toxicity study of the extract was performed on eighteen Wistar mice, while 30 rats were sacrificed for liver enzymes assay. The rats were divided into six clusters: each cluster has five rats, cluster 1 served as control and was given 2 mL/kg b.w - distilled water; clusters 2 – 6 were CCl₄ induced. Cluster 2 was untreated but served as the negative control while cluster 3 was given 0.025 mL or 25kg/kg B.W of Silymarin, which was a regular medicine and aided as the ordinary control. Rats in clusters 4 – 6 were administered - 100, 200 and 500 mg/kg, respectively of ethanol extract for fourteen days. The acute toxicity results of *A. boonei* extract showed relative fortification due to no death or adversarial responses after 24 hours of the extract administration. A substantial ($P \geq 0.05$) surge in ALT action after administering 500 mg/kg proves lesser toxicity was greater dosage. At low dosage of the extract, a non-significant ($p \geq 0.05$) rise in AST action specifies that the extract was moderately harmless with no hepatotoxic magnitude at low medication. The substantial reduction of alkaline phosphatase action in cluster 3 rats induced with CCl₄ and given with Silymarin, together with clusters 4 – 6 that were CCl₄ induced and administered with graded doses of *A. boonei* stem bark extract suggest hepatoprotective properties.

Keywords: *Alstonia boonei*, CCl₄, Liver-enzymes, Silymarin, Wistar albino rats

Introduction:

The practice of using specific medicinal plants for the management of definite ailments was beginning to gain recognition. The decrease in efficiency and side effect of artificial medicines and increasing contraindications of their usage/dosages automatically advanced the utilization of beneficial natural plants as drugs. Researches by scientists propose that plant-based drugs and extracts are healthier and safer in managing growing health complications. Therefore, no plant is exclusively unusable to man¹.

Alstonia boonei is known as “ahun” in the Yoruba language of Nigeria, “ogbu-ora” in the Igbo language of Nigeria and “ukhu” by the Urhobo people of Southern Nigeria². The herbals have several medicinal mixtures including, N α -formylechitamidine, boonein, loganin, lupeol, frolic acid, and b-amyryn including triterpenoids³. Over 12 species of *Alstonia* are dispersed across the world, where two are indigenous to Africa and are known to the traditional healers in West Africa⁴, Fig. 1.



Figure 1. *A. boonei* plant⁵.

The bark of *A. boonei* plant is an important traditional medicine in West and Central Africa⁶. Therapeutically, the steam bark holds anti-rheumatic, anti-inflammatory, and antibiotic properties⁷. It is used in ejecting reserved substance of formation and afterbirth given to lactating women⁸, and applied as a therapy for dysentery, typhoid, gonorrhea, and asthma⁶.

The liver is the prime interior organ - 1.5kg in adult human and varies 110 – 140g in rats. The liver is also the most energetic and complex organ. Accordingly, the replenishing powers of the liver confer its ability to metabolize, secrete, excrete, store, and decontaminate the body⁹. Notwithstanding its countless interesting tasks, the liver is prone to a lot of damage, caused by various types of liver disorders¹⁰. This marks the malfunctioning of the liver by hindering its capacity to function well. There are numerous liver disorders such as hepatic cirrhosis, hepatocellular carcinoma, hemochromatosis, autoimmune hepatitis, fulminant hepatitis, and hepatic encephalopathy, which are caused by excessive intake of alcohol, drugs (acetaminophen), and chemicals such as thioacetamide and carbon tetrachloride¹¹. This study scrutinized the influence of *Alstonia boonei* stem bark extracted with ethanol on the liver enzymes after induction of carbon tetrachloride (CCl₄) to Wistar albino rats.

Materials and Methods:

Herbal material

The stem bark - *Alstonia boonei* was utilized.

Experimental animals

Eighteen 18 mice and thirty albino rats were utilized.

Chemicals

All chemicals were of analytical grades and are products of British Drug House (BDH).

Gathering of herbal material

The stem bark - *A. boonei* was obtained from botanical garden at Michael Okpara University of

Agriculture, Umudike, Abia State (MOUAUAS), Nigeria, and was authenticated by Dr. K. N. Ibe.

Extract Preparation

The fresh bark - *A. boonei* was hand-picked from their bole and dried at room temperature. Thereafter, the dried sample was ground into a fine powder, weighed and stored in a sterile container for extraction. The weight of the extract was determined as follows:

$$\text{Volume of extraction (mL) to be administered} \\ = (\text{weight of animal (kg)} \times \text{dose (mg/kg)})$$

Concentration of extract (mg/mL)

Mining of plant material

A 500g of pulverized sample was soaked in 1.5 L of absolute ethanol for 72 hours in a sterile container. The mined plant sample was sieved with a mesh fabric and clarified with What-man No. 1 paper. The deposits were placed in a water bath at 60°C and allowed to evaporate completely. The extract was weighed to calculate the percentage yield.

Preparation of CCl₄

The carbon tetrachloride (CCl₄) was mixed with olive oil (2:1 v/v). A 2 mg/kg body weight of carbon tetrachloride was injected intraperitoneally to induced liver failure in rats after 24 hrs fasting before treatment commencement.

Preparation of standard drug

Disinfection of the top of dose vial with 70% alcohol and gauze was carried out. A needle gauge of 24g and 10 mg/kg body weight, for example, a rat that weigh 250 grams, was given 0.025 mL of the standard drug - silymarin. This was drawn into the syringe and injected to the rats.

Experimental animal for the study

Eighteen mice and 30 albino rats were purchased from the University of Nigeria, Nsukka. The rats were housed at the (MOUAUAS) for seven days involving twelve hours.

Experimental design

The research involved the use 30 male rats and eighteen mice. The eighteen male albino mice remained separated into 2 sets of 9 mice. The Two sets were separated into 3 where each set has 3 mice, for the phase I and phase II to test for acute toxicity, as shown in Tab. 1.

Table 1. Experimental design of clusters and administration of extract into the rats

Clustering of rats	Administration of extract to the rats
Cluster 1 (regular control)	Received 2 mg/kg of distilled water for 14 days for body weight.
cluster 2 (Negative control)	Received 2 mg/kg B.W of CCl ₄ , with regular animal feed and water in 14 days.
cluster 3 (Standard control)	Received 2 mg/kg B.W - CCl ₄ and was given with 25 mg/kg B.W of silymarin via oral administration for 14 days.
cluster 4	Took in 2 mg/kg B.W - CCl ₄ and was given with 100 mg/kg B.W of <i>A. boonei</i> stem bark extract via oral rout for 14 days.
cluster 5	Received 2 mg/kg body weight - CCl ₄ and was given with 200 mg/kg B.W - <i>A. boonei</i> stem bark extract orally in 14 days.
cluster 6	Took in 2 mg/kg body weight - CCl ₄ and was given with 500 mg/kg B.W. - <i>A. boonei</i> stem bark extract orally in 14 days.

Induction of Carbon tetrachloride

The rats were induced with hepatotoxicity via intraperitoneal injection of 2mg/kg body weight in overnight-fasted animals and allowed for 72 hours without treatment before inducing CCl₄.

Acute toxicity studies (LD₅₀)

The acute toxicity test was determined by the method of ¹². The test was carried out in two phases, with phase I and II were further clustered into three clusters each. Phase I received the extract doses of 10, 100, 200 and 500 mg/kg body weight, respectively while phase II received extract dosages - 1000, 2900, and 5000 mg/kg B.W., respectively. Both phases were observed for a period of 24 hours during which they were checked for manifest signs of toxicity, drowsiness, nervousness, in-coordination or death. The LD50 was calculated with the geometric mean to the square root of the maximum non-lethal dose and the lowest lethal dose.

Action of Alanine aminotransferase (ALT)

Alanine aminotransferase was assayed using¹³. Alanine aminotransferase was quantified by observing pyruvate hydrazone concentration formed with 2, 4-dinitrophenylhydrazine. The intensity of the color was measured against the blank at 540 nm. A volume, 0.1 mL of serum was pipetted into the tubes. To this, a 0.5 mL of phosphate buffer, L-alanine, and α -oxoglutarate was added. The mixtures were thoroughly mixed and incubated for

30 minutes at 37°C, and pH 7.4. A 0.5 mL of 2,4-dinitrophenylhydrazine was later added into both test tubes while 0.1 mL of the sample was added into the blank test tube. The tubes were incubated for 20 minutes at 25°C. A 5 mL of NaOH solution was added into each test tube, the mixture was read at 540 nm against the blank after 5 minutes.

Aspartate aminotransferase (AST) activity

Aspartate aminotransferase activity was assayed according to the method prescribed by¹⁴. Aspartate aminotransferase is quantify by checking the oxaloacetate hydrazone concentration which formed with 2,4-dinitrophenylhydrazine. The color intensity was quantified together with the blank at 546 nm. A 0.1 mL of serum was pipetted into the test tubes, and a 0.5 mL of reagent 1 was pipetted into the sample and blank tube. The solutions were mixed and incubated for 30 minutes at 37°C and pH 7.4. A 0.5 mL of reagent-2 - 2,4-dinitrophenylhydrazine was added, trailed by a 0.1 mL of sample into the blank test tube. The test tubes were mixed and incubated for 20 minutes at 25°C, while a 5.0 mL of NaOH solution was thereafter added to each test tube and mixed.

Alkaline phosphatase (ALP) activity

The method of ¹⁴ was used to assay for alkaline phosphatase activity. The principle lies on the response between alkaline phosphate and phenolphthalein monophosphate (colorless substrate), which gave phosphoric acid and a pink phenolphthalein. A 0.05 mL - distilled water was pipetted into the blank test tube. A 3.0 mL of the substrate was pipetted into each tube and read for the first absorbance - 405 nm. Alkaline phosphatase activity was calculated using equation below.

$$\text{The activity of ALP } \left(\frac{U}{L}\right) = \frac{\text{Absorbance of sample}}{\text{absorbance of standard}} \times 3300$$

Statistical analysis

Data are presented in mean \pm standard deviation while the mean was compared for Duncan's multiple comparison post hoc tests (LSD). A significant difference was taken using a One Way Analysis of Variance (ANOVA), while the suitable degree of consequence was $P \leq 0.05$.

Results

The percentage yield of ethanol extraction of *A. boonei* stem bark

After extraction and concentration of 500g of finely ground plant material sample, 23% yield was obtained.

LD₅₀ of *A. boonei* stem bark

The result of LD₅₀ studyof *Alstonia boonei* stem bark extract is presented in Tab. 2. There were no death and adversarial responses in the mice within 24 hours.

Table 2. Stage I and II of the LD₅₀ study of the *Alstonia boonei* stem bark extract

	Prescription (mg/kg B.W)	Mortality
Stage I		
Cluster 1	10	0/3
Cluster 2	100	0/3
Cluster 3	500	0/3
Stage II		
Cluster 4	1000	0/3
Cluster 5	2900	0/3
Cluster 6	5000	0/3

Effects of *Alstonia boonei* stem bark on alkaline aminotransferase

In cluster 2, rats that were CCl₄ induced and were untreated, together with rats from clusters 4 and 5 which were CCl₄ induced and given 100 and 200 mg/kg b.w of ethanol extract of *A. boonei* stem bark respectively, showed significantly ($P < 0.05$) rise in ALT activity. Meanwhile, the rats in clusters 3 and 6 that were CCl₄ induced but given standard drug (Silymarin) and 500 mg/kg b.w of *A. boonei* stem bark, had no substantial ($P > 0.05$) upsurge in ALT action likened to normal control (cluster 1) that was not CCl₄ induced. In Fig. 2, it was also observed that cluster 3 that was CCl₄ induced but given Silymarin, and cluster 5 and 6 which were CCl₄ induced but given 200 and 500 mg/kg b.w. *A. boonei* stem bark showed meaningful ($P < 0.05$) decrease in ALT compared with cluster 2, although cluster 4 CCl₄ induced but given 100 mg/kg b.w of *A. boonei* stem bark extract had no major ($P > 0.05$) decrease in ALT action likened to that of cluster 2 that were CCl₄ induced but untreated. Furthermore, when comparing the ALT action of cluster 3 that was CCl₄ induced but was given Silymarin, it was seen that cluster 4 CCl₄ induced but given 100 mg/kg b.w of *A. boonei* stem bark exhibited major ($P < 0.05$) rise, as cluster 5 that was CCl₄ induced but given 200 mg/kg b.w of *A. boonei* stem bark had no substantial ($P > 0.05$) increase, while cluster 6 CCl₄ induced but given 500 mg/kg b.w of *A. boonei* stem bark gave no significant ($P > 0.05$) decrease in their respective ALT actions.

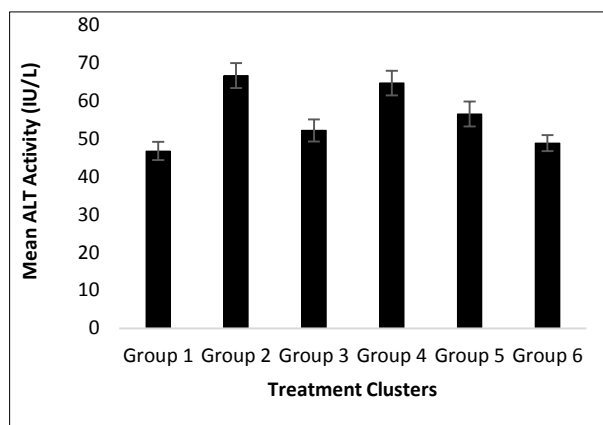


Figure 2. Alanine aminotransferase (ALT) activity of carbon tetrachloride (CCl₄) induced male Wister albino rats administered with *Alstonia boonei* extract.

Effects of *Alstonia boonei* stem bark extract on Aspartate aminotransferase

The data in Fig. 3 represent the aspartate aminotransferase (AST) activity of carbon tetrachloride (CCl₄) induced male Wistar albino rats. Results showed that cluster 2 that was CCl₄ made but was not given the extract, cluster 3 that was CCl₄ made but was given Silymarin, together with cluster 5 and 6 that were CCl₄ made but were given 200 and 500 mg/kg b.w of *A. boonei* stem bark, had a substantial ($P < 0.05$) increase in AST action, whereas cluster 4 that was CCl₄ made but was given low dose of *A. boonei* stem bark revealed no major ($P > 0.05$) rise in AST action relative to cluster 1 not CCl₄ induced. However, it was detected that the AST activities of rats in cluster 3 that were CCl₄ induced but given standard drug - Silymarin and cluster 4 - 6 that were CCl₄ made but were given 100, 200 and 500 mg/kg b.w *A. boonei* stem bark extract, separately revealed substantial ($P < 0.05$) decrease relative to cluster 2 that was CCl₄ induced and were not given. The AST activities of the rats in cluster 3 that was CCl₄ induced but given - Silymarin was compared with that of cluster 4 that was CCl₄ induced but given 100 mg/kg b.w of *A. boonei* stem bark, there was a substantial ($P < 0.05$) decrease, as the AST action of cluster 5 that was CCl₄ induced but was given 200 mg/kg b.w of *A. boonei* stem bark exhibited no substantial ($P > 0.05$) increase, despite the fact cluster 6 that was CCl₄ induced and given 500 mg/kg body weight of *A. boonei* stem bark revealed no significant ($P > 0.05$) decrease in AST action.

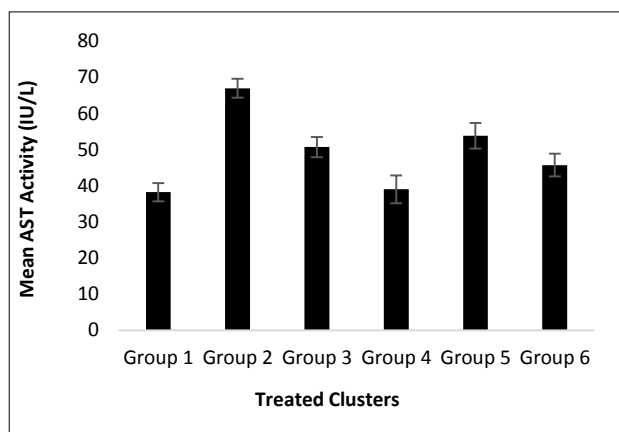


Figure 3. Aspartate aminotransferase (AST) activity of carbon tetrachloride (CCl₄) induced male Wister albino rats administered with *Alstonia boonei* extract.

Effects of *Alstonia boonei* stem bark extract on alkaline phosphatase (ALP) activity

Fig. 4 illustrates the alkaline phosphatase (ALP) activity of male rats induced with CCl₄. The ALP action of cluster 1 induced with CCl₄ was compared with other clusters, it was witnessed that only cluster 2 rats CCl₄ induced but untreated showed substantial ($p < 0.05$) increase in ALP action, while cluster 3 induced with CCl₄ but given Silymarin and clusters 4 – 6 CCl₄ induced but given classified dosages of *A. boonei* stem bark extract showed substantial reduction in their respective ALP activities. Nevertheless, it was perceived that the ALP activities of rats in cluster 3 that was CCl₄ induced but given Silymarin, and cluster 4 – 6 that was CCl₄ induced and given 100, 200 and 500 mg/kg body weight of *A. boonei* stem bark extract, showed substantial ($P < 0.05$) decrease corresponding with cluster 2 that was CCl₄ induced but untreated. Rats in cluster 4 that were CCl₄ induced but given 100 mg/kg b.w of *A. boonei* stem bark had no significant ($P > 0.05$) reduction in ALP activity, as cluster 5 that was CCl₄ induced but given 200 and 500 mg/kg b.w of *A. boonei* stem bark showed substantial reduction in ALP actions compared to ALP activity of cluster 3 that was CCl₄ induced but was given Silymarin only.

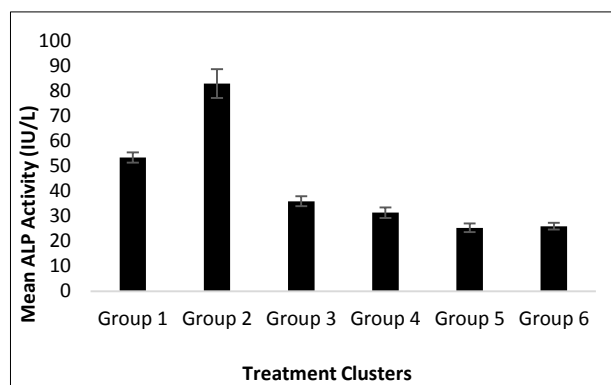


Figure 4. Alkaline phosphatase (ALP) activity of carbon tetrachloride (CCl₄) induced male Wister albino rats administered with *Alstonia boonei* stem bark extract.

Discussion

The study evaluated the outcome of *Alstonia boonei* stem bark extracted with ethanol on the liver enzymes and hematological properties after inducing carbon tetrachloride (CCl₄) in male Wistar albino rats. The liver is the main organ for metabolism and detoxification of drugs as well as environmental chemicals¹⁵. The liver cells are unprotected against significant concentrations of chemicals, thus, the function of the liver may be affected adversely¹⁶. The impairment to the liver could lead to elevated levels of liver enzymes and their activities in the extrahepatic tissues that may ultimately lead to the development, progression, and complication of various hepatic disorders. There are several conditions of liver disorders that can be induced by different means such as alcohol liver disease, fatty liver disease, hepatitis and several others¹⁷.

The no death or adversarial behavior saw in the LD₅₀ study of *A. boonei* stem bark extract suggests safeties at a high dose of 500 mg/kg body weight. The effect was ascribed to the moderately non-toxic or that the extract possesses low toxicity potential. However, most substances may be chronically toxic in that they may not be toxic but manifest signs of toxicity on continued consumption. The absence of adverse behavior may also be attributed to the extract containing a very low concentration of possible toxicants, with lack of sufficient concentration and interval for the expression of toxicity.

Alanine aminotransferase (ALT) is a liver-specific enzyme used in identifying liver damage as it is rarely seen except in liver disorder¹⁸. The substantial rise in ALT activity in all the rats induced with CCl₄ but untreated; together with those induced but given with a small amount of the ethanol extract indicate toxicity and potential liver damage. This might be as a result of the outflow of

the enzyme into the extrahepatic surroundings caused by a compromised plasma membrane resulting in the continuous administration of the extract. The upsurge in ALT action detected, corroborate with the findings of ¹⁹, who reported that increased activities of ALT are associated with hepatitis and other liver disorders. The non-significant ($P > 0.05$) upsurge in ALT action saw in cluster 3 CCl₄ induced but given standard drug (Silymarin) as well as cluster 6 that were CCl₄ induced but were given 500 mg/kg b.w of *A. boonei* stem bark was credited to the hepatoprotective effects of *A. boonei* stem bark, this implied that the extract may not be very toxic or have lesser toxic impact at a high prescription of 500 mg/kg b.w, and may not have affected the ALT production. Thus, administration of Silymarin and a high dose of the extract respectively caused the liver to gradually recover from the damage caused by CCl₄ induction, hence preventing leakage of ALT to extrahepatic tissues as indicated by a decrease in its activity.

Aspartate aminotransferase catalyzes the reductive transfer of an amino group from aspartate to 2-oxo-glutarate to produce oxaloacetate and glutamate. It helps in detecting the hepatocellular disorder, at the same time; it is well-thought-out a less specific biomarker-enzyme for hepatocellular damage ²⁰. The non-substantial rise in AST action of clusters that received a low dose of *A. boonei* extract shows moderate safety with no hepatotoxic outcome at low dose. However, the substantial rise in AST action in untreated clusters that were given standard drug and those administered high categorized dosages, suggest that the extract may possess hepatotoxic outcome at high concentrations. Also, a rise in AST action may be due to the liver damage ²¹.

The substantial reduction in alkaline phosphatase action in cluster 3 rats that were CCl₄ induced but given with the standard drug (Silymarin), together with clusters 4 – 6 that were CCl₄ induced but administered graded doses of *A. boonei* stem bark suggest the extract possess hepatoprotective properties²². In other words, a noticeable ($P < 0.05$) upsurge in ALP action of rats that were CCl₄ induced but untreated may be ascribed to CCl₄ action on the liver that impaired the liver integrity. These toxic influence may lead to membrane and liver cells damage occasioned by a rise in permeability of the liver membrane causing an elevation in ALP action at the extrahepatic cell.

Conclusion:

The administration of 500 mg/kg body weight for acute toxicity study shows that the plant

is less toxic as no death was recorded. The *Alstonia boonei* stem bark influence the activity of the liver enzymes in a dose-dependent perspective. This suggests that at high dosage, the activities of the liver enzymes is greatly regulated, conferring its capacity to protect the hepatocytes against injuries.

Acknowledgments

We are thankful to the laboratory analyst Mr. Bassey Udo for his time and knowledge which he spends to ensure that this investigation is successful.

Funding

This investigation did not attract grant or sponsorship from any agency or organization.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Cross River University of Technology.

Authors' contributions statement:

Okpashi V.E. and Robert I. Uroko conceived the idea. Okpashi V.E. developed the theory and performed the computations. Joy Ogana and A. Agbafor verified the analytical methods and collation of literature. C. P. Nwuke encouraged Okpashi V.E. to investigate liver marker enzymes and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Author's contribution:

All authors performed equally during this research.

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تأثير مستخلصات لحاء جذع ألتونيا على نشاط إنزيمات المصنعة بالكبد في الفئران التي تستحقها CCL4

فيكتور إي أوكباشي¹ روبرت أ. أوروكو² جوي أوجاتا³ أ. أغبافور²
سي بي نووك²

¹قسم الكيمياء الحيوية ، جامعة كروس ريفر للتكنولوجيا ، كالابار
²قسم الكيمياء الحيوية ، جامعة ميشيل أوكبارا للزراعة ، نيجيريا
³قسم الكيمياء الحيوية التطبيقية ، جامعة نامدي أزيكوي ، أوكا ، ولاية أنامبرا

الخلاصة :

بحثت هذه الدراسة في نتائج لحاء جذع ألتونيا بوناي على إنزيمات الكبد بعد تحريض فئران ويستار البيضاء باستخدام رباعي كلوريد الكربون (CCl4). تمت مقارنة تأثير المستخلصات النباتية مع سيليمارين - وهو دواء شائع الاستخدام لعلاج اضطراب خلايا الكبد المزمن. تم استخلاص العينة النباتية مع الإيثانول. تم إجراء دراسة السمية الحادة للمستخلص على ثمانية عشر فأر من Wistar ، بينما تم التضحية بـ 30 جرذاً لفحص إنزيمات الكبد. تم تقسيم الجرذان إلى ست مجموعات: كل مجموعة بها خمسة فئران ، و 1 culster بمثابة مجموعة تحكم وأعطيت 2 مل / كجم من وزن الجسم - ماء مقطر ؛ المجموعات 2-6 كانت مستحثة من CCl₄. لم يتم علاج المجموعة 2 ولكنها كانت بمثابة عنصر تحكم سلبي بينما أعطيت المجموعة 3 0.025 مل أو 25 كجم / كجم من وزن الجسم من سيليمارين ، والذي كان دواءً عاديًا ومساعدًا كعنصر تحكم عادي. تم إعطاء الجرذان في المجموعات 4-6 - 100 و 200 و 500 ملغم / كجم على التوالي من مستخلص الإيثانول لمدة أربعة عشر يومًا. أظهرت نتائج السمية الحادة لمستخلص A. boonei التحسين النسبي نتيجة عدم حدوث موت أو استجابات عدائية بعد 24 ساعة من إعطاء المستخلص. زيادة كبيرة (P 0.05) في عمل ALT بعد إعطاء 500 مجم / كجم يثبت أن السمية الأقل كانت جرعة أكبر. عند جرعة منخفضة من المستخلص ، يشير الارتفاع غير الهام (p 0.05) في تأثير AST إلى أن المستخلص كان غير ضار إلى حد ما مع عدم وجود سمية كبدية عند تناول الأدوية المنخفضة. يشير الانخفاض الكبير في تأثير الفوسفاتيز القلوي في المجموعة 3 من الفئران المستحثة باستخدام CCl₄ والمُعطاة مع Silymarin ، جنبًا إلى جنب مع المجموعات 4 - 6 التي تم تحفيزها باستخدام CCl₄ وتم إعطاؤها بجرعات متدرجة من مستخلص لحاء جذع A. boonei ، مما يشير إلى وجود خصائص واقية للكبد.

الكلمات المفتاحية : ألتونيا بوناي ، CCl₄ ، إنزيمات الكبد ، سيليمارين ، فئران ويستار البيضاء