Tikrit Journal for Agricultural Sciences (2024) 24 (2): 298-310
DOI: https://doi.org/10.25130/tjas.24.2.21

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

Evolution the effectiveness of *Vernonia amygdalina* in some physiological parameters against hepatic and renal injury in male rats

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KEY WORDS:
liver cancer, kidney injury, anti-cancer, anti-inflammation

Received: 18/4/2024
Revision : 22/05/2024
Proofreading: 30/05/2024
Accepted: 18/06/2024
Available online: 30/06/2024

ABSTRACT

In this study we investigate the efficacy of Vernonia *amygdalina (VA)* leafs phenolic extracts against hepatic tumor and renal injury induced by thioacetamide (TAA). Forty adult males rats were used for this study and divided randomly into four equal groups (10 animals for each group) , First negative (G1) Group was given distilled water, Second positive group (G2) was intraperitoneally (IP) injected with 200 mg/kg inter of TAA twice a week, While the third group (G3) was gavaged with 100 mg/kg of VA, The last group (G4) taken 200 mg/kg of TAA and 100 mg/kg (*VA)* , After 14 weeks , the following parameters were measured ALP , ALT , AST , TB , Urea , Cr , TGF-β , A-II , Renin, ACE , ,MDA , GSH, SOD , ,TNF-α and IL-10 to evaluation the effectiveness of *Vernonia amygdalina*(*VA*) leafs extracts . The results showed that treatment with *VA* enhance normal liver and kidney function as well as show its effectiveness against liver cancer and renal abnormalities.

تقييم كفاءة نبات الفيرنونيا *Vernonia amygdalina* في بعض المعايير الفسيولوجية ضد الإصابة الكبدية والكلوية في ذكور الجرذان

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الخلاصة

في هذه الدراسة قمنا بدراسة كفاءة المستخلص الفينولي لأوراق نبات (VA) *Vernonia amygdalina* ضد الاورام الكبدية والتلف الكلوي المستحث بمادة الثيواسيتاميد (TAA). في هذه الدراسة تم استخدام أربعين جرذ من ذكور الجرذان البالغة وتم تقسيمهم عشوائياً إلى أربع مجاميع متساوية (10 حيوانات/ مجموعة)، مجموعة السيطرة السالبة (G1) جرعت بالماء المقطر، مجموعة السيطرة الموجبة (G2) تم حقنها تحت البريتون بجرعة 200 ملغم/كغم من مادة TAA مرتين في الأسبوع، في حين تم تجريع المجموعة الثالثة (G3) بجرعة 100 ملغم / كغم من المستخلص الفينولي من نبات VA ، في حين حقنت المجموعة الرابعة (G4) بجرعة 200 ملغم / كغم من TAA وجرعت بجرعة 100 ملغم / كغم من المستخلص الفينولي من نبات VA ، وبعد 14 أسبوع، تم سحب عينات الد لقياس المعايير التالية: , ALP , ALT , AST TNF-α, , SOD , GSH , MDA , ACE , Renin , A-II , TGF-β , Cr , Urea , TB و IL-10 لتقييم فعالية المستخلص الفينولي لنبات ((VA Vernonia amygdalina وأظهرت نتائج الدراسة الحالية أن المعاملة بـ VA يعزز وظائف الكبد والكلى الطبيعية بالإضافة إلى فعاليته ضد سرطان الكبد والتشوهات الكلوية.

الكلمات الافتتاحية: سرطان الكبد ، الاصابة الكلوية ، مضاد سرطان ، مضاد التهابات .

INTROUCTION

The *Vernonia amygdalina* (*VA*), is medical shrabe widely used in Africa for treating various diseases, many studies were done of its effectiveness (Ijeh and Ejike, 2011). It has bitter taste leaf because of its flavonoid contents, and it is cultivated in Nigeria and in many Asian country especially Singapore and Malaysia (Moga, 2018). *Amygdalina* belongs to Asteraceae family, the genus *Vernonia* is usually planted in the tropical climate and its economically significant (Abosi, and Raseroka, 2003; Tekou *et al.,* 2018) . It is shrub which has smooth wood the height is nearly ranging from 1 - 6 m (Ifedibalu *et al.*,2020). Many studies have proven that *VA* Leaf has anti-inflammatory, anti-oxidant, anti-cancer, anti-parasitic and antibacterial activities (Farombi and Owoeye, 2011; Ahmed *et al.,* 2023).

Firstly, the anticancer activity of VA was demonstrated in nasopharynx cancer by using alcoholic leaf extract of *VA* (Morris *et al.*,1969). Many phenolic compounds, flavonoids sesquiterpene, lactones, saponins , and edotides have been found as a main components that exert

anti-cancer activity that make it pharmacologically important. Also it was found that water extract of *VA* has been used in treatment of different types of cancer (Yedjou *et al* ., 2018), reduce obesity (Atangwho *et al*., 2012) , relive typhoid fever (Fadimu *et al*., 2014) , treatment of inflammatory diseases, malaria , renal abnormalites , and many of gastrointestinal disorders (Atangwho *et al*., 2012 ; Akah and Ekekwe, 1995 ; Asante *et al*., 2019) . Additionally for its analgesic action (Abdulqader *et al*., 2022) , hepatoprotective and neuroprotective activity (Oladele *et al*., 2020), anti-allergic and antioxidant action (Ngatu *et al*., 2012) . *Vernonia amygdalina* also effective in treatment of urinary tract and sexually transported diseases (Fadimu *et al*., 2014). Hypertension, fevers, and coughs have been administer with *VA* (Amira and Okubadejo, 2007) . Another study opined that *V. amygdalina* has effectiveness in the relive symptomes of eczema and regulate blood glucose levels in blood . Despite that there is little data for toxicity of high dosage *V. amygdalina* leaves extracts (Njan *et al*., 2008).

The intent of the current study is to evolution the effectiveness and protective role of *V. amygdalina* phenolic leafs extracts against hepatic and renal injury induced by thioacetamide (TAA) in male rats .

MATERIAL AND METHODS

Animal Ethics

All experimental procedures involving animals were reviewed and approved by the Animal Ethics Committee of the University of Karbala.

This study was conducted in the College of Education for Pure Sciences / University of Karbala and the animal house of Pharmacy College, University of Karbala for the period from the beginning of November 2023 until February 2024 , and the Physiological tests were done in Al-Husseini Teaching Hospital in the Holy Karbala Governorate, Department of Clinical Chemistry.

This study included use of 40 white male rats *Ratuss norvegicus*, their ages ranged between 2-3 Months and their weights ranged between 200-240 g . Adult males rats were arranged equally into 4 groups , Negative control Group 1 (G1) was gavage distilled, Second positive group (G2) was IP injected with 200 mg/kg of TAA twice a week, while the third group (G3) was given 100 mg/kg *Vernonia amygdalina* leafs phenolic extracts, the last group (G4) gave 200 mg/kg of TAA with 100 mg/kg *VA* leafs phenolic extracts for 14 weeks . after

that the following parameters were measure: AST , ALP , ALT ,Total bilirubin (TB), Transforming Growth factor -β (TGF-β), , Urea (U) , Creatinine (Cr) , angiotensin- II (A-II), Renin (R) , angiotensin converting enzyme (ACE), MDA , SOD , TNF-α and IL-10 . Serum concentration of ALT and AST was measured according to (AL-Mashhadani et al., 2012), Serum ALP was measured according to method of (Engvall and Perlmann, 1972) , Concentration of TB was according to (Burtis and Ashwood, 1994), Urea (U) and Creatinine (Cr) were measured according to method of (Bishop *et al*., 2013) , TGF-β, A-II , ACE , Renin (R) TNF-α and IL-10 were measured by ELISA technique . MDA was measured according to (Muslih *et al* 2001).

Statistical Analysis

The results were performed and underwent by SAS Statistical program (SAS, 2001). ANOVA was used to compare between the means of the variables, and a significant variances were estimated by using Duncan's test (Duncan, 1955), and the significance level ($P<0.05$).

RESULTS AND DISSCUSION

The result of our study showed the administration of rats with TAA caused significant increase in liver enzyme which include AST, ALP and ALT and also significant increase in TB compared with control group. Administration of VA leaf extract in G4 groups relieve the toxic effect of TAA compared with control group. Rats in G2 groups showed significant increase in MDA and significant decrease in GSH and SOD compared with control group, whereas G4 group showed no significant differences in GSH, MDA and SOD .

Table 1: Effect of TAA and *Vernonia amygdalina* on AST , ALP, ALT and TB in male rats

Parameters	**Groups**			
	(G1)	**(G2)**	**(G3)**	**(G4)**
AST (U/L)	116.43 ± 8.45	327.68 ± 22.56	115.91± 12.97	122.63± 17.64
ALP (U/L)	225.15 ± 5.31	492.32 ± 29.68	227.82± 37.54	236.19 ± 8.22
ALT (U/L)	52.06± 9.98	128.47 ± 3.85	52.74± 2.61	59.18± 4.88
TB (mg/dl)	0.15± 0.02	0.73±0.12	0.14±0.04	0.19±0.06

Mean± standard error

Table 2: Effect of TAA and *Vernonia amygdalina* on GSH , MDA and SOD in male rats

Parameters	**Groups**			
	(G1)	**(G2)**	**(G3)**	**(G4)**
GSH (mg/dl)	15.69± 0.76	9.21 ± 2.14	23.49± 2.01	16.74 ±1.88
MDA (mg/dl)	0.34± 0.06	0.97± 0.03	0.21± 0.05	0.36± 0.06
SOD (mg/dl)	61.52± 7.02	31.19 ±6.32	69.10 ± 4.33	59.44± 1.39

Mean± standard error

Many studies improved the hepatoprotective efficiency of *VA* extracts to minimize many liver abnormalities in experimental animals , treatment by using *VA* extract reduce changes induced by TAA in liver function parameters . Administration of phenolic leaves extract elevated the activities of antioxidant enzymes due to higher ratio of flavonoid and other antioxidant that found in *VA* extract . Phytochemical test of the of *VA* extract display that it contains higher levels of alkaloids, flavonoids , saponins , tannins terpenoids , steroids and oils with different types of solvent used in extraction (Okafor and Anichie, 1983). Recently many research has proved many plants produce these active chemicals to protect themselves , humans and many living organisms from free radicals and diseases. Alkaloids have been extensively studied because of their bioactive and pharmacologic properties. Alkaloids possess antioxidant activity due to their ability to scavenge singlet oxygen and superoxide anions (Mariana *et al*., 2013). Many of research have also demonstrated that flavonoids is active antioxidant which can destroy superoxide anion, hydroxyl radicals and peroxy radicals (Cort, 2009). In this study, the rats which received TAA , indicate significant elevation in liver function enzymes , which contribute to induce hepatic irreversible injury. This is assign to action of TAA which subsequently lead in glutathione (GSH) depletion and increased creation of free radicals and ROS leading to destroy of hepatocytes and cell death (Katzung and Trevor, 2015) . Histopathological result was showing presence of tumor nodules and major hepatocellular damage compared with control group as well as many renal lesion and destroying of renal functional unit with shrinking and decay of glomerulus .

The result in table 3 showed that administration of rats with TAA caused significant increase in Urea ,Cr , Renin ,TGF , TNF-α and IL-10 and significant decrease in A-II and ACE compared with control group . Administration of VA leaf extract in G4 groups showed no significant differences in in Urea ,Cr , Renin ,TGF , TNF-α , IL-10 ,A-II and ACE .

Table 3: Effect of TAA and *Vernonia amygdalina* on Urea , Cr , A-II ,ACE ,Renin ,TGF-β , TNF-α and IL-10 in male rats

Parameters	**Groups**			
	(G1)	**(G2)**	**(G3)**	**(G4)**
Urea (mmol/L)	4.91 ± 0.41	9. 85 ± 0.84	3.22±0.77	5.13±0.53
Cr (µm/l)	38.41±6.32	89.93 ±5.62	32.03± 5.34	40.72 ± 5.22
A-II(µm/l)	4.32± 0.32	2.81± 0.28	4.49± 0.19	4.25± 0.36
ACE (nmol/ml)	1.36± 0.11	0.81± 0.09	1.33± 0.05	1.01±0.08
Renin (ng/ml)	25.90±2.96	38.96± 3.77	24.41±8.41	27.18± 0.83
TGF-β (ng/ml)	0.32 ±0.07	8.73± 0.85	0.28±0.06	0.41±0.06
TNF-α (ng/ml)	2.51 ±0.33	8.79±0.26	1.62±0.09	2.74±0.36
IL-10 (pg/ml)	13.42± 1.15	40.93±0.58	13.36± 0.17	14.22± 0.92

Mean± standard error

The administration of TAA caused hepatic tumor and damage of liver cells as well as many renal abnormalities in many experimental animals the elevated level of BUN ,Urea and Creatinine in TAA-treated group indicated the toxic effects of TAA in kidney. Many prior studies proved this toxic effect in generation case of tubular necrosis and destroying functional filtering unit . Tubular injury is the most common causes that responsible for the destruction glomerular filtering units . It was also found that it lead to destroying of renal tubules and many anomaly such as blockage of renal tubules which in turn caused inversely return of glomerular filtration fluids (Whelton and Solez, 1983) . And so on, renal failure in TAA-treated rats was due to the action of ROS (Leena and Alaraman, 2005). In this study, it was found that *VA* extract contribute to enhance renal functions, which contains higher ratio of antioxidant and vitamins

that could inhibit the apoptosis. TGF-β have been implicated in many disease such as atherosclerosis , cardiovascular disease, heart attack, hypertension, and cardiac enlargement . TGF-β is an active catalyst for collagen production , generating fibrosis. It has been found that Luteolin in *VA* plays a critical role in inhibiting the destroying and transformation of growth factor-β receptor 1 (TGFBR1) which found in vascular smooth muscle cells, and was active for prevent fibrosis and maintains normal body functioning . Another study has proved that rutin eliminate ROS and prevent its production and cell death , This mainly done by the deactivation the action of TGF-β1-p38 MAPK signaling pathway (An *et al*., 2009) .

Reduced antioxidant activity mainly catalase was noticed in the TAA group , due to ROS which caused depletion of an antioxidant stock in rats for this group. Moreover , IL-10 and TNF-α , were increased distinctively after TAA administration . Histologically Oxidative stress generation in many tissues by TAA has been proved in many researches (Fayyadh *et al*., 2021 ; Bayomy *et al*., 2016; Romualdo *et al*., 2017) . Many studies demonstrated that the TAA toxic substance may cause tissue damage , pathologies and many diseases. TAA-treated rats caused kidney to activate NFκB in ischemia reperfusion injury. Which in turn results in the activation of p38 MAPK, that could be lead to NFκB stimulation and increased TNF-α generation (Hsu *et al*., 2024) . Other researches have been recorded the increased of IL-10 concentration in process of development of acute renal injury (Simmons *et al*., 2004).

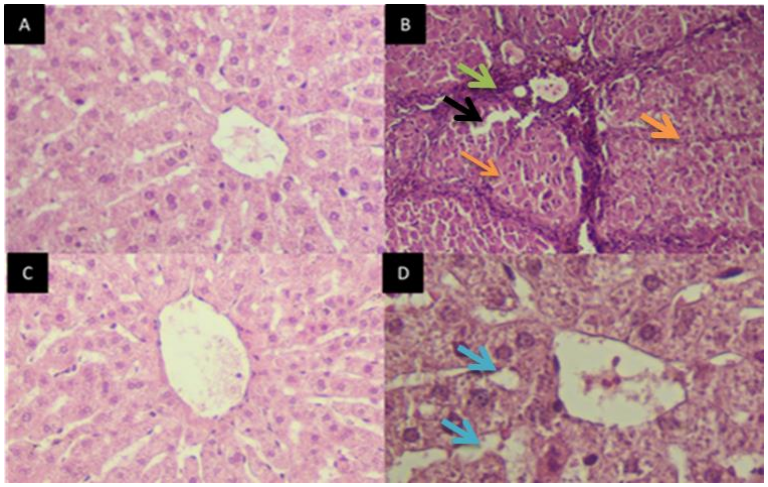

Figure 1: liver section photomicrograph A: control group exhibit normal liver architecture B: liver of rats treated with 200 mg/kg of TAA showing presence of tumor nodules (orange arrows) and filteration of inflamentary cells (green arrow) with degenerative change (black arrow) C: liver of rat treated with 100 mg/kg *Vernonia amygdalina* (VA) leafs phenolic extracts for 14 weeks showing normal liver architecture D: liver of rats treated with 200mg/kg showing TAA +100 mg/kg VA leaf extract showing present some vacuoles in tissue (blue arrow) .

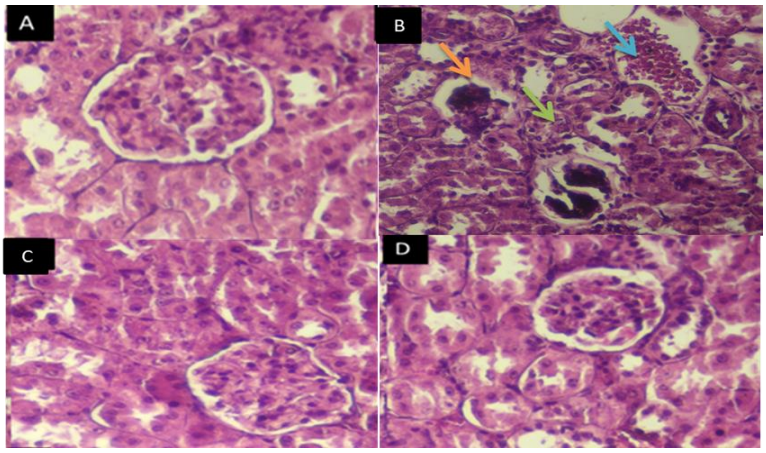

Figure 2: kidney section photomicrograph A: control group exhibit normal structure of glomerulus and renal tubules B: kidney of rats with 200 mg/kg of TAA showing degenerative changes in tissue and shrinking in glomeruli (orange arrow) with filteration of inflamentary cells in tissue (green arrows) with presence of blood congestion (blue arrow) C: kidney of rat treated with 100 mg/kg *Vernonia amygdalina* (VA) leafs phenolic extracts for 14 weeks showing normal liverarchitecture D: kidney of rats treated with 200mg/kg TAA +100 mg/kg VA leaf extract showing maintain normal structure to tissue

CONCLUSION

Vernonia amygdalina (VA) leafs phenolic extracts evolve the defense case against abnormalities and toxic effect of TAA in liver cancer and renal dysfunctions , due to elevated antioxidant contents and its ability to reform liver and kidney damage , it has great preventing importance in preserve normal liver and kidney tissue from damaged caused by TAA .

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the [Department, College or University] for their technical and general support.

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