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EFFECTS OF AQUEOUS EXTRACT OF *TALINUM TRIANGULARE* (LEAVES): EVALUATION OF ENZYMES ACTIVITIES IN TISSUE HOMOGENATES OF ALBINO RATS

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Abstract

The effect of water extract of Talinum triangulare was investigated on the activities of enzymes such as aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP), in the serum and tissue homogenates of an adult albino rats along with the serum total protein. The rats were randomly distributed into four treatment groups A-D; with groups B to D administered with 100, 200 and 400 mg/kg body weights orally and the control group A with water orally respectively. The aqueous extract was prepared using the air-dried leaves parts of the plant following a standardized method with the final yield been considered. The serum total protein (g/dl) was significantly different ($p<0.05$) relatively compared to control group. In the same vein, statistical data of the activities (u/l) of the enzymes was significantly different ($P<0.05$) in aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) when compared to control groups but significant different ($p>0.05$) in alkaline phosphatase (ALP) when compared to the control group.

Key words: Phytochemicals, Aspartate aminotransaminase, Alanine aminotransaminase, Alkaline phosphatase.

Introduction

The significance of phytomedicine is strikingly accepted world-wide with special consideration to the vast natural non-nutritive phytochemical components in plants with various biological activities they perform, both in humans and animals e.g. in strengthening immune system and in helping to lower the risk of many chronic diseases and infections [1]. Also, the general view among folks and in the medical community is that; plant-based products are reliably safer and healthier than synthetics when their protective abilities in the system are considerably compared [2, 3].

Transaminases are important markers in the assay for many injuries in organs, most prominently in the hepatic tissue [4]. There are different types of transaminases, but the two that are commonly measured medically are alanine transaminase (ALT) also called glutamate-pyruvate transaminase (SGPT) and aspartate transaminase (AST) also called glutamate-oxaloacetate transaminase (SGOT) [5]. Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) are well known diagnostic indicators of liver injury and they are considered the bio-markers for liver functions. Elevated ALT may also be caused by dietary choline deficiency [6]. Fluctuation of ALT levels is normal over the course of the day, and they can also increase in response to strenuous physical exercise [7]. They can also indicate liver necrosis or tumor, thyroid disease, diabetes the use of drugs that are toxic to the liver, mononucleosis, or shock [8, 9, 10]. Aspartate aminotransaminase (AST) can be elevated from conditions other than liver damage, since it is found in other parts of the body, such as the heart, muscle tissue, and kidneys. Also, elevated plasma alkaline phosphatase (ALP) activity can be an indication to pathological processes such as liver impairment and kidney dysfunction [11].

In the current study, we investigated the effects of the aqueous extract of *Talinum triangulae* on these enzymes activities in the tissues and in the serum after the its administration for the of 28 days *Talinum triangulare* is one of the most commonly found plants; it is a plant with an erect herb, swollen roots and succulent stems. The plant when mixed with other plant makes vegetable additives [12]. *Talinum triangulare* has also been found to be beneficial in preventing common neurodegenerative diseases including; Parkinson's and Alzheimer's diseases and it has been established to substantially reduce the risk of cardiovascular diseases and cancers [13].

Materials and Methods

Identification of the plant

Fresh water leaves, *Talinum triangulare* were bought in Ado-Ekiti, Ekiti State, Nigeria. Sample was taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria and was identified by a specialized Taxonomist Mr. Omotayo with herbarium number UHAE 2013/76.

Plant materials and preparation

The leaves parts of the plant were air-dried in a ventilated place at room temperature for 15 days and thereafter blended using a laboratory blender, the fine powders obtained was stored at moderate temperature of 37°C until further use. The powdered sample (50 g) was soaked in 500 ml distilled water for 48 h. It was then evaporated to dryness using rotary evaporator and water bath to obtain total yield of 20.9 g. The crude extract was later subjected to bioassay analyses.

Animal treatment

Albino rats of weight ranging between 90-150 g were used for these experiments. The principles of laboratory animal care (NIH) were followed. The rats were randomly distributed into four treatment groups of four rats each. Group A (control Group) consists of animals fed with standard animal feed. Group B, consists of animals administered with 100 mg per kg body weight dosage of the 10mg/ml concentration of aqueous extract of *Talinum triangulare* alone. Group C, consists of animals administered with 200 mg per kg body weight dosage of the 10 mg/ml concentration of aqueous extract of *Talinum triangulare*. Group D consists of animals administered with 400 mg per kg body weight dosage of the 10 mg/ml concentration of aqueous extract of *Talinum triangulare*. The animals were sacrificed by cervical dislocation and simply incising the jugular vein after 28 days of feeding and administration, the blood samples were collected into plain sample tubes for enzymatic and serum analysis respectively.

Preparation of serum

Blood samples for serum were allowed to stand at room temperature for 30 min. to form clot after which it was centrifuged at 3000 g (gravity) for 10 min. After centrifugation, the clot forms sediment at the bottom of the centrifuge and the supernatant

which is the serum was collected using a Pasteur's pipette. The serum, thus obtained were appropriately labeled and stored at favorable temperature.

Preparation of tissue homogenates

After the animals have been sacrificed and dissected, tissues of interest (liver, kidney and brain) were removed and washed with 0.25 M sucrose solution. The isolated tissues were weighed and immediately stored on ice-cold 0.25 M sucrose solution. The brain, liver and pair of kidneys that were cut with a clean scalpel were then subjected to homogenization using Teflon homogenizer in ice-cold 0.25 M sucrose solution (1:5 w/v). The supernatants were stored in the freezer at -5^{o}C.

Determination of total serum protein

The protein concentration in the serum of the animals was assayed for at 540 nm, using Biuret method as described by Peter et al. (1982) [14]. Activities of the enzymes in the serum and in the tissues were also estimated from the standard calibration curves using standard Randox kits with spectrophotometric method as described by Reitman and Frankel (1957) [15].

Data analysis

One way analysis of variance was used to analyze the results and Duncan multiple tests was applied for the *post hoc* [16]. Statistical package for Social Science (SPSS) was used for the analysis. The p value < 0.05 was considered statistically significant in the analytical data.

Results

The table below shows total protein (TP) (g/dl) in the serum of the albino rat administered with *Talinum triangulare* aqueous extract for 28 days.

In this table 1, control group shows significant relative difference (7.253 ± 0.244) in total protein (g/dl) when compared to other groups in the table ($p>0.05$) and there is moderate significant difference in their total protein (g/dl) level as indicated in the table within the groups ($p<0.05$) after treatment with *Talinum triangulare* aqueous extract for 28 days (Table 1). Alkaline phosphatase (ALP) activity (u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days. In this table, there are indications that, the activities of Alkaline phosphatase activity (u/l) observed varies significantly within the groups and the organs respectively. Considering the activity of Alkaline phosphatase (ALP) (u/l) in the liver and the serum of the groups considered as indicated in the table, little significant difference ($p<0.05$) was observed within the group in the serum, while variations were observed within the groups in the kidney, with significant difference ($p>0.05$) when compared to that of control groups both in the serum and in the kidney. Also, the level of significance observed in the brain is different significantly within the groups ($p<0.05$) when compared to the level of significance ($p>0.05$) observed in the control group (Table 2).

Aspartate aminotransaminase activity (AST) (u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days. From the table 3, it is shown that aspartate aminotransaminase (AST) activity (u/l) observed in the liver, kidney, brain and serum indicates that the groups are significantly different from one and the other ($p<0.05$) when compared to that of the control (Table 3). Alanine aminotransaminase (ALT) activity (in u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days. Table 4 below shows significant difference ($p>0.05$) in the level of Alanine aminotransaminase (ALT) observed in the groups when compared to the control group as indicated in the liver and serum and significantly ($p<0.05$) different in the kidney and brain across the group when compared to that of control group (Table 4).

Discussion

The results of this study are as clearly shown in the Table 1-4, the total serum protein observed after 28 days administration of *Talinum triangulare* is shown from Table 1, it shows an observable moderate reduction ($p < 0.05$) in the serum total protein levels among the groups when compared to that of the control group in dose dependent manner after the administration window.

From the table, the control group shows serum protein value of 7.253 ± 0.244 g/dl while group A that received oral administration of 100 mg/kg, showed little reduction ($p < 0.05$) with 6.098 ± 0.438 g/dl while group B and C that received 200 and 400mg/kg dosage showed moderate reduction ($p < 0.05$) when compared to that of group A and the control group with 5.953 ± 0.190 g/dl and 5.053 ± 0.349 respectively. This study clearly agrees with a report by Arit et al. (2007) [17] and this could be deduced that, the plant shows no tendency to

increase serum protein level but shows little ability to reduce serum total protein moderately in dose dependent manner after the administration window of 28 days. Activities of maker enzymes are shown in the Table 2-4, from the table, it is indicated that there were observable changes in the levels of activities of the enzymes in the tissue homogenates and also in the serum.

The enzyme alanine aminotransaminase (ALT-) and L-aspartate (AST) are important enzymes which are often checked for when evaluating the level of damage(s) done to the liver [18]. Aspartate aminotransaminase also called serum glutamic oxaloacetic transaminase or aspartate aminotransferase is similar to alanine aminotransaminase in that, it is another enzyme associated with liver cells, it is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle, so is not specific and limited to the liver alone [19; 20].

Taking into consideration Table 2, the levels of alkaline phosphatase (ALP) in (u/l) reduced significantly ($p<0.05$) in the liver homogenates of the tested groups when compared to that of control group as indicated in the group C and group D with ALP activity values of 193.32 ± 37.1 and 165.28 ± 22.5 u/l respectively but the value is high significantly ($p>0.05$) in the group A with ALP activity values of 221.10 ± 11.9 u/l when compared to that of the control group. Also, in the kidney, alkaline phosphatase (ALP) levels were observed to be increased significantly ($p>0.05$) in dose dependent manner in the kidney even at lower concentration as indicated in the group A, B and C with values 351.60 ± 6.93, 368.50 ± 51.5 and 397.65 ± 26.6 u/l respectively when compared to that of the control group with 90.3 ± 6.46 u/l, the increase in the levels of alkaline phosphatase (ALP) observed in the kidney could be traced to the fact that oxalate (oxalic acid) content contained in *Talinum triangulare* is high compared to other phytochemicals as described by the report of Tesleem et al*,* (2008) [21] and the can affect the kidney adversely. Concurrently, the level of significance of alkaline phosphatase (ALP) was also considered in the brain as indicated in the Table 2. From the table, reduction was observed in the levels of ALP activities significantly ($p<0.05$) in all the groups when compared to that of the control group after 28 days administration of aqueous extract of *Talinum triangulare.* Serum levels of ALP activities (in u/l) were also reduced significantly ($p<0.05$) in dose dependent manner across the tested groups but significantly ($p>0.05$) different from that of the control group. It could be deduced from the study that the plant aqueous extract has protective ability on the other organs except on the kidney. The activities of Aspartate aminotransaminase (AST) in u/l was also assayed for after 28 days administration of aqueous extract of *Talinum triangulare* and the result is shown in the Table 3,. Aspartate transaminase catalyzes the inter-conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, AST relies on Vitamin B6 as a cofactor to transfer the amino group from aspartate or glutamate to the corresponding ketoacid. In the process, the cofactor shuttles between Vitamin B6 and the pyridoxamine phosphate (PMP) form [22].

The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. As indicated in the Table 3, there appeared a level of significant ($p<0.05$) difference in Aspartate aminotransaminase (AST) activities in the liver of the tested rats across the experimental groups when compared to that of the control group, with group A, B and C showing values of 85.93 ± 1.42, 100.50 ± 6.95 and 99.65 ± 8.51 u/l respectively when compared to that of the control group with 103.68 ± 3.41 u/l. From the results, it could be clearly inferred that no amount of damage(s) was done on the liver tissue. In the same vein, the activities of AST (in u/l) was significantly ($p<0.05$) reduced in the kidney homogenate when compared to that of the control group. This was also reduced significantly ($p<0.05$) in the brain homogenate of the tested group A and B with 92.66 ± 8.26, 87.90 ± 5.88 respectively but significantly ($p>0.05$) different from that of group C with 61.31 ± 2.48 u/l when compared to that of the control group. Serum levels of Aspartate aminotransaminase (AST) were also reduced ($p<0.05$) significantly in the groups when compared to the control group.

Alanine aminotransaminase (ALT) as maker enzyme in u/l was also assayed for as shown in Table 4, this enzyme catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate, it require the activity of coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid, so, Alanine aminotransaminase (ALT) is commonly used as a way of evaluating hepatocellular injuries [23,

24]. From the table, there was significant (P>0.05) difference in the values of ALT activities in dose dependent manner as indicated across the groups tested when compared to that of control group with 42.64 ± 3.29 u/l, group A, B and C having ALT values in u/l of 38.80 ± 2.94, 48.85 ± 1.84 and 52.24 ± 4.18 u/l respectively. In the kidney, the levels observed within the groups showed no significant (p<0.05) difference as the values are moderately altered within the group compared to that of the control group. Also, from the Table 4, the groups tested revealed significant (p<0.05) difference of ALT activities in the brain tissue, group A, B and C showed ALT activities values of 38.93 ± 3.47, 37.06 ± 2.48 and 25.85 ± 1.05 u/l with group C that received 400mg/kg dosage orally exhibited reduction in the level of Alanine aminotransaminase (ALT) activity when compared to the control group with 38.31 ± 2.83 u/l. In the serum, the group C that received 400mg/kg dosage showed considerable reduction than other tested groups significantly (p<0.05) with 16.49 ± 1.85 u/l when compared to that of the control group with Alanine aminotransaminase (ALT) activity of 29.96 ± 4.93 u/l, its indicates that; *Talinum triangulare* aqueous extract at higher dosage may not be hepatotoxic and could be protective to the organs as indicated in the tested animals.

Conclusion

The current study has really shown a strong indication that aqueous extract of *Talinum triangulare* is biologically active and when dietary intake is considered, it could serve as a remedy in the guide against hepatocellular injuries and inflammation as indicated in the organs that were considered in this study.

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Table 1. The table below shows total protein in (g/dl) in the serum of the albino rat administered with *Talinum triangulare* aqueous extract for 28 days.

Control	Group A (100mg/kg)	Group B (200mg/kg)	Group C (400mg/kg)
7.253 ± 0.244^{c}	6.098 ± 0.438^{b}	5.953 ± 0.190 ^{ab}	5.053 ± 0.349^{a}

* Mean values of n=4 within a row and ±S.E with different subscripts (a, b, c) that shows significant difference

Table 2. Alkaline phosphatase activity (in u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days.

Tissues	Control	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)
Liver	195.80 ± 11.6^{a}	221.10 ± 11.9^{a}	193.32 ± 37.1^{a}	165.28 ± 22.5^{a}
Kidney	90.3 ± 6.46^{a}	351.60 ± 6.93^{b}	368.50 ± 51.5^{b}	397.65 ± 26.6^{b}
Brain	308.40 ±29.7^{b}	305.45 ±23.17^{b}	224.80 ± 9.86^{a}	201.50 ±25.6^{a}
Serum	90.48 ± 9.83^{a}	148.44 ± 22.1^{a}	122.80 ± 26.1^{a}	100.98 ± 19.7^{a}

*****Values are expressed as mean of four determinations within a row ± S.E and different subscripts (a, b, c) that indicates significant difference.

Table 3. Aspartate aminotransaminase activity (in u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days.

Tissues	Control	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)
Liver	103.68 ± 3.41[a]	85.93 ± 1.42[a]	100.50 ± 6.95[a]	99.65 ± 8.51[a]
Kidney	99.65 ± 0.95[b]	83.98 ± 1.59[a]	91.07 ± 7.01[ab]	90.23 ± 3.12[ab]
Brain	89.06 ± 5.86[b]	92.66 ± 8.26[b]	87.90 ± 5.88[b]	61.31 ± 2.48[a]
Serum	71.17 ± 2.18[a]	60.79 ± 8.91[a]	60.99 ± 2.86[a]	62.26 ± 3.27[a]

*****Values are expressed as mean of four determinations within a row ± S.E and different subscripts (a, b, c) that indicates significant difference.

Table 4. Alanine aminotransaminase activity (in u/l) of liver, kidney, brain and serum of rats administered with *Talinum triangulare* aqueous extract for 28 days.

Tissues	Control	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)
Liver	42.64 ± 3.29[ab]	38.80 ± 2.94[a]	48.85 ± 1.84[ab]	52.24 ± 4.18[b]
Kidney	42.54 ± 0.34[b]	36.21 ± 1.12[a]	36.44 ± 2.96[a]	36.83 ± 1.82[a]
Brain	38.31 ± 2.83[b]	38.93 ± 3.47[b]	37.06 ± 2.48[b]	25.85 ± 1.05[a]
Serum	29.96 ± 4.93[b]	29.39 ± 3.25[ab]	23.22 ± 0.57[ab]	16.49 ± 1.85[a]

*****Values are expressed as mean of four determinations within a row ± S.E and different subscripts (a, b, c) that indicates significant difference.