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Biochemical and Histological Study of Aminoacylase-1 Purified from Amniotic Fluid in Rats with Oxidative Stress Induced by Lead Acetate

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Abstract:

This work involves separating and studying the aminoacylase-1 (ACY1) of amniotic fluid from healthy pregnant, mainly one peak with higher activity has been isolated by DEAE-Cellulose ion exchange from the proteinous supernatant produced by deposition of proteins using ammonium sulfate (65%) after dialysis. The purification folds reaching to 19 folds also gave one protein peak when injected into the gel filtration column, a high ACY1 purity was obtained, with 38 folds of purification. It was found that the molecular weight of the isolated ACY1 was up to 46698 Dalton when using gel chromatography technique.

The effect of ACY1 isolate was studied on rats with oxidative stress caused by lead acetate(LA) at 40 mg / kg body weight and compared with normal rats by measuring the selected biochemical parameters which included: Glutathione (GSH), malondialdehyde (MDA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) through liver and kidney tissue examination. The results showed a significant increase in the levels of (MDA, AST, ALT) and a decrease in the level of GSH compared with the control group, Also it has been observed there that was a significant decrease in the levels of (MDA, AST, ALT) and high level of GSH when injecting the ACY1 isolate in a dose of 4 mg / kg of rat weight with LA at 40 mg/kg. The results of the tissue examination demonstrated high pathological changes in the liver tissue of rats treated with LA at 40 mg/kg of rat weight when compared with normal rats. The liver and kidney tissue improved when treated with isolate at 4 mg / kg rat weight and LA. These results demonstrate the role of ACY1 in protecting from oxidative stress then can reduce the severity of various diseases.

Key words: Aminoacylase-1, Amniotic fluid, Isolation, Lead acetate, Rats.

Introduction:

Aminoacylase-1 [EC 3.5.1.14] (ACY1) is a soluble protein found in many tissues of mammals, plants and microbiology (e.g. bacteria and fungi). It was noticed that an isolated ACY1 from different tissues has a molecular weight that varies depending on the organism and the tissue isolated from it (1). ACY1 catalyzes the hydrolysis of N-acylated L-amino acids to yield the corresponding organic acid and amino acid(2). The ACY1 reduces the effects of toxic substances resulting from oxidation processes, and has a role in the pharmaceutical industry and in the production of free amino acids used in protein synthesis. Also, it acts as a regulator of the urea cycle through its work as an allosteric regulator of carbamoyl phosphate synthetase and then gets rid of the increase in ammonia in the body by converting it to urea (3). The ACY1 has a vital role in

inhibiting the development of cancer cells(4) through interacting with sphingosine kinase 1 which inhibits apoptosis of tumor cells (5). Deficiency in ACY-1 can cause neurological disorders, growth delay, encephalopathy, brain atrophy, unspecific psychomotor delay, muscle weakness, myelin deficiency, hyperactivity and mental retardation (6). ACY1 has a role in the production of important amino acids, like methionine produced from N-acetyl methionine which is an important compound in methylation process, as well as in the synthesis of choline (Precursor of acetylcholine) which is an important substance in the nervous system for the transmission of nerve signals in addition to the acid itself that is a raw material for the synthesis of cysteine, as the methionine turns into cysteine, and the latter participates in the formation of glutathione

(GSH) which is an important antioxidant that protects the cell from the effects of oxidant compounds and the occurrence of oxidative stress (OS). Besides, N-acetylcysteine is an important antioxidant that can work to remove OS, and may serve as an L-cysteine produced as an important amino acid. It becomes an antioxidant through its entry into the production of various antioxidants like glutathione which is involved in the removal of toxic chemical compounds resulting from various oxidative processes or from various environmental pollutants (7).

Oxidative stress can lead to different effects on cell components including mitochondria dysfunction, as well as loss of maintenance of the electrical and chemical membrane of the inner membrane of mitochondria, and changes in the electron transport chain function, these changes lead to a decrease on the production of energy in cell as ATP, as well as the cause of some diseases for example, heart failure, albuminuria, atherosclerosis and diabetes (8).

The researcher explained the decrease of the ACY1 activity in pregnant women with congenital malformations and encephalopathy, as there is a relationship between enzyme deficiency and autism, as N-acetyl-glutamate has a vital role in learning, perception and memory and that changing its level causes a defect in many brain functions (9).

The research aims to isolate ACY1 from the amniotic fluid using different biochemical techniques and determine its molecular weight then studying the biochemical parameters and tissue effect of ACY1 on rats with OS induced by LA.

Materials and Methods:

Samples of 100 ml of amniotic fluid were obtained from healthy pregnant women between the ages of (26-35) years from Al-Khansa teaching hospital in Mosul city and under the supervision of specialized physicians, taking into account the fact that they are non-smokers and do not take medication of various types during the period of sampling. Subsequent purification steps were carried out, using different techniques as follows:

Step I: Enzyme precipitation: ACY1 was precipitated from the amniotic fluid using 65% ammonium sulfate saturation with continuous movement of the electric motor at 4°C, and then the solution was left for 24 hours in the refrigerator to complete precipitation of proteins (10).

Step II: Cooling ultracentrifuge separation: The suspension produced from step I was centrifuged at 9000 g for (30) min at -4°C to avoid denaturation. The protein in precipitate and supernatant is determined (11). ACY1 activity was determined in

each fraction by using Peterson method (12), then the precipitate protein and the supernatant were kept at -20°C until used in subsequent steps.

Step III : Dialysis: This is done by using a cellophane (Semi permeable) membrane with M.wt. cut off (<10000) Dalton the sac containing solution from (Step II) using 0.1M ammonium bicarbonate to prevent Donnan's effect and left overnight at 4°C(13) with stirring, the solution was changed three times (per 3 hours)(14). The protein of the suspension was used to estimate ACY1 activity by modifying Lowry method (11) and also measuring the ACY1 activity in the solution resulting from dialysis by using the Peterson method (12), then keeping the solution at a temperature of (-20)°C until it was used in the next step.

Step IV: Ion-Exchange Chromatography: A sample (10) mL of the ACY1 fractions, which was prepared in (III), was applied to column (2.2 × 45) cm which contained DEAE-Cellulose (Diethyl amino ethane-cellulose) anion exchange to (40)cm height (15), which has been equilibrated with sodium phosphate buffer (10) mM of pH=7.1 (13), fractions of (5)mL volume were collected. Flow rate was approximately 1 mL/min. The protein in each fraction was detected by absorbance at (280) nm using UV/visible spectrophotometer. The protein peak containing the high aminoacylase-1 activity (12) was also inferred. It has been observed that there is only one protein pack resulting from the ion exchange process.

Step V: Gel filtration chromatography using Sephadex G-100: In the present study, the column of dimension (2.2 × 45) cm contained a gel sephadex height of (40) cm. The exclusion limit for sephadex G-100 is (150000) Dalton(10). A sample (5) mL obtained from (Step IV), was applied on column using 0.1 M sodium phosphate, pH 7 (13). Elution is of a flow rate (48) mL/hour. The fractions were collected by using a fraction collector apparatus working on minute system. The protein compounds in each fraction were detected by absorbance at (280) nm by using UV/visible spectrophotometer and determined by ACY1 in each fraction (10). The protein parts containing the highest ACY1 activity were then collected, and also determined for the standard substances injected into the column for the purpose of determining the standard curve (16) to determine the approximate molecular weight of ACY1.

Step VI: Lyophilization stage: The ACY1 from step (V) was dried using a freeze-dryer to obtain a solid. The ACY1 was frozen at -20°C to be used in the subsequent step of the research (15).

ACY1 Assay:

Peterson method (12) was used for the determination of ACY1 which depends on the principle of the hydrolysis of the aliphatic amino acids containing the acetyl group such as used N-acetyl-L-methionine by determining its production of L-methionine and the determination of L-methionine using ninhydrin method was described by Rosen method (17).

Animals Used and How to Deal with them:

Twenty-four white Albino female rats (200-300) gm were obtained from Veterinary College, University of Mosul. Rodent food rich in nutrient and tap water were used as bedding. The rats were distributed into three groups with eight for each and for 30 days each group was treated as follow: **Group I** was given drinking water for the duration of the trial and was considered a healthy control group. **Group II** was given drinking water by special bottles and contained LA (40 mg/kg) for the duration of the trial, and was considered an infected group that was not treated. **Group III** was given drinking water containing LA at a concentration (40 mg/kg) and injected with the aminoacylase-1 (ACY1) purified in the intraperitoneal and at a dose (4 mg/kg) of rat weight throughout the trial, and was considered an infected group treated by ACY1 purified.

Collecting Blood Samples from Experiment Animals:

Blood samples were collected from animals upon times: Time zero (before treatment) and day 30 (after treatment). The animals were numbness by pure ether, blood was collected directly by orbital sinus (Intraocularly) and put into tubes and then left to clot. The tubes were then placed in a centrifuge at 3000 xg for 15 minutes to separate the coagulated part from the serum. The serum was used within 2 days for the purpose of estimating levels of: glutathione(GSH) (13), malondialdehyde (MDA) (18) and enzyme carriers of the AST and ALT

group (13) and upon completion of the treatment period for the three groups the animals were killed by dislocation. The rats in each group were killed and part of the liver and kidney tissues were extracted for the purpose of studying tissue examination (Histology). The statistical program SPSS-25 was used to determine the mean and standard deviation (SD) and t-test was chosen to compare two parameters and find the difference between the values, the P-values equal or less than 0.05 were considered to be significantly different.

Histology:

Luna method (19) was used for fixation, parts of liver tissue and kidney were taken from all groups after killing at the end of the treatment and were installed with the previously prepared stabilized solution, then washed with distilled water to remove the remaining fixation. The installer was then passed through a gradual series of ethyl alcohol starting from 70%, 90%, 100% and then passed to the solution of the xylene then transferred to the wax of the molten paraffin for half an hour after which the wax molds were poured to be ready to cut in the microtome rotary hang out as they were cut into strips of tissue with a thickness of five micrometers. The slides were dyed with hematoxylin and eosin and then the exact tissue diagnosis was performed by the histologist.

Results and Discussion:

ACY1 purification

As it was noted in the previous studies that the ACY1 was isolated from the tissues of different mammals, as it is found in the liver, brain, kidneys, muscles, pancreas and in the blood serum and has not been isolated from the amniotic fluid (20). The ACY1 was found in (65)% (Table 1), and increased the specific activity after dialysis because of removal of the molecules (Below 10000 Dalton) and increasing the folds of purification for ACY1.

Table 1. Isolation steps for ACY1 from the amniotic fluid.

Purification stage	Volum (ml)	Protein conc. (mg/ml)	Activity×10 ⁻³ (U*/ml)	Total activity (U)	Sp.activity (U/mg protein)	Folds of Purification	Recovery %
Amniotic fluid	100	3.22	4.92	492	1.527	1.0	100
Precipitate by (NH ₄) ₂ SO ₄ (65%)	42	4.55	10.21	428.82	2.243	2	87.1
Supernatant	58	0.94	0.23	13.34	0.244	0.2	2.7
Dialysis	44	2.32	9.1	400.4	3.922	3	81.4
DEAE-cellulose (Fractions) Peak	15	0.44	12.9	193.5	29.318	19	39.3
Sephadex G-100 (Fractions) Peak	14	0.162	9.37	131.18	57.839	38	26.7

U*: a amount of aminoacylase-1 (ACY1) catalyzing the formation of one micromole for L-methionine from N-acetyl-L-methionine per mint under optimum conditions.

Ion-Exchange Chromatography: Selective adsorption and elution of proteins from the polydextran derivatives anion exchange diethylaminoethyl (DEAE-cellulose) have also been extremely successful for extensive and rapid purification. Specific activity of ACY1 was increased after the use of DEAE-cellulose exchanger when passing the protein solution resulting from the dialysis process, where it was found that there is one peak of ACY1, Fig. 1 explains the elution profile of purified ACY1 by ion exchange chromatography. It obtained a one peak at elution volume (175) mL with a specific activity (29.3) U/mg protein and (19) folds of purification.

Gel Filtration Separations: This technique was applied to separate the protein as a source of ACY1, which was obtained after ion exchange using a column containing sephadex G-100 gel as shown in (step V). The result (Fig. 2) indicated that there is mainly one peak for ACY1 in sephadex G-100. The elution volume of peak was (132) mL. The specific activity of the ACY1 peak was (57.8 U/mg protein) and 38 folds of purification compared to initial extract, (Table 1).

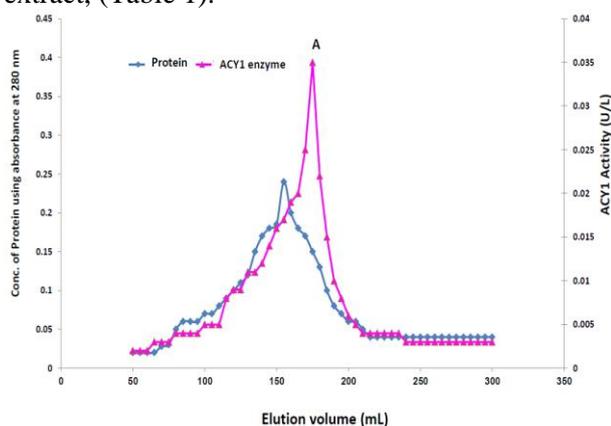


Figure 1. Purification of ACY-1 by DEAE-cellulose chromatography.

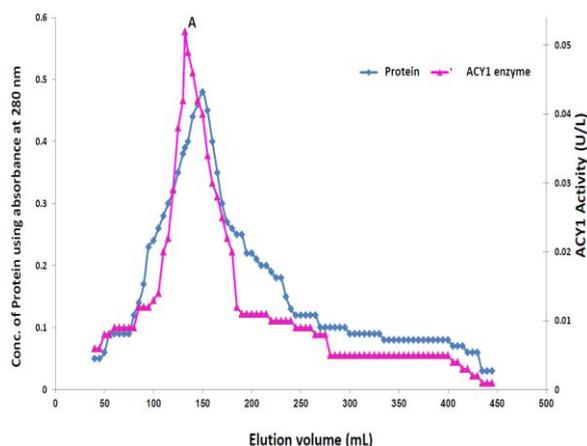


Figure 2. Elution profile of ACY-1 for amniotic fluid on sephadex G-100.

Molecular Weight Determination of ACY-1 by Gel Filtration:

The molecular weight (M.wt) determined in step V by column (2.2 × 45) cm calibrated with known proteins that were listed in Table 2. Estimating the approximate M.wt for ACY1 gave the highest specific activity of the ACY1 amounting to 57.8 U/mg protein and purity reached to 38 times. Therefore, it was used to estimate the M.wt of the ACY1 by passing a number of known M.wt compounds whose M.wts range between (204-2000000 Dalton) on the separation column for the purpose of determining the properties of the column in terms of internal volume (V_0) that were up to (217 mL) and (57 mL) respectively. Table 2 shows the materials that were ordered on the separation column containing the sephadex gel with its fractional weight and elution volumes.

The M.wt of unknown protein separated by the column in (step V) was determined from the standard curve, which is represented by Fig. 3. The comparative M.wt of peak as a source of ACY1 is approximately equal to (46698.18) Dalton, and is close to what Zhong and others (21) found for the enzyme extracted from human blood, which reached 47000 Dalton when separated and evaluated by the electrophoresis. Furthermore, it matches the M.wt found by Sommar and others (2) who indicated that the M.wt of ACY in the mammals is approximately 46000 Dalton. It was also observed that the ACY1 isolated from the rats kidney has a M.wt around 43000 Dalton (22), and using ion exchange technology has isolated enzyme by Zhong and others (21) and also from rats kidney who found that his M.wt up to 43000 Dalton.

Table 2. Elution volumes of standard compounds.

Materials	M.wt (Dalton)	Elution volume (mL)
Blue dextran (Void volume(V_0))	2000000	57
Hexokinase	100000	68
Bovine serum albumin	67000	78
Amylase	58000	129.5
Albumin from egg	45000	139
Pepsin	36000	159.8
Insulin hormone	5750	191.8
Tryptophan (Internal volume(V_i))	204	217
Unknown (peak A)	46698.18	*132

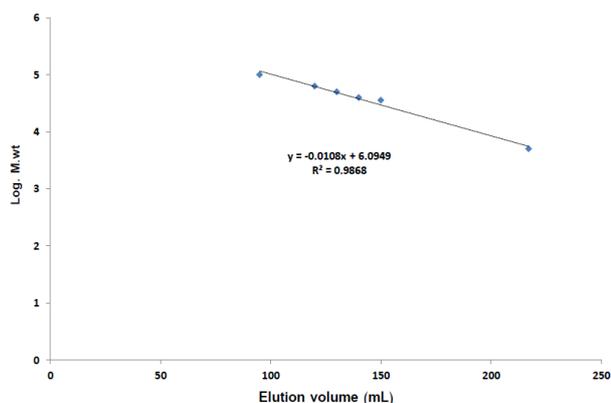


Figure 3. A plot of the logarithm molecular weights of known proteins versus elution volume on a sephadex G-100.

The Effects of ACY1 Isolated on Biochemical Parameter in Animals with Oxidative Stress Induced with LA:

The results shown in Table 3 indicated that there is a significant decrease ($p \leq 0.001$) in the level of GSH in the serum of rats treated with LA at a concentration of 40mg / kg of weight compared to the control group. These results are consistent with those of the previous studies that have indicated a low level of GSH in the serum of rats treated with lead reduction (23). The reason for this decrease is the consumption of GSH to remove free radicals, which are increasingly produced as a result of exposure to high levels of lead, and causes the production of oxidative compounds of different types and lead to inhibit many important enzymes in the production of erythrocytes, such as aminolevulinic acid dehydratase (ALAD). It then inhibits the process of hematopoiesis (24) as a result of the accumulation of high amounts of gamma-aminolevulinic acid, which is auto-oxidized and then produces high amounts of reactive oxygen species (ROS). This leads to an increase in oxidative stress, which causes protein oxidation and changes its function, in addition to an increase in lipid peroxidation and oxidation of nucleic acids that cause many mutations that lead to cancer. Moreover, lead interferes with ferrous ions in protoporphyrin IX, which is responsible for the formation of heme and cannot be capable of carrying oxygen. These causes stimulate increased oxidative compounds production and thus increase the consumption of glutathione and decrease its level as a result of its participation in the removal of those compounds through direct removal or through its use as a substance for antioxidant enzymes such as glutathione S-transferases (GSTs) and glutathione peroxidase (GPx)(25).

Table 3. The effect of the biochemical parameters for animals treatment with LA compared to the control group during the 30-day treatment period.

Parameters	(Mean ± Standard deviation)	
	Control group	Animals treatment with LA
Glutathione (µm/L)	17.9± 0.15	11.2±0.11**
Malondialdehyde(µm/L)	2.3±0.1	4.8±0.13**
AST (U/L)	50.4±0.3	67.8±0.5**
ALT (U/L)	44.8±0.8	56.5 ±0.3**

** Significant difference at $p \leq 0.001$

On the other hand, it was noted from the results in Table 4 that there were no significant differences between rats treated with LA and ACY1 at a dose (4 mg/kg of rat weight) compared to the control group. This indicates the participation of the isolated ACY1 in protecting the body from these harmful compounds resulting from lead contamination. The results also indicated that there is a significant increase $p \leq 0.001$ in the level of glutathione in the rat serum treated with lead and ACY1 in a dose (4 mg/kg of rat weight) compared to experimental animals treated with lead-only (Table 4 and 5). As to value of glutathione, it returned as in the group of normal animals (Table 4). The high level of GSH value may be due to the effectiveness of ACY1 in removing the acetyl group from N-acetyl methionine to give methionine and producing cysteine from N-acetylcysteine thus increasing its GSH level (20).

Table 4. The effect of the biochemical parameters for animals treatment with LA (40 mg/ kg) body weight and ACY1 isolated from the amniotic fluid (4 mg/ kg) compared to the control group during the 30-day treatment period.

Parameters	(Mean ± Standard deviation)	
	Control group	Animals treatment with LA (40 mg/kg)as well as treated with ACY-1 isolated from amniotic fluid(4 mg/kg)
Glutathione (µm/L)	17.9± 0.15	16.8±0.12
Malondialdehyde (µm/L)	2.3±0.1	2.5±0.14
AST (U/L)	50.4±0.3	52.6±0.7
ALT (U/L)	44.8±0.8	49.9 ±0.4

Table 5. Comparison of the effect of the biochemical parameters for animals treatment by LA (40 mg/kg) with animals treatment by ACY1 isolated from the amniotic fluid (4 mg/kg) during the 30-day treatment period.

Parameters	(Mean ± Standard deviation)	
	Animals treated with LA only	Animals treated with LA as well as treated with ACY-1 isolated from amniotic fluid(4 mg/kg)
Glutathione (µm/L)	11.2±0.11	16.8±0.12**
Malondialdehyde (µm/L)	4.8±0.13	2.5±0.14**
AST (U/L)	67.8±0.5	52.6±0.7**
ALT (U/L)	56.5 ±0.3	49.9 ±0.4**

** Significant difference at $p \leq 0.001$

The results shown in Table 3 indicate that there is a significant increase $p \leq 0.001$ of the MDA level in the serum of rats treated with LA compared to the control group. These results are consistent with those of the previous studies that have indicated a rise in the level of MDA in the serum of rats treated with LA (26). The rise is due to the increased lipid peroxidation, due to the ability of lead to stimulate oxidation and then raise the level of MDA, which is one of the important products of the lipid peroxidation and the resulting interactions of these compounds modulate and toxic within the cells and tissues of animals. Exposure to heavy metals such as lead causes a reduction in the construction of many antioxidants as a result of inhibition of the necessary enzymes in the construction process, such as the gamma- Glutamyl-cysteine synthase and glutathione synthase enzyme, which are involved in the synthesis of glutathione, thus reduces the level of antioxidants that are involved in the process of removing oxidation and increases in turn the oxidation compounds that lead to increased lipid peroxidation process and the production of MDA(27). The results in Table 4 also show that there were no significant differences between rats treated with lead in concentration of 40 mg/kg and the isolated ACY1 at a dose (4 mg/kg of rat weight) compared to the control group, which gives a clear indication that the enzyme worked to protect the body from those harmful compounds resulting from lead contamination and repair damage through the production of antioxidant compounds. It was demonstrated that there was a significant decrease at $p \leq 0.001$ of the MDA level in the rat treated with LA and the isolated ACY1 at a dose (4 mg/kg rat weight) compared to test animals treated with lead-only (Table 5) and returned to the MDA level of control group (Table

4). This decrease in MDA level is due to the effectiveness, role and participation of the ACY1 as an antioxidant, as well as the direct removal of oxidative compounds, causing a decrease in the process of lipid peroxidation and thus a decrease in the MDA level in rat serum. The results in Table 3 manifests increase $p \leq 0.001$ for the of enzymes activity (AST and ALT) in the serum of rats treated with LA compared to the control group. These results correspond to the results of previous studies (23), this rise is due to the fact that exposure to lead leads to accumulation in the liver tissue and causes liver damage and thus leads to the release of enzymes from f the necrosis liver tissue to the bloodstream (25). The release of different substances from the liver and endothelial cells causes blood flow to the tissues, including the liver which is the source of these enzymes, as well as in the heart, muscles and kidneys where increases enzymes activity(28). Moreover, the high level of enzymes activity are associated with an increased oxidative stress as a result of the increased oxidant compounds resulting from the exposure to lead pollutants that can damage liver and heart cells and tissues.

The results shown in Table 4 indicate that there were no significant differences in the enzymes activity (AST, ALT) in the serum of animals with LA lets at a concentration of 40 mg/kg and the isolated ACY1 at a dose (4 mg/kg) compared to the control group. This gives a clear indication that the ACY1 worked to protect the body from those harmful compounds resulting from lead contamination and repair the damage to liver and heart cells and tissues through the production of antioxidant compounds as well as through the production of structural materials for the repairing process. It has been observed that there is a significant decrease $p \leq 0.001$ of the enzyme activity level (AST and ALT) in the serum of rats treated with LA and the isolated ACY1 at a dose (4 mg/kg rat weight) compared to test animals treated with lead-only reductions (Table 5). This is due to the role of the ACY1 in increasing the production of amino acids, especially methionine, which turns into cysteine, which is an antioxidant and has a role in direct removal of oxidative compounds or through being one of the components of glutathione as antioxidant, which protects cells from the toxicity of substances and pollutants.

Effect of ACY1 on the Liver and Kidney Tissue Sections of the Liver and Kidneys of Rats with a Case of Oxidative Stress Caused by LA:

The tissue examination of the liver and kidney treatment at a dose of 40 mg/kg of body weight for

30 days compared to the tissue examination of the control group (Fig. 4a and b) indicate the presence of tissue changes represented by the expansion of the central veins, thrombotic necrosis of the hepatocytes, as well as the presence of focal infiltration of inflammatory cells and congestion of blood vessels in the portal region. This is due to the ability of lead to destroy the cell walls, which helps in the liberation of many chemical media that work to attract inflammatory cells in the hurt zone (29). It has been observed that there is follicular degeneration in the group having LA at a dose of 40 mg/kg of body weight for 30 days, as well as injected with ACY1 in the intraperitoneal at a dose of 4 mg/kg of rat weight, but significantly less severe when compared with the group having lead-only as observed from (Fig. 4c). This results demonstrate the role of the ACY1 separated in order to protect the body from oxidative stress. As previously pointed out, oxidative stress is one of the factors that cause many diseases and lead to damages in cellular materials and changes cellular

compositions and results in increased production of free radicals and reduced availability of antioxidants, as it inhibits the ability of antioxidant enzymes as well as inhibits the action of antioxidants containing thiol group such as glutathione, cysteine, and others, as well as a change in calcium balance and thus activate the process of producing reactive oxygen species, this leads to increased processes of lipid peroxidation and cellular damage by LA(30). One of the products of oxidative processes is the damage of cellular components, which is observed through (Fig. 4 (b)) compared to the form control group (Fig. 4 (a)). However, after the use of the isolated ACY1 and injected with LA, it was noted that it has the ability to improve the condition of the animal by reducing the oxidative processes and toxicity resulting in lead contamination on cells, liver tissues and kidney form (6), by increasing the production of methionine and the production of glutathione, and to preserve the proteins containing the thiol group (31).

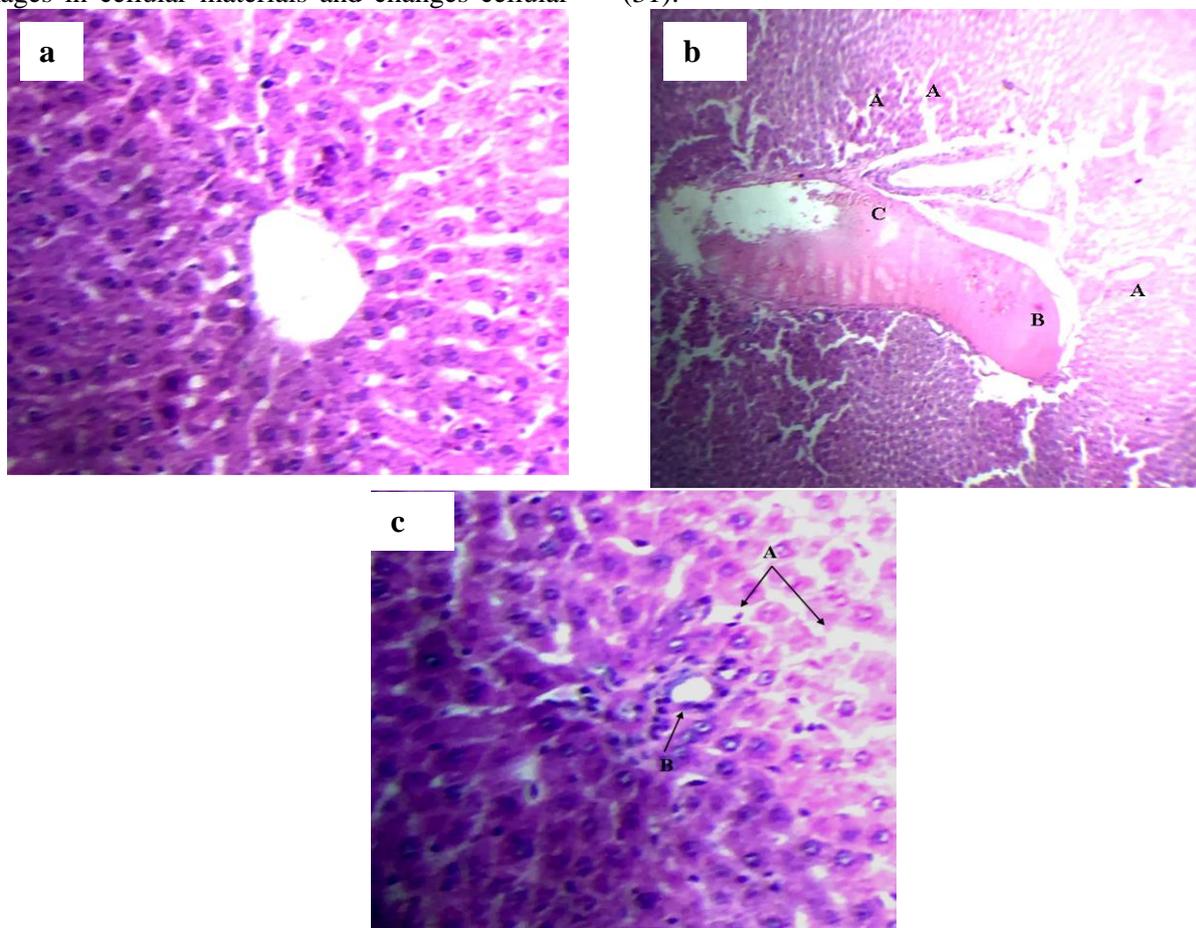


Figure 4. A cross section of the liver tissue, using hematoxylin and eosin dye and 165x magnification power:

- a- Without any treatment, only given regular drinking water for the duration of the trial.
- b- Which treat with LA during the 30-day treatment period, where it is observed: A-Thrombotic necrosis. B-Congestion of the blood vessels in the papyratic area. C- Inflammation of inflammatory cells in the door yard.
- c- Which treat with LA, as well as treatment with aminoacylase-1, at a dose of 4 mg/kg over a period of 30 days, where it is observed: A- Follicular degeneration is much less severe when compared to the group treated with lead acetate only. B- The portal vein expands significantly less frequently when compared to the lead acetate group only.

The results of the kidney tissue examination treated with LA at a dose of 40 mg/kg body weight for 30 days compared to the tissue examination of the control group (Fig.5a and b) indicated the presence of tissue changes represented by bleeding in the interstitial tissue and epithelial cells hyperplasia and the bulge, resulting in narrowing of the lumen of the renal tubule, and degeneration of the cells lining the renal tubules. The shrinkage of the glomerular lumen and the expansion of the Bowman space with the clarity of the glomerular

lumen, are due to the fact that the kidneys are the target organ of lead toxicity, because of their ability to reabsorb and collect lead in cells nearby tubular (31). On the other hand, it has also been observed that there is hemorrhage with vascular congestion in the cortex area of the group treated with lead letters at a dose of 40 mg/kg body weight for 30 days, as well as injected with ACY1 in the intraperitoneal at a dose of 4 mg/kg of rat weight, but much less severely when compared with the treatment group with lead-only decreases as observed from Fig. 5c.

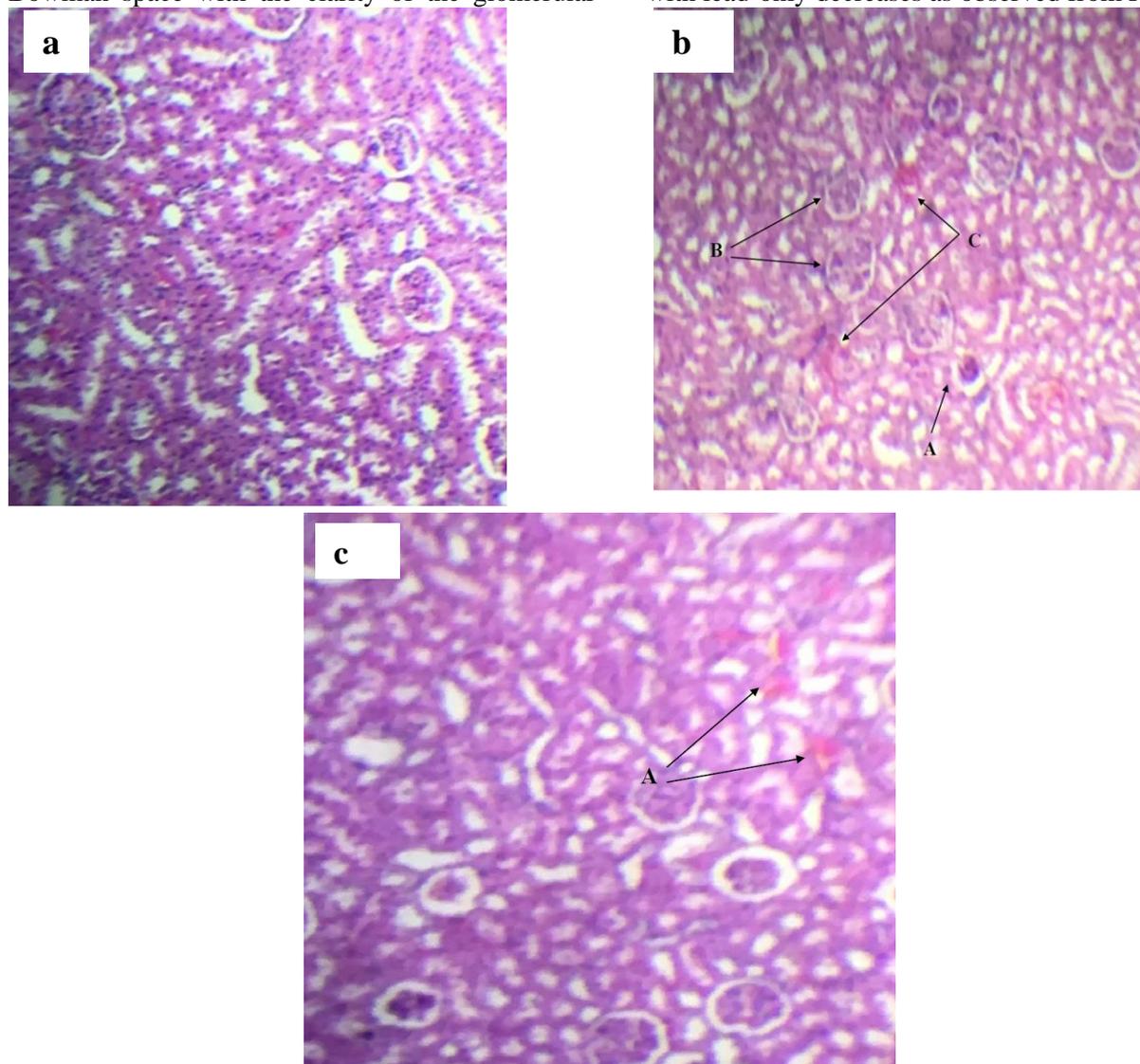


Figure 5. A cross section of the kidney tissue, using hematoxylin and eosin dye and 165x magnification power:

- a-Without any treatment, only was given drinking water for the duration of the experiment.
- b-Which is treat with LA during the 30-day treatment period, where it is observed: A - Shrinkage and curvature of the glomerular, and expand the Bowman's space. B - Hepatitis osteoarthritis. C - Bleeding in the interstitial tissue.
- c- After being treated with LA, as well as treatment with the amniocyclase-1, at a dose of 4 mg/kg over a period of 30 days, as there is bleeding with blood congestion in the crust area (A) but significantly lower when compared with the treatment with lead-only.

Conclusion:

From the results of this study, it is concluded that the role of isolated ACY1 from the amniotic fluid in protecting from oxidative stress is

induced by LA and then can reduce the severity of various diseases.

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Ethical approval:

Animals were used in this study according to institutional, national and international guidelines for the care and use of animals. Also, all ethical standards of Mosul University employed carefully in studies involved animals.

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 re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mosul.

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دراسة كيموحيوية ونسجية لانزيم أمينواسيليز-1 المنقى من السائل الامنيوني في الجرذان المصابين بحالة الإجهاد التأكسدي المستحدثة بخلات الرصاص

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الخلاصة:

تضمن البحث فصل ودراسة إنزيم أمينواسيليز-1 (ACY1) من السائل الأمينيوني للحوامل الاصحاء، إذ تم فصل حزمة رئيسية واحدة فقط ذات فعالية عالية بتقنية التبادل الأيوني السالب نوع DEAE-cellulose للراسب البروتيني الناتج عن ترسيب البروتينات باستخدام كبريتات الأمونيوم (65%) بعد عملية الفرز الغشائي، إذ وصلت عدد مرات التنقية إلى (19) مرة، وعند استخدام عمود الترشيح الهلامي باستخدام سيفادكس من نوع G-100 تم الحصول على نقاوة عالية للإنزيم وصلت عدد مرات التنقية له إلى (38)، وقد وجد أن الوزن الجزيئي للإنزيم المعزولة كان بحدود 46698 دالتون عند استخدام تقنية كروماتوغرافي الترشيح الهلامي. درس تأثير إنزيم ACY1 المعزول على الجرذان المصابين بالإجهاد التأكسدي المستحدث بخلات الرصاص (LA) بجرعة 40 ملغم/كغم من وزن الجرذ وتمت مقارنتها مع الطبيعيين وذلك من خلال قياس المتغيرات الكيموحيوية المختارة والتي تضمنت: الكلوتاتايون (GSH) والمالوندايالديهيد (MDA) وإنزيم اسبارتيت أمينوترانسفيريز (AST) وإنزيم ألانين أمينوترانسفيريز (ALT) وكذلك من خلال الفحص النسيجي للكبد والكلية. إذ أظهرت النتائج ارتفاعاً معنوياً في مستويات (MDA وAST وALT) وانخفاضها في مستوى (GSH) مقارنة مع مجموعة السيطرة السالبة، كما لوحظ أن هناك انخفاضاً معنوياً في مستويات (MDA وAST وALT) وارتفاع في GSH عند حقن إنزيم ACY1 4 ملغم/كغم من وزن الجرذ مع LA بجرعة 40 ملغم/كغم. وأظهرت نتائج الفحص النسيجي وجود تغيرات مرضية عالية لأنسجة الكبد للجرذان المعاملة بمادة LA 40 ملغم/كغم من وزن الجرذان عند مقارنتها مع الطبيعيين، لقد حصل تحسن لأنسجة الكبد والكلية عند استخدام إنزيم ACY1 4 ملغم/كغم من وزن الجرذان مع LA، وهذه النتائج تثبت دور إنزيم ACY1 في الحماية من الإجهاد التأكسدي والتقليل من حدة الأمراض المختلفة.

الكلمات المفتاحية: أمينواسيليز-1، السائل الامنيوني، عزل، خلات الرصاص، الجرذان.