International Journal of Food and Nutritional Science

ISSN: 2377-0619

Review

OMMEGA Publishers

OPEN ACCESS

DOI: 10.15436/2377-0619.19.2416

Copper (I) Nicotinate complex Abrogates Acrylamide Induced Hepatotoxicity in Male Rats: Biochemical and Histological Studies

Nagwa M. Elsawi[1*], Wejdan, A. Aldajani[2], Mounir A. A. Mohamed[1], Ahmed. M. Ali[3], Gamal .S.A[4], Soad Shaker Ali[5], Sabra A. Abd El lah[1]

1Department of Chemistry, Department Faculty of Science, Sohag University, Egypt
2Department of Biochemistry, Department Faculty of Science, Sohag University, Egypt
3Department of Chemistry, Department Faculty of Science, Al-Azhar University, Assiut Branch, Egypt
4Department of Zoology, Department Faculty of Science, Sohag University, Egypt
5Department of Medical Biology, Department Faculty of Medicine, King Abdulaziz University, Jeddah 21551, Saudi Arabia

***Corresponding author:** Nagwa M. Elsawi, Chemistry, Department Faculty of Science, Sohag University, Egypt;
E-mail: elsawinagwa@yahoo.com

Abstract

This study was conducted as a trail to abrogates acrylamide induced hepatotoxicity in male rats by administration of copper (I) nicotinic acid complex. Animals were divided into 4 groups ;GI: control kept on balanced diet and tap water , GII :Acrylamide group received acrylamide in drinking water (7.5 mg/kg b.w.)for 30 days .GIII: treated with copper (I)-nicotinate complex (400 µg/kg b.w.) after acrylamide intoxication for 30 days and GIV the complex then acrylamide (prophylactic). Results showed significant increase in serum levels of AST, ALT,ALP,GGT and total antioxidant capacity (TAC) in acrylamide group. Moreover a significant increase in tumer markers AFU and TNF-Alpha. while Levels of each of S. total protein and S. albumin were significantly decreased in ACR group compared with control. Histological study showed congestion of central vein in liver section and vacuolation of hepatocytes in ACR group. Anticancer activity using in-vitro cytotoxic assay of Cu(I) Nicotinate complex ;proves the anticancer properties of it comparing with doxorubicin .Treatment with Copper (I)-Nicotinate Complex restored tissue and serological indices concomitantly towards normal levels. These results revealed that Copper (I)-Nicotinate Complex is able to significantly alleviate the hepatotoxicity induced by AA in rats and could be utilized as a potent food additives.

Keywords: Cu(I) Nicotinic acid complex; Acrylamide; Anticancer activity and liver functions.

Introduction

Food safety form environmental chemical and biological contamination is a vast subject area for recent researches[1]. Among them, Acrylamide (AA) is a low molecular weight[2], water soluble vinyl monomer figure 1 its odorless, colorless, rapidly polymerizable and crystalline substance[3,4]. Acrylamide is found in carbohydrates-rich food prepared at high temperatures such as potato chips and French fries which are consumed by humans[5]. Its metabolite a glycidamide; an epoxide derivative, is more reactive toward proteins and DNA than acrylamide; the parent compound[6,7]. Acrylamide piles up at higher levels in the blood than any other tissues following exposure via oral ingestion, inhalation, or via the dermis[8]. It is well known that acrylamide was classified as one of the potent genotoxic substance that predispose to cancer development (Group 2A) and this raised the concern that it must be considered a risk for many health problems[9]. Furthermore, It has been shown that AA is neurotoxin[10], gonadtoxic[11] and implicated in carcinogesis in experimental animals.

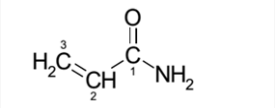


Figure 1: Chemical structure of Acrylamide (AA) with carbon numbers indicated

Received Date: January 22, 2019
Accepted Date: April 22, 2019
Published Date: April 25, 2019

Citation: Elsawi, N.M., et al. Copper (I) Nicotinate complex Abrogates Acrylamide Induced Hepatotoxicity in Male Rats: Biochemical and Histological Studies. (2019) J Food Nutr Sci 6(1): 21- 31.

The experimental research demonstrates that exposure to acrylamide in specific doses and periods may have toxic consequences on animals and humans[12,13]. Occupational exposure to AA and dose of 0.3-2.0 µg/kg b.w. as an average daily consumption were reported in literature. Cytochrome P450 is known to be involved in AA metabolism in the liver changing it to glycidamide , the active genotoxic product of AA[14] which can be abolished by administration of antioxidants for detoxification such effect[15]. Antioxidants were now used to scavenge reactive oxygen species (ROS) inhibit advanced glycation end-products (AGEs) formation and used widely as additives in food industry for such purpose[16]. Copper (I) - Nictitate figure (2) was known for its antioxidant activity and therapeutic uses as anti-inflammatory in modulating gastric ulcer[17], Newcastle diseases[18], reducing hepatotoxicity of 5-Fluorouracil[19] and besides its effectiveness in preventing fatty liver in experimental animals[20].

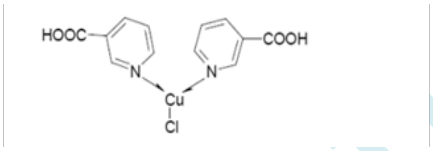


Figure 2: Chemical structure of Cu(I) Nicotinic acid complex.

The present study was designed to evaluate the effectiveness of Cu(I) Nicotinic acid complex in antagonizing AA induced hepatotoxicity in rat based on evidences provided by biochemical ,cell line culture cancer of Cu(l) Nictitate complex and histological study of liver tissue.

Material and Methods

Acrylamide, ≥ 98.0 % (GC), CAS: 79-06-1, MW: 71.08 g / mol, mp: 81-87 ºC and P Code: 101601204 were obtained from SIGMA-ALDRICH chemical Co.

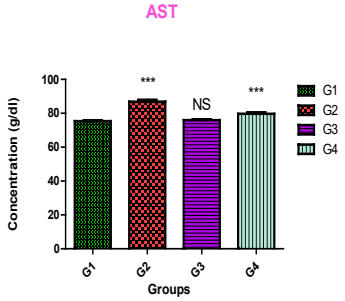


Figure 3a: AST levels in the sera of different groups

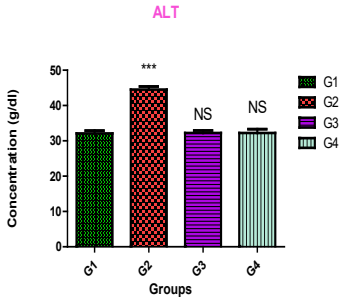


Figure 3b: ALTlevels in the sera of different groups

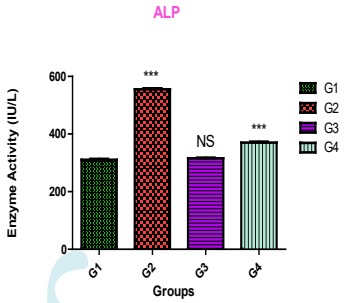


Figure 3c: ALP levels in the sera of different groups

Preparation of Cu (I)- nicotinate complex

The [Cu (I)-(nicotinic acid) $_2$] Cl - complex was prepared accord to method provided previously[21], where nicotinic acid (1.45 g) was dissolved in boiling distilled water (50 ml) and then added to 0.85 g, 40 ml of an ethanol solution of $CuCl_2$–$_2H_2O$. After cooling the mixture, L-(1)-ascorbic acid (0.5 g) was added (at room temperature) to obtain clear orange–red crystals, clear orange–red crystals were obtained. The crystals were purified in ethanol in a boiling water bath for 5 min, an infrared spectrum used to verify the chemical structure as [Cu $(HNA)_2$]1 Cl and pointed to the chemical composition of Cu (I)-(nicotinic acid)$_2$ Cl–$2H_2O$, with 18.4 %. Cu content and 345 Da molecular weight, the daily requirement of Cu is 73.6 mg / kg and 285.2 mg / kg for nicotinic acid as recommended by the National Research Council 2000. Accordingly; the dose used in this study was calculated.

Animals and study design

The experiment was performed upon forty healthy male albino rats, (120-150 gm). Animals were provided by animal house in Sohag University, faculty of science. Rats were kept in the experimental room two weeks before starting the experiment for

Table 1: Effect of acrylamide (7.5 mg/kg b.w) on liver enzyme.

Parameter	G1	G2	G3	G4
AST (U/ml)	75.41± 0.360	86.88±0.995***	75.98±0.509^{NS,a***}	79.71±0.731^{***,b***}
ALT(U/ml)	32.10± 0.794	44.51±0.899***	32.21±0.742^{NS,a***}	32.25±1.062^{NS,b***}
ALP(IU/L)	310.6± 2.876	555.1± 3.598***	315.8± 1.619^{NS}	370.2± 2.761***

Table 2: Effect of acrylamide (7.5 mg/kg b.w) on serum proteins

Parameter	G1	G2	G3	G4
Total protein(g/dl)	6.348± 0.076	5.916± 0.038***	6.222±0.016^{NS,a***}	6.206± 0.037^{NS,b***}
Albumin(g/dl)	4.233± 0.022	3.667±0.058***	4.176±0.0376^{NS,a***}	4.378±0.098^{NS,b***}

acclimatization. They were kept in metal cages under hygienic conditions with free access to standard rat diet .The animals were sorted to 4 groups, (N = 10). The first group (G1) served as control kept on balanced diet and tap water, GII; daily administrated with freshly prepared acrylamide dose of acrylamide (7.5 mg / kg / B.W in drinking water .) for 30 days which is less than lethal dose the LD_{50} of acrylamide in rats (150 mg / kg/ BW.) according to[22], GIII; treated with the Copper (I)-Nicotinate complex (400 µg/kg b.w.) after acrylamide intoxication for 30 days according to[23] and last GIV; Copper (I)-Nicotinate complex then acrylamide (prophylactic).

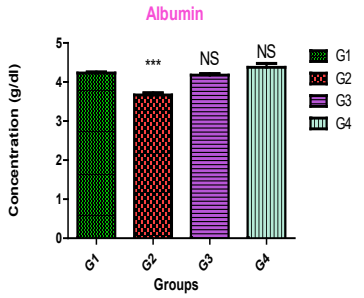


Figure 4a: ALB levels in the sera of different groups

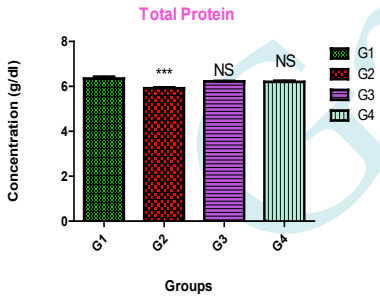


Figure 4b: TP levels in the sera of different groups

Biochemical study

Serum was collected from blood samples taken from the heart of deeply ether anesthetized animals via in plain tubes and centrifuged at 5000 *rpm* for 10 min, stored at - 20°C till analysis .

The biochemical testes included Serum alanine transaminase (ALT), aspartate transaminase (AST)[24], serum alkaline phosphatase (ALP)[25], Albumin[26], Protein[27], g-glutamyl transpeptidase (GGT)[28], AFU[29] and TAC[30] were obtained from (diagnostic kit by Biodiagnostic company, Egyptian company). And Rat Tumor necrosis factor α (TNF-α) ELISA Kit No. 18, Keyuan Road, DaXing Industry Zone, Beijing, China from (biovisioncampony, Egyptian company).

Anticancer activity using *in-vitro* cytotoxic assay

The cells lines that have been used is HepG-2 cells (human Hepatocellular carcinoma) was obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Viability assay using MTT: Cell viability was determined by measuring the mitochondrial activity of live cells by the metabolism of a tetrazolium substrate, 3-(4, 5-dimethyl-2 thiazolyl)- 2, 5-diphenyl-2H-tetrazolium bromide (MTT). The assay is based on the reduction of the yellow, water-soluble MTT to purple water-insoluble formazan crystals in the mitochondria of living cells. During this process, the MTT reagent is reduced by means of NADH in a succinate-dependent dehydrogenase reaction and occurs only in living cells.

Since this reaction can only occur in viable cells, the production of formazan crystals can directly be correlated to cell viability. Accordingly, MTT assay can give a preliminary idea of the impact of a treatment on cell viability and provides therefore general information on cytotoxicity[31].

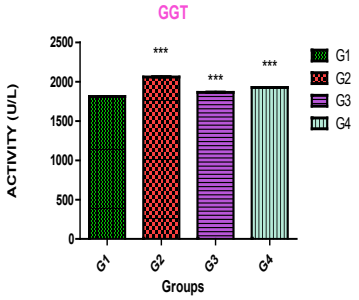


Figure 5a: GGTlevels in the sera of different groups

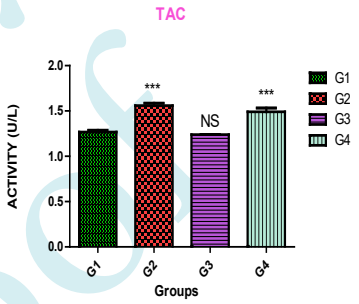


Figure 5b: TAClevels in the sera of different groups

Histological study

Preparation of Liver Section and Histopathological Examination

Liver samples 2 × 2 mm from the main large lobe were thoroughly rinsed by saline, fixed in 10% neutral buffered formalin for further paraffin processing, 0.5 µm thick sections stained with routine haematoxylin and eosin are examined and photographed using digital camera, attached to Olympus CX21 light microscope and connected to computer.

Statistical Analysis

Statistics was performed using the statistical using Graph pad Prism software (San Diego, CA. USA). One way analysis of variables (ANOVA) was used posted by Newman-keuls test. All results are expressed as mean ± SE and the level of significance between groups were *p < 0.05, **p < 0.01, ***p < 0.0001.

Results and Discussion

Hepatotoxicity upon exposure to acrylamide was reported both in human and experimental animals[32,33] that associated with alteration in liver function tests, lipid profile and body antioxidants capacities.

Biochemical study

The present study of acrylamide has focused on its effect on liver function, ALT, AST, ALP, Total protein, Albumin, GGT, TAC, AFU, TNF-Alpha and histological studies.

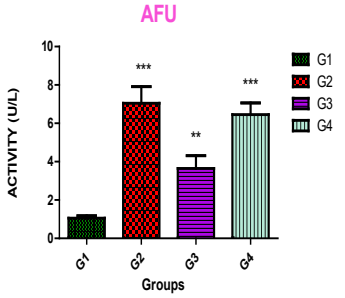


Figure 6a: AFUlevels in the sera of different groups

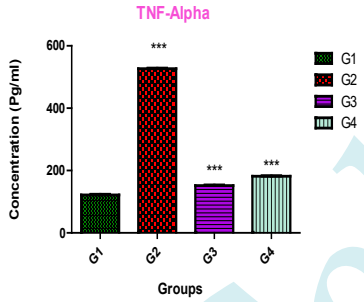


Figure 6b: TNF-Alpha levels in the sera of different groups.

Liver Enzymes: ALT (U / ml), AST (U / ml) and ALP (IU / L): The data in table 1 indicated that AST, ALT and ALP in serum levels were significantly high in G2 (AA group) ($P < 0.001$) when compared to G1:control. This pointed to hepatocyte cellular damage[34] reviewed the mechanism by which AA intoxication may lead to production of free radicles via its epoxide glycidamide produced in the liver by Cytochrome P450. This metabolite which is more reactive and can attack and denature DNA and proteins leading to cellular damage[35].

AA and its byproduct glycid amide were reported to conjugate with glutathione (GSH) in the liver[36] followed by elimination in the urine[37].

This will result in reduction in natural serum level of glutathione thus enhancing the levels of Reactive Oxygen Species (ROS) produced by AA and consequent oxidative cellular damage.

AA and its byproducts were known to cause lipid peroxidation, loss of membranous integrity and further cellular damage due to oxidative stress following reduction and elimination of natural body antioxidant such as Glutathione (GSH) as reported by[36]. The reduction in GSH levels may enhance the levels of reactive oxygen species (ROS), which are widely known as key mediators of cell. After one month, may be attributed to acrylamide that induced free radical production leads to liver damage. On the other hand, the value of liver enzymes in G3 decreased to near the value of control group by administration of Cu-N complex.

Hepato protective drugs[38] and natural antioxidants were used to modulate free radical induced oxidative stress cellular damage via increased the level of glutathione (GSH) and superoxide dismutase (SOD). In the present study, Cu-N complex was selected as a hepato protective owing to its antioxidant properties and radical scavenging effect[23].

Serum Proteins and albumin

The result of Total protein and albumin levels (g / dl) in serum are shown in a Table (2).

The level showed highly significant decrease ($P < 0.001$) in the mean values in AA group compared with the control.

The level of serum total protein is low the range that usually reflect low albumin level and it due to liver disease or acute infection[39]. low levels could reflect impaired in synthesis of protein[40]. The total serum protein or serum albumin levels were highly reduced by acrylamide due to inhibition of protein synthesis in hepatic tissue[41,42].

In the present data it was surprising to observe that total proteins and albumin levels in G3, G4 were no significant in the mean values compared to the control group. Administration of the complex either as protective or curative looked to show improvement that confers a hepato-cellular protective as well as antioxidant effect of copper (I) nicotinate complex[17,19].

But in G3 the total protein was still high, but albumin was increased than control group.

GGT and total antioxidant capacity

In Table (3) there was highly significant increase in GGT(U / L) ($P < 0.01$) in the mean values following acrylamide administration (G2) where in G3 and G4 which received the complex were highly significant decrease in GGT level ($P < 0.001$) compared to AA group with superior effect in G3 where the complex is given as therapeutic agent. The mean value in G3 (Therapeutics) is the best value compared to G4 (Prophylactic) and G3 appeared improvement than G4 compared to the control. Gamma Glutamyl Transferase (GGT) is one of a broad group of enzymes that catalyze the transfer of amino acids from one peptide to another amino acid or peptide. This enzyme is sometimes referred to as a "transpeptidase" but is more appropriately included in the amino acid transferase group[43].

GGT was considered as a cumulative biomarker of various environmental chemicals; and was needed for glutathione (GSH) conjugation and metabolism; a process needed for conjugation diverse toxic chemicals so is recently used as an indicator for exposure to mixed chemicals at very low levels[43]. And also, GGT is as a marker for glutathione depletion in the liver[44]; so this increase reflected the presence of oxidative stress and confirms the presence of biochemical data of increasing liver enzymes denoting hepatocellular necrosis observed in hepatic tissues prepared from the same samples.

GGT levels were found to be correlated with histo pathological alteration in many hepatic disorders[45]. The increase in GGT in the present study was an indicator for damaging effect of AA on rat hepatic tissue this could be based on previous reports that find an increase in GGT levels in both human and animals exposed to hepatitis inducing agents, special medication, or chemical toxicant insult[46,47].

The present results are also in agreement with those in[48]; when liver (but no bone or intestine) ALP is elevated, GGT will be generally elevated[49].

Analysis of TAC concentration (mmol / l) on serum

of both treated and control group were shown in Table (3) and figure (5b) which revealed that the concentration has significant increase ($P < 0.001$) after injected of acrylamide but in G3 showing non-significant compared to the control, but on the other hand in G4 there was highly significant increase compared to the control. High level of total antioxidants in liver serum in G2 may reflect a body defense against oxidative toxicant[50,51]. It is suggested that increase in antioxidant level may be defense mechanism by liver to prevent effect of acrylamide toxicity. But in G3 the concentration of TAC decreased compared to control group. As expected, there was a decrease in the TAC levels after administration of a drug that can be potentially hepatotoxic. However, in G4 there was high significant increase in TAC concentration compared to control and non-significant compared to G2 this may back to defense mechanism.

AFU and TNF-Alpha

The analysis of AFU (U / L) and TNF-Alpha (Pg / ml) in table 4 revealed that the mean value of the two parameters in G2: were highly significantly increased ($P < 0.001$) than control group, but in G3:it was highly significantly decreased and non-significantly decreased in G4 compared with non-treated G2. Alpha-L-fucosidase (AFU) belongs to the lysosome acidic hydrolase group[52]. Hepatic disorders such as hepatotoxicity, cirrhosis , early hepatic cancer and hepatitis are found to be associated with increased serum AFU[53,54], Thus increasing AFU (U/L) in G2 receiving acrylamide could be due to AC induced hepatotoxicity. Mutagenic effect or with subsequent hepatocyte transformation[55]. It was reported in literature that serum level of AFU was beneficial as a diagnostic index for primary hepatic carcinoma[54,56], also[57] said that AFU was a valuable biomarker in HCC detection because it can reveal the case 6 to 9 months earlier than ultra sonographic visualization. AFU in the present study was decreased in G3 treated by Copper (I) Nicotinate Complex that indicated a hepato-cellular protective effect as an antioxidant agent[19], also in G4 it was insignificantly decreased in the mean value compared to G2 which points to its benefit as treatment rather than prophylactic drug.

In the present study ,the elevated in serum concentration of TNF-Alpha in G2 that received ACR points to hepatic tissue damage as TNF alpha can signal apoptotic, necrotic, or aponecrotic cell death or, on the other hand, proliferation and secondary cytokine production[58]. On the other hand, the concentration of TNF-Alpha in G3 was decreased compared to acrylamide group. The primary role of TNF-Alpha is in the regulation of immune cells. TNF-Alpha is able to induce apoptotic cell death, to induce inflammation and to inhibit tumorigenesis and viral replication. So, dysregulation of TNF-Alpha production has been implicated in a variety of human diseases as well as cancer[58-60]. TNF -Alpha can be thought to be crucial for liver injury in acrylamide or its metabolite in experimental rodents[61-63]. It was observed that this toxicity was prevented by administration of natural antioxidant. As Copper (I) Nicotinate Complex was considered as an antioxidant drug[64], so the decrease in TNF-Alpha in G3 and G4 was most probably attributed to this effect.

In conclusion: Acrylamide hepatotoxicity was similar to what was observed previously in literature most probably due to oxidative stress based on elevation of serum markers, on the other hand the above results indicated that treatment by Copper (I) Nicotinate Complex can be used as both therapeutic as well as protective agent against risk of acrylamide hepatotoxicity in susceptible exposed persons.

Data are expressed as mean ± SE

Significant change in comparison between groups: *p<0.05; **p<0.01; ***p<0.001, N.S Non significant ($P > 0.05$).

[a] Significant change in comparison between G1 & G2;

[b] Significant change in comparison between G1 & G3

where :

G1: control kept on balanced diet and tap water;

GII: Acrylamide group received acrylamide in drinking water (7.5 mg/kg b.w.) for 30 days.

GIII: Treated with Copper (I)-Nicotinate complex (400 µg/kg b.w.) after acrylamide intoxication for 30 days;

GIV; Copper (I)-Nicotinate complex then acrylamide(prophylactic)

Anticancer activity using *in-vitro* cytotoxic assay

Copper is a fundamental element for most aerobic organisms especially human[65,66]. Reports show that copper complexes have anti-cancer, anti-inflammatory, antiviral, antifungal and antibacterial properties[67]. Many Cu-complexes have been examined as cytotoxic agents and exhibited anti-cancer activity in several in vitro experiments and a few in vivo tests[66,68]. In the present study, we examined the anticancer properties of a novel Copper (I) Nicotinate Complex on proliferation of HepG2 cells was examined by MTT assay. The anti-proliferative activities of the Copper (I) Nicotinate Complex evaluated against human cancer cell line HepG-2 cells. The cells were treated at concentrations 11000 µg / mL, 5500 µg / mL, 2750 µg / mL, 1375 µg / mL, 687.5µg / mL, 343.75µg / mL, 171.87µg / mL and 85.93µg / mL for 24h. The anticancer drug, Doxorubicin, was used as a positive control .The results demonstrated that Copper (I) Nicotinate Complex decreased the viability of HepG2 cells compared to

Table 3: Effect of acrylamide (7.5 mg/kg b.w) on serum Antioxidant and GGT

Parameter	G1	G2	G3	G4
GGT(U/L)	1810±1.892	2059±1.919***	1863±1.760***,a***	1925±1.531***,b***
TAC(mmol/l)	1.267±0.020	1.559±0.027***	1.239±0.001NS, a**	1.490±0.043***, b NS

Table 4: Effect of acrylamide (7.5 mg/kg b.w) on serum tumer markers

Parameter	G1	G2	G3	G4
AFU (U/L)	1.052 ± 0.125	7.050 ± 0.862***	3.650 ± 0.665**, a**	6.451 ± 0.612***
TNF – Alpha (Pg/ml)	121.9 ± 1.976	526.6 ± 1.867***	151.9 ± 1.549***,a***	181.6 ± 1.383***

doxorubicin which DOX is a cytotoxic anthracycline used successfully for the treatment of several malignancies, such as liver cancer[69,70].

In the current work IC_{50} was 432.92µg / ml as a positive result of anticancer for liver compared to IC_{50} of doxorubicin (106.60 µg / ml).

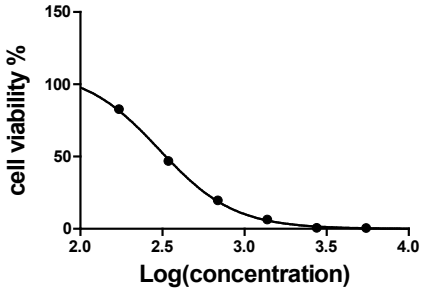


Figure 7: relationship between different doses of cu(I) Nicotinate complex (85.93µg/ml -11000µg/ml) and cell viability of HepG-2 using MTT assay.

In conclusion our search for this complex might produce more specific anticancer effects is ongoing[71] and copper is an essential element for cell growth many drugs used in clinical practice and has ability and display cytotoxicity[72].

Table 5: Anticancer activity using *in-vitro* cytotoxic assay

Cancer cell line	IC_{50} (µM), mean ±SD	
	copper (I)-nicotinate complex	Doxorubicin
Liver —HepG-2	432.91±14.1	106.6 ±17.32

Acryl amide-induced DNA genotoxicity, adduction and mutagenesis

Direct interaction of acrylamide with DNA through Michael-type addition reaction is considerably slow[73], where the interaction of glycidamide with DNA through Michael-type addition is relatively fast as concluded from the semi-empirical MOPAC calculations[74]. The reaction mechanism was suggested to proceed via a preliminary nucleophilic attack of the guanine NH group onto the acrylamide double bond or oxirane ring in glycidamide resulting in the formation of 7-(2-carbamoyl-2-hydroxyethyl)-guanine (N7-GA-Gua), figure 8.

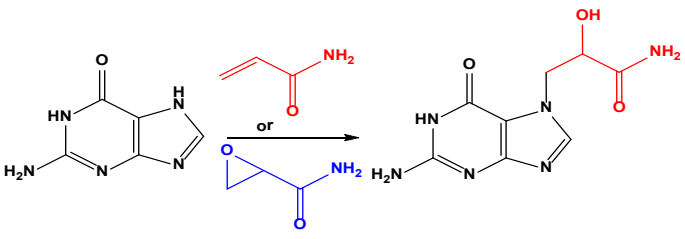


Figure 8: Chemical structures of acrylamide-induced DNA adduct and identified as (N7-GA-Gua).

Treatment with Copper nicotinate complex may alternate this (N7-GA-Gua) bonding system by replacing acrylamide/glycidamide by nicotinic acid, figure 9.

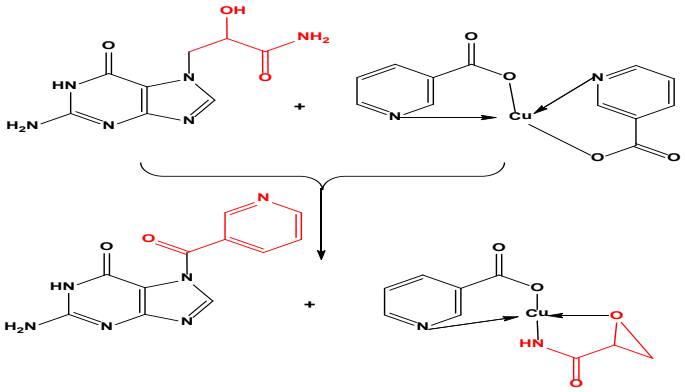
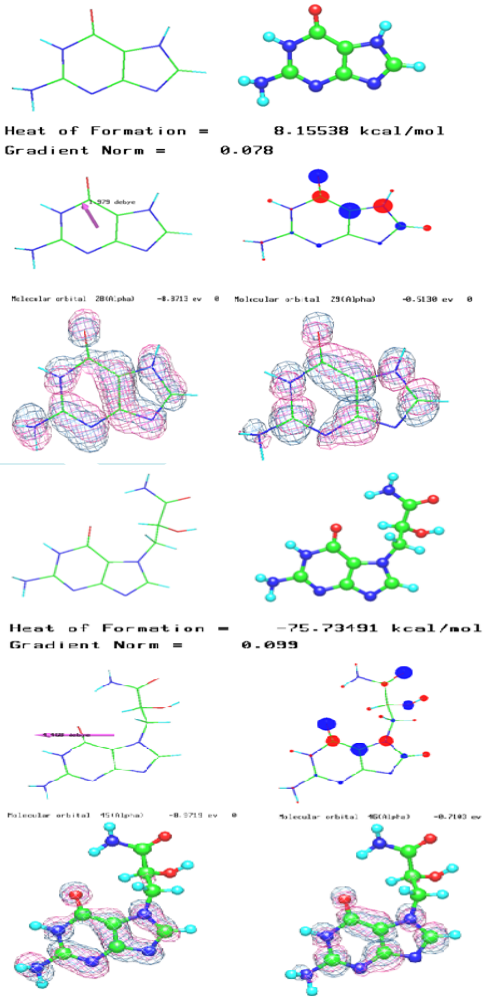


Figure 9: Interaction of Copper nicotinate complex with DNA-acrylamide system.



Frontier molecular orbital analysis: Calculations were performed oh PC computer (Pentium III, 733 MHz) using MOPAC2000[74] with WinMOPAC 2.0[75] as a graphic interface. At the beginning, structure the starting material was optimized with the eigenvector-following routine (EF)[76] using the semi-empirical PM3 method[77,78]. The input file for the geometry optimization of the starting material should contain keywords that request an explanation of the electronic state of the chemical system, what kind of computational study should be performed (method-PM3) and how we can deviate from the stationary point on the potential energy surface (GNORM = 0.1) and it is particularly good when the gradient is small[76].

The frontier orbital (HOMO and LUMO) of acryl amide and/or glycidamide as well as their adducts with N7-GA-Gua part of DNA are very important in defining its reactivity[79,80]. Higher value of HOMO of a molecule has a tendency to donate electrons to good acceptor molecule with low energy, empty molecular orbitals. The highest occupied molecular orbital (HOMO) energies, the lowest unoccupied molecular orbital (LUMO) energies Figure 10, hardness (η), ionization energy (IE), total energy and dipole moment have been calculated and are given in Table (6).

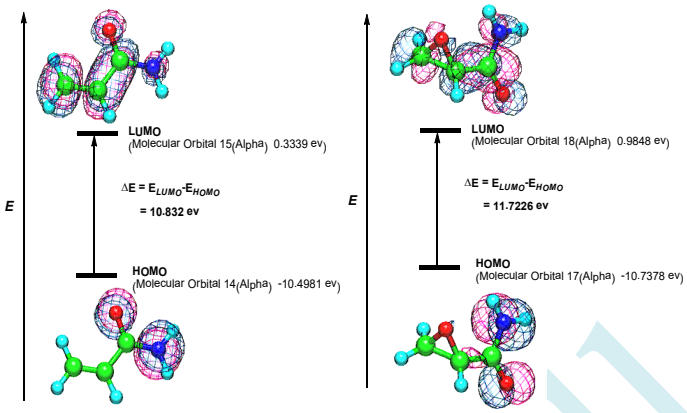


Figure 10: Molecular orbital surface and HOMO-LUMO energy gap for HOMO and LUMO of acrylamide and glycidamide obtained by PM3 semi-empirical method.

Based on PM3 optimized geometry, the total energy of acrylamide and glycidamide has been calculated, which are -24.6087 and -47.3681 Kcal/mol respectively. An electronic system with a larger HOMO-LUMO gap should be less reactive than one having smaller gap[81]. The hardness (η) corresponds to the gap between the HOMO and LUMO orbital energies. The larger the HOMO-LUMO orbital energy gap, the harder the molecule. The hardness has been associated with the stability of the chemical system. In the present study, the HOMO-LUMO gap of acrylamide and glycidamide is 10.832 and 11.7226 eV respectively as shown in Table 6, which clearly indicates that the molecule is very stable. The calculated dipole moment values show that the molecule is highly polar in nature.

The induced DNA adducts suggested of acrylamide and glycidamide, including a predominant adduct, N7-GA-Gua, Figure 11.

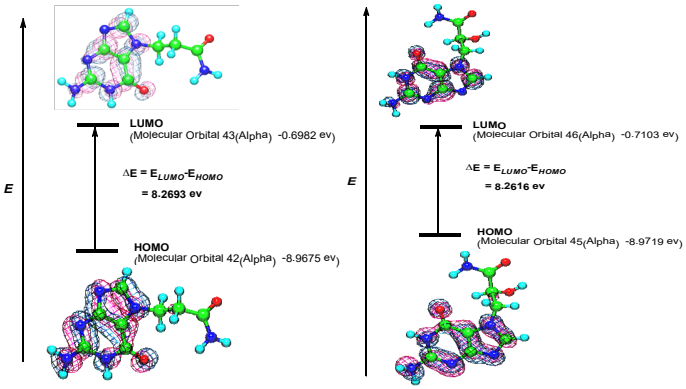


Figure 11: Molecular orbital surface and HOMO-LUMO energy gap for HOMO and LUMO of N7-GA-Gua adduct with acrylamide and glycidamide obtained by PM3 semi-empirical method.

The obtained results were denoted that CuCl $(HNA)_2$ complex undergoes ligand exchange with both acrylamide and glycidamide from DNA and this leads to protection to DNA from damage or mutation.

Histological Results:

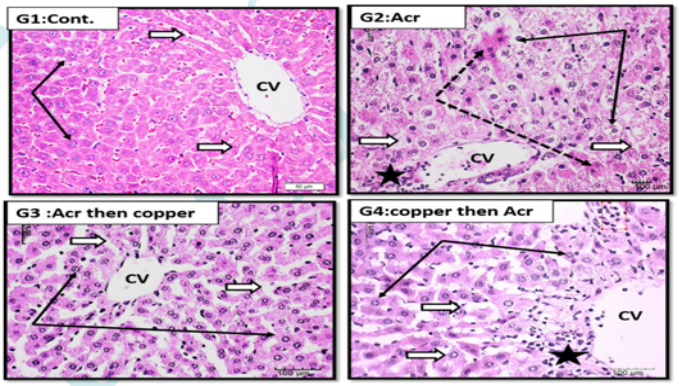


Figure 12:

G1: is a photograph of part of control rat liver at central vein region (CV) showing normal hepatocytes with their acidophilic stained cytoplasm and active vesicular nuclei (black arrows) . They are arranged in the form of cords or plates radiating from the central vein (CV) and separated by thin blood sinusoids (white arrows),

G2: Acr (acrylamide group) in a dose of 7.5 mg kg / BW result in alteration of hepatic tissue. Cells near the central veins underwent process of programmed cell death or what was known as apoptosis where the cells are shrinking and have dark cytoplasm and nuclei. Cells at the periphery underwent hydropic degeneration (dotted arrows) where they lost their outlines and their cytoplasms become unstained (vacuolation). Blood sinusoids are dilated (white arrows).

G3: where rats were given acrylamide for 4 weeks and then treated with copper nicotinate,the tissue showed complete preservation of normal hepatocytes (black arrows).

Table 6:

Comp. No.	ΔH (kcal/mol)	Dipole moment (μ, debye)	E_{HOMO} (ev)	E_{LUMO} (ev)	ΔE (E_{HOMO}-E_{LUMO})	(χ)	(η)	(σ)
Acylamide	-24.6087	3.388	-10.4981	0.3339	10.832	5.0821	5.416	0.1846
Glycidamide	-47.3681	2.428	-10.7378	0.9848	11.7226	4.8765	5.8613	0.1706

(χ) = Absolute Electronegativity, (η) = Global Hardness, (σ) = Softness

G4: where copper nicotinate was given 30 days prior to acrylamide intoxication there was moderate preservation of normal hepatocyte structure(black arrows) .However, there was necrotic changes in hepatocytes located near central veins leaving only aggregation of degenerated nuclei (black stars).

Histological Results

Histological study in the present work was done to give evidences for biochemical results. Compard to cnotrol (Figure 12 –G1) which showed normal hepatocytes with their acidophilic stained cytoplasm and active vesicular nuclei radiating from the central vein(CV) and separated by thin blood sinusoids (white arrows) ;hepatocytes of acrylamide (G-2) administrated rats showed alteration of hepatic tissue. Cells near the central veins underwent process of programmed cell death or what was known as apoptosis where the cells appeared to have dark acidophlic cytoplasm and dark pyknotic nuclei. Other cells are swollen compressing and obliterating blood siusoids. They showed unstained cytoplasm and small dark nuclei (hydropic degneration). In G3 where rats were given acrylamide for 4 weeks and then treated with copper nicotinate histological examination showed complete preservation of normal hepatic parenchyma where hepatocyte showed active vesicular nuclei and normal stained cytoplasm. In G4 where copper nicotinate was given 30 days prior to acrylamide intoxication there was also preservation of normal hepatocyte structure but still there was few necrotic changes in hepatocytes located near central veins leaving only aggregation of degenerated nuclei (black stars). In both treated cases blood siusoids between hepatocyte cell cords looked dilated (white arrows). Corrlation of those results with biochemical finding showed that alteration of hepatocytes strcture by AA was the underlying cause of increasing serum level of liver enzymes. The toxicity of AA is attributed to the fact that it biotransforms to a more potent and highly reactive molecule that initiates cellular toxicity. Therefore, the most important pathogenic pathway is the oxidative biotransformation of AA by cytochrome P450 2E1 (CYP2E1)[14]. Hepatic cytochrome P450 2E1 (CYP2E1) was known to take role in oxidative biotransformation of AA and its exhaustion by repeated toxic exposure may alter liver function and damaged hepatocytes and this on repeated administration may result in altering both structural and functional integrity of mammalian liver as proved by many previous researches[82-84].

Conclusion

Both biochemical and histological study proved the drastic effect of acrylamide on rat liver functions and structure. Which were markedly ameliorated by administration of copper –nicotinic complex.

Funding

We would like to express our sincere thanks to academy of scientific research and technology (ASRT SNG cycle 5) for helping me.

References

1. Erkeoglu, P., Baydar, T. Toxicity of acryl amide and evaluation of its exposure in baby foods. (2010) Nutr. Res Rev 23(2): 323-333.
Pubmed | Crossref | Others
2. Al-Serwi, R.H., Fatma, M.G. The impact of vitamin E against acryl amide induced toxicity on skeletal muscles of adult male albino rat tongue: Light and electron microscopic study. (2015) J Microscopy Ultra structure 3(4): 137-147.
Pubmed | Crossref | Others
3. Sekeroglu, Z.A., Aydın, B., Şekeroglu, V. Argan oil reduces oxidative stress, genetic damage and emperipolesisin rats treated with acrylamide. (2017) Biomed Pharmacother 94: 873-879.
Pubmed | Crossref | Others
4. Lin, C.Y., Lee, H.L., Chen, Y.C., et al. Positive association between urinary levels of 8- hydroxydeoxyguanosine and the acryl amide metabolite N-acetyl-S- (propionamide)-cysteine in adolescents and young adults. (2013) J Hazard Mater 261: 372-377.
Pubmed | Crossref | Others
5. Ghorbel, I., Maktouf, S., Kallel, C., et al. Disruption of erythrocyte antioxidant defense system, hematological parameters, induction of pro-inflammatory cytokines and DNA damage in liver of co-exposed rats to aluminium and acryl amide. (2015) Chem Biol Interact 236: 31-40.
Pubmed | Crossref | Others
6. Barber, D.S., Stevens, S., LoPachin, R.M. Proteomic analysis of rat striatal synaptosomes during acryl amide intoxication at a low dose rate. (2007) Toxicol Sci 100(1): 156-167.
Pubmed | Crossref | Others
7. Martins, C., Oliveira, N.G., Pingarilho, M. Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: correlation with specific glycidamide–DNA adducts. (2007) Toxicol Sci 95(2): 383-390.
Pubmed | Crossref | Others
8. Shipp, A., Lawrence, G., Gentry, R., et al. Acrylamide: review of toxicity data and dose- response analyses for cancer and noncancer effects. (2006) Crit Rev Toxicol 36(6-7): 481-608.
Pubmed | Crossref | Others
9. Ji, K., Kang, S., Lee, G., et al. Urinary levels of N-acetyl-S-(2-carbamoylethyl)- cysteine (AAMA), an acrylamide metabolite, in Korean children and their association with food consumption. (2013) Sci Total Environ 456–457: 17–23.
Pubmed | Crossref | Others
10. Seale, S.M., Feng, Q, Agarwal A.K., et al. Neurobehavioral and transcriptional effects of acryl amide in juvenile rats. (2012) Pharmacol Biochem Behav 101(1): 77–84.
Pubmed | Crossref | Others
11. Ma, Y., Shi, J., Zheng, M., et al. Toxicological effects of acryl amide on the reproductive system of weaning male rats. (2011) Toxicol Ind Health 27(7): 617-627.
Pubmed | Crossref | Others
12. LoPachin, R.M., Balaban, C.D., Ross, J.F. Acryl amide axonopathy revisited. (2003) Toxicol Appl Pharmacol 188(3): 135-153.
Pubmed | Crossref | Others
13. Tareke, E., Rydberg, P., Karlsson, P., et al. Acrylamide: a cooking carcinogen. (2000) Chemical Res Toxicol 13(6): 517-522.

Pubmed | Crossref | Others

14. Semla, M., Goc, Z., Martiniaková, M., et al. Acrylamide: a Common Food Toxin Related to Physiological Functions and Health. (2017) Physiol Res 66(2): 205-217.
Pubmed | Crossref | Others
15. Milenda, S.Y., Kensler, T.W. Detoxication of Chemical Carcinogens and Chemoprevention. (2011) Chemical Carcinogenesis: 59-179.
Pubmed | Crossref | Others
16. Crozier, A., Jaganath, I.B., Clifford, M.N. Dietary phenolics: Chemistry, bioavailability and effects on health. (2009) Nat Prod Rep 26(8): 1001–1043.
Pubmed | Crossref | Others
17. EL-Saadani, M.A., Nassar, A.Y., Soad, H., et al. The protective effect of copper complexes against gastric mucosal ulcer in rats. (1993) Bio chem Pharmacol 46(6): 1011-1018.
Pubmed | Crossref | Others
18. Musa, S.A., Hafez, A.H., Nassar, A.Y., et al. Copper (I) nicotinic acid complex: An immunepoptentiator in chickens vaccinated against New Castle diease. (1987) Biology of Copper Complexes: 343-350.
Pubmed | Crossref | Others
19. Sorenson, J.R.J. Biology of copper complexes. (1987) Humana Press: 343-350.
Pubmed | Crossref | Others
20. Saadani, M.A. A combination therapy with copper nicotinate complex reduces the adverse effects of 5-flourouracil on patients with hepato cellular carcinomas. (2004) J Exp Ther Oncol 4(1): 19-24.
Pubmed | Crossref | Others
21. Salama, R.H., Nassar, A.Y., Nafady, A.A. A novel therapeutic drug (copper nicotinic acid complex) for non-alcoholic fatty liver. (2007) Liver Int 27(4): 454-464.
Pubmed | Crossref | Others
22. Gohar, M., Dratovsky, M. Synthesis and infrared examination of copper (I) halid complex with nicotinic acid and its ethyl ester. (1975) Coll Czech Chem 40: 26-32.
Pubmed | Crossref | Others
23. Zenick, H., Hope, E., Smith, M.K. Reproductive toxicity associated with acryl amide treatment in male and female rats. (1986) J Toxicol Environ Health 17(4): 457-472.
Pubmed | Crossref | Others
24. Ahmed, R., Shatat, H.M., Saad, E., et al. Protective Effects of Copper (I)-Nicotinate Complex against Aflatoxicosis. (2013) The Open Toxicol J 6: 1-10.
Pubmed | Crossref | Others
25. Reitman, A., Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. (1957) Am J Clin Pathol 28(1): 56-63.
Pubmed | Crossref | Others
26. Belfield, A., Goldberg, D.M. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. (1971) Enzyme 12(5): 561-573.
Pubmed | Crossref | Others
27. Doumas, B.T., Watson, W.A., Biggs, H.G. Albumin standards and the measurement of serum albumin with bromcresol green. (1971) Clin Chim Acta 31(1): 87-96.
Pubmed | Crossref | Others
28. Gornal, A.C., Bardawill, C.J., David, M.M. Determination of serum proteins by means of the biuret reaction. (1949) J Biol Chem 177(2): 751.
Pubmed | Crossref | Others
29. Saw, M., Stromme, J.H., London, J.L., et al. International Federation of Clinical Chemistry, (IFCC), Scientific Committee, Analytical Section. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for g- glutamyl transferase [(g-glutamyl)-peptide: amino acid g-glutamyl transferees, EC 2.3.2.2]. (1983) J Clin Chem Acta 21(10): 633-646.
Pubmed | Crossref | Others
30. El-Houseini, M.E., Mohammed, M.S., Elshemey, W.M., et al. Enhanced detection of hepatocellular carcinoma. (2005) Cancer Control 12(4): 248-253.
Pubmed | Crossref | Others
31. Koracevic, D., Koracevic, G., Djordjevic, V., et al. Method for the measurement of antioxidant activity in human fluids. (2001) J Clin Pathol 54(5): 356-361.
Pubmed | Crossref | Others
32. Gomha, S.M., Riyadh, S.M., Mahmmoud, E.A., et al. Synthesis and Anticancer Activity of Arylazo thiazoles And 1, 3, 4-Thiadiazoles Using Chitosan-Grafted-Poly (4-Vinylpyridine) As A Novel Copolymer Basic Catalyst. (2015) Heterocycles 51(11): 1030-1038.
Pubmed | Crossref | Others
33. AL-Mosaibih, M.A. Effects of Monosodium Glutamate and Acryl amide on The Liver Tissue of Adult Wistar Rats. (2013) Life Sci J 10: 35-42.
Pubmed | Crossref | Others
34. Al-Qahtani, F.A., Arapaho, M., Sharma, B., et al. Effects of alpha lipoic acid on acryl amide-induced hepatotoxicity in rats. (2017) Cell Mol Biol 63(6): 1-6.
Pubmed | Crossref | Others
35. Ehsan, Z., Mohammad, S., Marjan, F., et al. A review of acryl amide toxicity and its mechanism. (2017) Pharma Biomed Res 3(1): 1-7.
Pubmed | Crossref | Others
36. Xiaomei, Z., Caob, J., Jiang, L., et al. Protective effect of hydroxytyrosol against acryl amide-induced cytotoxicity and DNA damage in $HepG_2$ cells. (2009) Mutat Res 664(1-2): 64-68.
Pubmed | Crossref | Others
37. Dixit, R., Mukhtar, H., Seth, P.K., et al. Conjugation of acryl amide with glutathione catalysed by glutathione-Stransferases of rat liver and brain. (1981) Biochem Pharmacol 30(13): 1739-1744.
Pubmed | Crossref | Others
38. Riboldi, B.P., Vinhas, Á.M., Moreira, J.D. Risks of dietary acrylamide exposure: A systematic review. (2014) Food Chem 157: 310-322.
Pubmed | Crossref | Others
39. Senthil kumar, M.K., Rajkapoor, B., Kavimani, S. Protective effect of enicostemmalittorale. Against CC_{l4}-induced hepatic damage in rats. (2005) Pharm Biol 43(5): 485–487.
Pubmed | Crossref | Others
40. Sanchez, M.R., Lopez-Toeerjon, G., Pascual, C.Y., et al. Vicilin and convicilin are potential major allergens from Pea. (2004) Clin Exp Allergy 34(11): 1747-1753.
Pubmed | Crossref | Others

41. Ekpenyong, C.E., Akpan, E.E., Udoh, N.S. Phytochemistry and toxicity studies of Telfairiaoccidentalis Aqueous leaves extract on liver biochemical indices in wistar rats. (2012) American Journal of medicine and medical sciences 2(5): 103-110.
Pubmed | Crossref | Others
42. Sharma, R.P. Immunotoxicity of mycotoxins. (1993) J Dairy Sci 76(3): 892-897.
Pubmed | Crossref | Others
43. Abdel-Wahhab, M.A., Nada, S.A., Khalil, F.A. Physiological and toxicological responses in rats fed aflatoxin- contaminated diet with or without sorbent materials. (2002) Anim Feed Sci Tech 97(4): 209-219.
Pubmed | Crossref | Others
44. Lee, D.H., Jacobs, D.R. Is serum gamma-glutamyl transferase a marker of exposure to various environmental pollutants? (2009) Free Radic Res 43(6): 533-537.
Pubmed | Crossref | Others
45. Gerald, K., Stephanie, S. Gamma-Glutamyl transferase: A Predictive Biomarker of Cellular Antioxidant Inadequacy and Disease Risk. (2015) Disease Markers.
Pubmed | Crossref | Others
46. Eminler, A.T., Irak, K., Ayyildiz, T., et al. The relation between liver histopathology and GGT levels in viral hepatitis: more important in hepatitis B. (2014) Turk J Gastroenterol 25(4): 411-415.
Pubmed | Crossref | Others
47. James, E.E., Elizabeth, C.W. Association of γ-glutamyl transferase (GGT) activity with treatment and clinical outcomes in chronic hepatitis C (HCV). (2013) Hepatology 57(5): 1725-1733.
Pubmed | Crossref | Others
48. Mehmet, Z.K., Nurullah, I., Suleyman, O., et al. Oral Amiodarone-induced Liver Injury with Gamma Glutamyl Transferase Elevation. (2018) A Case Report 2(2): 117-119.
Pubmed | Crossref | Others
49. Qusti, S.Y., El-Sawi, N.M. Effect of Nigella sativa L. oil on roridin E toxin administration on liver of male mice. (2007) J Appl Anim Res 31(2): 161-164.
Pubmed | Crossref | Others
50. El-Sawi, N.M., El-wassimy, M.T., Youssef, O.A. Biochemicals studies of verrucarin J in male mice liver. (2000) Toxin Rev 19(3-4): 265-273.
Pubmed | Crossref | Others
51. Harfenist, E.J., Murray, R.K. Plasma proteins, immunoglobulins, and clotting factors. (1991) In: Harper's biochemistry: 611.
Pubmed | Crossref | Others
52. Nabawia, M.I., Ahmed, M.A., Nagwa, M.E., et al. Biochemical and Spectral Analysis of Roridin A Toxin and Copper (I) Nicotinate Complex as Antidote on Male Rat Liver. (2017) J Pharm Appl Chem 3(2): 127-133.
Pubmed | Crossref | Others
53. Zeyu, S., Yize, M., Guohe, L. et al. Alpha-L-Fucosidase Serves as a PrognosticIndicator for Intrahepatic Cholangio carcinoma and Inhibits Its Invasion Capacity. (2018) Bio Med Research Int 25(1-7).
Pubmed | Crossref | Others
54. Gan, Y., Liang, Q., Song, X. Diagnostic value of alpha-L-fucosidase for hepatocellular carcinoma: a meta-analysis [J]. (2014) Tumour Biol 35(5): 3953-3960.
Pubmed | Crossref | Others
55. Tara, B., Sitki, C.M. Biomarkers for Hepato cellular Carcinoma, (2012) Int J Hepatology.
Pubmed | Crossref | Others
56. Besaratinia, A., Pfeifer, G.P. A review of mechanisms of acryl amide carcinogenicity, (2007) Carcinogenesis 28(3): 519-528.
Pubmed | Crossref | Others
57. Guo, W., Li, N., Shi, J., et al. Alpha-1-fucosidase as a prognostic indicator for hepatocellular carcinoma following hepatectomy: a large-scale, long-term study. (2014) Br J Cancer 110(7): 1811–1819.
Pubmed | Crossref | Others
58. Pillai, A.A., Fimmel, C.J. Emerging serum biomarkers of HCC. (2012) Clin Gastro enterol 27: 247-262.
Pubmed | Crossref | Others
59. Pryhuber, G.S., Heidie, L.H., Roper, J.M. et al. Acute Tumor Necrosis Factor--Induced Liver Injuryin the Absence of Tumor Necrosis Factor Receptor Associated Factor 1 Gene Expression. (2005) Am J Pathol 166(6): 1637-1645.
Pubmed | Crossref | Others
60. Osawa, Y., Nagaki, M., Banno, Y., et al. Possible involvement of reactive oxygen species in D-galactosamine-induced sensitization against tumor necrosis factor-alpha-induced hepatocyte apoptosis. (2001) J Cell Physiol 187(3): 374–385.
Pubmed | Crossref | Others
61. LockSleg R.M., Killeen, N., Lenardo, M.J. The TNF and TNF receptor super families : integrating mammalian biology. (2001) cell 104(4): 487- 501.
Pubmed | Crossref | Others
62. Zhang, L., Wang, E., Chen, F., et al. Potential: protective effects of oral administration of allicin on acrylamide-induced toxicity in male mice. (2013) Food Funct 4(8): 1229-1236.
Pubmed | Crossref | Others
63. Zhang, L., Zhang, H., Miao, Y., et al. Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo. (2012) Food Chem Toxicol 50(9): 3306-3312.
Pubmed | Crossref | Others
64. Wang, E.T., Chen, D.Y., Liu, H.Y., et al. Protective effect of allicin against glycidamide-induced toxicity in male and female mice. (2015) Gen Physiol Biophys 34(2): 177-187.
Pubmed | Crossref | Others
65. Halliwell, B., Gutteridge, J.M. Free Radicals in Biology and Medicine. (2015) Oxford University Press.
Pubmed | Crossref | Others
66. Harris, Z.L., Gitlin, J.D. Genetic and molecular basis for copper toxicity. (1996) Am J ClinNutr 63(5): 836–841.
Pubmed | Crossref | Others
67. Santini, C., Pellei, M., Gandin, V., et al. Advances in copper complexes as anticancer agents. (2014) Chem Rev 114(1): 815–862.
Pubmed | Crossref | Others
68. Płotek, M., Dudek, K., Kyzioł, A. Selected copper (I) complexes as potential anticancer agent. (2013) Chemik 67(12): 11-15.

Pubmed | Crossref | Others

69. Ruiz-Azuara, L., Bravo-Gomezm, M. E. Copper compounds in cancer chemotherapy. (2010) Curr Med Chem 17(31): 3606-3615.
Pubmed | Crossref | Others
70. Arcamone, F. Cassinelli, G., Fantini, G., et al. Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from s. Peucetius var. Caesius. (1969) Biotechnol Bioeng 11(6): 1101–1110.
Pubmed | Crossref | Others
71. Maeng, J.H., Lee, D.H., Jung, K.H., et al. Multifunctional doxorubicin loaded superparamagnetic iron oxide nanoparticles for chemotherapy and magnetic resonance imaging in live cancer. (2010) Biomaterials 31(18): 4995-5006.
Pubmed | Crossref | Others
72. Chen, D., Frezza, M., Shakya, R., et al. Inhibition of the proteasome activity by gallium (III) complexes contributes to their anti–prostate tumor effects. (2007) Cancer Res 67(19): 9258-9265.
Pubmed | Crossref | Others
73. Wang, T., Yun, F., Tengfei, H., et al. Copper ion attenuated the antiproliferative activity of Di-2pyridylhydrazonedithiocarbamate derivative; however, there was a lack of correlation between ROS generation and antiproliferative activity. (2016) Molecules 21(8): 1088.
Pubmed | Crossref | Others
74. Friedman, M. Chemistry, biochemistry, and safety of acrylamide. A review. (2003) J Agr Food Chem 51(16): 4504-4526.
Pubmed | Crossref | Others
75. Stewart, J. Mopac Manual, Fujitsu Limited. (2000) Tokyo Japan.
Pubmed | Crossref | Others
76. Mopac, W. User Manual, Fujitsu Limited. (1997) Tokyo Japan.
Pubmed | Crossref | Others
77. Baker, J. An algorithm for the location of transition states. J. (1986) Comp Chem 7(4): 385.
Pubmed | Crossref | Others
78. Stewart, J. J. P. Optimization of parameters for semiempirical methods I. Method. J. Comp. (1989) Comp Chem 10: 209.
Pubmed | Crossref | Others
79. Stewart, J. J. P. (1991) J Comp Chem. 12: 320.
Pubmed | Crossref | Others
80. Fukui, K., Yonezaw, T., Shingu, A. Molecualr Orbital Theory of Reactivity in Aromatic Hydrocarbons. (1952) J Chem Phy 20: 722.
Pubmed | Crossref | Others
81. Huizar- Mendoza, L.H., Reyes-Rios, C.H. Chemical Reactivity of Atrazine Employing the Fukui Function. (2011) J Mex Chem Soc 55(3): 142.
Pubmed | Crossref | Others
82. Kurtaran, R., Sinem, O., Azizoglu, A., et al. Experimental and Computational study on [2, 6-bis (3, 5- dimethyl-Npyrazolyl) pyridine] - (dithiocyanato) mercury (II). (2007) Polyhedron 26(17): 5069-5074.
Pubmed | Crossref | Others
83. Totani, N., Yawata, M., Ojiri, Y., et al. Effects of trace acrylamide intake in Wistar rats. (2007) J Oleo Sci 56(9): 501-506.
Pubmed | Crossref | Others
84. Shler, A.F. M., Kawa A.M. A., Shilan F.M. S. Effect of Acrylamide on Liver and Kidneys in Albino WistarRats. (2015) Int J Curr Microbio App Sci 4(5): 434-444.
Pubmed | Crossref | Others