Egypt. J. Chem. **Vol. 65**, No. 4, pp. 341 – 350, (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Role of Trace Elements as a Cancer Diseases Diagnostic Tools and Melanin for prevention and control

Rasha M. Almutairi[a], Mohammed S. Al-Ayed[a], Mohamed A. Abdelhalim[b] and Magdy M. Ghannam[c,*]

[a] *King Saud University, College of Science, Physics & Astronomy Dept., 11451Riyadh, KSA.*

[b] *Helwan University, Faculty of Science, Biophysics Department, Egypt*

[c] *Cairo University, Faculty of Science, Biophysics Department, 12613 Giza, Egypt*

Abstract

The presented study aims to investigate the beneficial role of melanin for cancer treatment. Moreover, the study aims to assess the concentration of trace elements of zinc (Zn), copper (Cu), magnesium (Mg), iron (Fe), and chromium (Cr) in cancerous and non-cancerous tissues of rat's liver and their role in liver cancer. Forty rats were randomly divided into four groups (n=5 each). Group 1 was taken as normal group. The other groups was treated with DEN and 2-AAF to induce liver cancer. Group 2 was taken as cancerous group. Group 3 after one month it was treated with a single dose of melanin (0.1 g/kg body weight) and animals were sacrificed post one week of melanin treatment (0.1 g/kg body weight) Group 4, it was treated with a single dose of melanin (0.1 g/kg body weight) along with Diethyl nitrosamine (DEN) and 2-Acetylaminofluorine (2-AAF) treatment. Liver and blood were taken from all studied groups. Elements have measured using an inductively coupled plasma mass spectrometry (ICP- MS). The concentration of ingredients was found to be higher in cancer tissues than that of normal. All measured parameters indicating a remarkable improvement in all liver parameters after melanin treatment. Melanin showed that various sources exhibit significant antioxidant activities, and owing to its antioxidant ability reducing DNA damage and oxidative stress by creating a cap cover round the nucleus. It has a role in a stabilization of plasma membrane as well as repair of hepatic tissue damaged caused by the concentration of trace and heavy elements.

Key words, Trace Elements, Cancer Diagnostic, Melanin

1. Introduction

Cancer is considered a leading cause of death in the world. It constitutes about 25% of all the yearly mortalities [1]. Cancer incidence is usually related to many factors that range from behavior, genetic, occupational, nutritional and trace elements. There are a lot of examples of the damage due to metal such as carcinoma, neurological diseases such as Parkinson's disease, cardiovascular disorders, an autoimmune disorder, and skin disorders. The significance of the essential metals is indisputable due to their positive roles at a specific concentration. There are three main predominant mechanisms related to metal genotoxicity: Production of oxidative stress which cause oxidative DNA damage or trigger signaling cascade that may leads to stimulate the malignant growth, the ability to inhibit the major DNA repair mechanisms which may result in genomic instability and accumulation of critical mutations and the ability to inactivate the growth controls such as tumor suppressor genes by induction of signaling pathways [2, 3]. So, trace element concentration are suitable biomarker for the diagnosis of cancer [4].

Instance of colorectal disease with trace elements concentrations in malignant and non-malignant tissues shows that middle groupings of zinc, chromium, copper, and lead in carcinogenic tissues were altogether higher than those of control tissues [5]. However, the median concentrations of iron were lower in malignant tissues than in non-malignant tissues [6-8].

*Corresponding author e-mail: magdighannam@yahoo.com

Receive Date: 29 August 2021, Revise Date: 01 October 2021, Accept Date: 07 October 2021

DOI: 10.21608/EJCHEM.2021.88501.4259

The high serum levels of Fe in colorectal disease might be related to─biochemical alteration and inflammatory status in which a variation in Fe metabolism exists as initiators. Furthermore, inflammation may play an important role in developing cancer. It was reported that the characterization of melanin from a strain of Aspergillus abridger have been examined by Kumar et al., [17], who indicated that melanin exhibits significant free radical scavenging activity. Melanin secures pigmented cells and contiguous tissues by adsorbing possibly hurtful substances, which are then concentrations of copper, iron, Magnesium, and lead Cr, were significantly elevated in cancerous sites of colorectal tissues, while the cadmium level was significantly lower in tumor tissues as compared to normal tissues [7-13-].

A few investigations have presumed that Zn levels are reduced in the serum of people with liver cirrhosis [11, 14, 15]. Cabré et al. [16] concluded that, breast cancer patients showed a significant lower concentration of Cu and Zn than that of control groups. Patients and control groups had similar plasma Fe concentrations. On the other hand, Olaiya et al. [17] concluded that trace element concentrations in cancerous whole blood and malignant breast tissues were significantly or relatively higher than the normal.

Melanin is a well - known biopolymer that is widely distributed in most living organisms which playing an important rules as photo protector, metal ion chelation, antibiotic, anti-inflammatory, thermoregulation, free radical scavenging and some involvement in nervous system [18, 19].

The antioxidant activity and physicochemical characterization of melanin from a strain of Aspergillus abridger have been examined by Kumar et al., [20], who indicated that melanin exhibits significant free radical scavenging activity. Melanin secures pigmented cells and contiguous tissues by adsorbing possibly hurtful substances, which are then gradually discharged in nontoxic focuses [21].

The presented study aims to investigate the beneficial roles of melanin for cancer treatment. Moreover, the study aims to assess the concentration of trace elements of zinc (Zn), copper (Cu), magnesium (Mg), iron (Fe) and chromium (Cr) in cancerous and healthy tissues of rat's liver and their role in liver cancer.

2. Materials and Methods

2.1. Chemicals and drugs

Diethyl nitrosamine (DEN) and 2-Acetylaminofluorine (2-AAF) were purchased from Tokyo Chemical Industry UK Ltd (United Kingdom).

2.2. Animals

Male Wister rats (180-200 g weight and 4-6 weeks old) were obtained from Central Animal House, College of Pharmacy, King Saud University, Saudi Arabia. The animals were housed in cages with free access to laboratory rats diet and tap water, then allowed them to acclimatize for a week before starting the experiment. All the procedures described were reviewed and approved by the king Saud university animal ethics committee.

2.3. Experimental design

The experimental design and treatment protocol was as follows:

Rats were randomly divided into four groups (n=5 in each):

Group 1 (G1): Normal group (n=5).

Group 2 (G2): DEN (200 mg/kg body weight) + 2-AAF (150 mg/ kg body weight), (n=10). The dose of DEN given to rats was administered intraperitoneal. After one week, 2-AAF was given orally three days for three weeks alternatively. Five animals were sacrificed at the end of 30 days.

Group 3 (G3): Isolation 5 rats from G2 after one month to treat them with a single dose of melanin (100 mg/kg body weight), and animals were sacrificed after a week of melanin.

Group 4 (G4): DEN (200 mg/kg body weight) + 2-AAF (150 mg/ kg body weight) + melanin (100 mg/kg body weight), (n=5). The dose of DEN given for rats administered intraperitoneal. After one week, 2-AAF will give orally for three days for three weeks, and after that, was given a single dose of Melanin and animals were sacrificed after a week of melanin.

Liver and blood were taken from all groups, G1, G2, G3, and G4. Trace elements level and liver function criteria were measured [22].

2.4 Blood sampling and tissue separation

Blood samples were collected from the heart from each animal in all groups in sterilized centrifuge tubes for serum separation. Serum was separated by centrifugation at 3000 rpm for 10 min and stored in Eppendorf at −20°C for the different biochemical estimations. Livers were collected through a midline incision, cut into small pieces and immersed in 10% neutral buffered formalin for histological examinations.

2.5 Biochemical analysis

Assessment of serum Alanine transaminase (ALT) and Aspartate aminotransferase (AST) activity were measure using diagnostic kits which purchased from United Diagnostics Industry UDI, KSA.

2.6 Assessment of trace elements levels in liver

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is one of the most important mass spectrometric techniques that have multi-element capabilities for the characterization of samples in materials science. ICP-MS offers extremely high sensitivity for a wide scope of components [23].

For sample preparation, 0.5 g of the homogenized samples were placed in Teflon vessels and dissolved in 7 ml 70% HNO3 + 1 ml 30% H2O2 acid solution heated at 80 °0 for 25 min. Then, 2 ml of deionized water was added, and samples were placed in a microwave digestion system. Solutions were then transferred to volumetric flasks and diluted to 25 ml using deionized water. Solutions were then passed through a 0.45 µm syringe filter. For trace elements analysis, homogenized samples were used directly for analysis [24].

2.7 Statistical analysis

Data are presented as mean ± SE. One-way ANOVA statistical analysis was used to calculate the significant differences of the G1, G2, G3, and G4.

3. Results

3.1. ALT and AST effect:

The activity of *liver* ALT and AST is summarized in table.1 and represented in Fig. 1 and Fig 2 respectively.

It is noticed that, a disturbance in the activity of liver ALT lead to a significant elevation p≤ 0.05 by 56.31±3.2 u/l in G2 as compared to the control group 25.23±1.8 u/l. Treated rats in G2 by melanin recorded Significant decreases in activities of ALT p≤ 0.05 by 50.02±3.05 in G3 compared to the G2. Inject melanin with DEN+2-AAF group G4, at the same time lead to decreases in activities of ALT by 43.81±3.1 compared to the G2. In addition, AST is high significantly increased p≤ 0.05 by 4.04±0.27 u/l u/l in G2 compared to control rats 1.52±0.20 u/l. G3 recorded decreases of AST by 2.83±0.25 u/l compared to G2. G4 has shown significant decreases p≤0.05 by 1.93±0.23 u/l as compared to G2.

3.2. Concentration of trace elements in liver of rats:

Results represented in fig.3 and table.2 showed the Zn concentrations in liver tissues in rats of G1, G2, G3 and G4. The Zn concentration of the G1 rats was 4.04±0.258 µg/g. Zn concentration in the G2 increased significantly p≤ 0.05 by 9.52±0.39 µg/g. The administration of melanin in G3 resulted in decreasing in Zn concentration to 8.32±0.13 µg/g. The administration of melanin in G4 resulted in decreased in Zn concentration to 7.02±0.40 µg/g.

(Table.1): Levels of serum ALT and AST (Mean± SE) in Control (G1), (DEN+2-AAF) (G2), (DEN+2-AAF+ Melanin after five weeks) (G3) and (DEN+2-AAF+Melanin) (G4) in rat liver (n=5).

Groups	Control **(G1)** (n=5)	DEN+ 2-AAF **(G2)** (n=5)	(G2) + Mel after 5 weeks **(G3)** (n=5)	DEN+2 -AAF + Mel **(G4)** (n=5)
ALT (U/L)	25.23 ±1.8	56.31 ±3.2	59.88 ±3.05	48.81 ±3.1
AST (U/L)	1.52 ±0.21	4.04 ±0.35	2.83 ±0.31	1.93 ±0.28

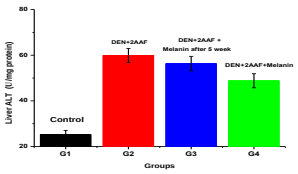

Fig.1: Levels of serum ALT (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

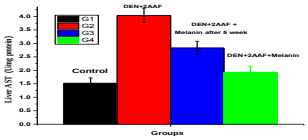

Fig.2: Levels of serum AST (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

Table.2: Concentrations of trace elements (Zn, Mg, Cu, Cr, and Fe) in Control (DEN+2-AAF), (DEN+2-AAF+ Melanin after five weeks) and (DEN+2-AAF+Melanin) in rat liver (n=5).

Groups	Control (G1) (n=5)	DEN +2-AAF (G2) (n=5)	(G2) + Mel after 5 weeks (G3) (n=5)	DEN +2-AAF + Mel (G4) (n=5)
Zn (µg/g)	4.04 ±0.258	9.52 ±0.39	8.32 ±0.13	7.02 ±0.40
Mg (µg/g)	75 ±5.60	117.46 ±14.29	86.82 ±0.34	78.70 ±1.54
Cu (µg/g)	0.90 ±0.07	2.66 ±0.17	2.10 ±0.10	1.50 ±0.13
Cr (µg/g)	1.28 ±0.20	4.34 ±0.14	3.48 ±0.14	2.12 ±0.05
Fe (µg/g)	22.76 ±1.75	39.30 ±4.80	28.58 ±0.36	25.86 ±0.33

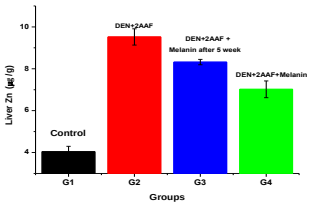

Fig.3: concentration of Zn in the liver of rats (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

As shown in Fig.4 and table.2, there is a significant increase (p≤0.05) in the Mg concentrations in liver tissues in rats by 117.46±14.29 µg/g as compared to the control rats 75±5.60 µg/g. G3 treated rats with melanin recorded decreased by 86.82±0.34 µg/g compared to the G2. In addition, G4 rats have shown a significant decrease (p≤0.05) in the Mg concentrations as compared to G2 by 78.70±1.54 µg/g

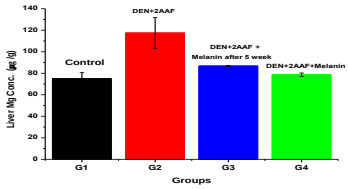

Fig.4: Concentration of Mg in the liver of rats (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

Fig.5 and Table.2 in the G2 have shown a significant increase in the Cu concentrations in liver tissues with p≤0.05 by 2.66±0.17 µg/g as compared to control group 0.90±0.07 µg/g. Treated rats with melanin in G3 recorded a decreased concentration of Cu by 2.10±0.10 µg/g compared with G2. In addition, Melanin co-administered rats in G4 lowered the Cu concentration with p≤0.05 significant difference as compared to G2 by 1.50±0.13 µg/g.

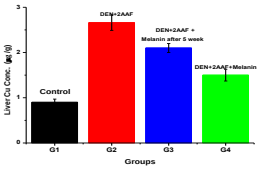

Fig.5: Concentration of Cu in the liver of rats (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

A significant increase of Cr concentrations in liver tissues was recorded after intraperitoneal administration of DEN and 2-AAF in G2 when compared to control rats with p≤0.05, 4.34±0.14 µg/g G2 vs 1.28±0.20 µg/g G1. Treated rats with melanin in G3 recorded a decreased concentration of Cr by 3.48±0.14 µg/g compared with G2. Co-administration of melanin a along with DEN and 2-AAF in G4 efficiently minimized the Cr concentrations comparing with G2 near to the control by 2.12±0.05 µg/g. (Fg.6, Table.2).

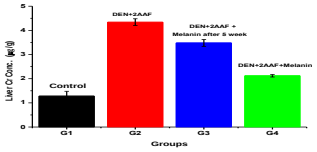

Fig.6: Concentration of Cr in the liver of rats (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

As shown in fig.7 and table.2 there is a significant increase with p≤ 0.05 in the Fe concentration in the G2 rats by 39.30±4.80 µg/g as compared to the control rats by 22.76±1.75 µg/g. Treatment of G2 by melanin (G3) resulted in a significant decrease in Fe concentration with p≤ 0.05 by 28.58±0.36 µg/g as compared with the G2. The melanin co-administered rats in G4 has shown a significant decrease with p≤ 0.05 in the Fe concentrations as compared to G2 rats 25.86±0.33 µg/g.

4- Discussion

The liver plays a major role in the regulation of different physiological processes in the body such as carbohydrate metabolism and glucose storage, bile formation, protein synthesis, hormone production,

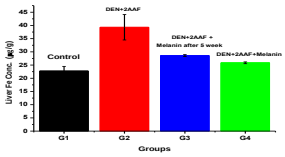

Fig.7: Concentration of Fe in the liver of rats (Mean± SE) in: G1: Control, G2: (DEN+2-AAF), G3: (DEN+2-AAF + Melanin after five weeks) and G4: (DEN+2-AAF+Melanin). Treated rats (n= 5).
Values of * p ≤ 0.05 considered as significant.

besides being the most important organ involved in the detoxification of different drugs in our body [25]. Hepatocarcinomatous is a major health problem in developing countries [10, 26-28].
Appropriate nutrition of all the metabolically active cells and tissues is essential for preserving the health of the human body as a whole. Micronutrients, including trace elements, vitamins, and antioxidants, play a vital role in continuously occurring regenerative processes, coping with ongoing oxidative stress in the body tissues, and sustaining immunity against pathogens [26-30]. Several models have been developed to study multistage carcinogenesis in the liver [31-34].
In this study, the combination of DEN and 2-AAF is used to develop hepatic tumorigenesis in rats. The results showed that a single intraperitoneally dose of DEN to rats induced changes in hepatocytes, and these cells were able to abnormal grow vigorously when used with another carcinogen like 2-AAF as a promoter. The levels of some important biochemical parameters in serum are used as diagnostic markers of liver injury.

ALT and AST activities in serum were used as biochemical markers for hepatic damage, which are sensitive indicators for liver injury are produced by hepatocytes. Changes in cell permeability, hepatocellular degeneration, and inflammation can cause the release of ALT and AST from hepatocytes and subsequent increase of their serum values.
It has been reported that these enzymes (AST and ALT) exhibit higher activity in abnormally functioning liver [35].

Zn plays an anti-carcinogenic role through structural stabilization of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and the ribosome. It is also important in the functions of several transcription factors and proteins that are involved in recognition of specific DNA sequences and regulation of gene transcription. Moreover, It has a protective effect against free-radical injury [36]. Zinc is a component of SOD (superoxide dismutase), an enzyme that removes free radicals,

and since it is also necessary for activating ion of DNA repair enzymes, zinc has the opposite effect and protects against carcinogenesis. The study of Pramoolsinsap et al, [37], reported that serum Zn concentrations were significantly decreased in patients with hepatocellular carcinoma, and changes in Zn status directly affect gene expression. Zn deficiency may also induce oxidative stress and/or modulation of selected signaling in the liver that causes DNA damage [38,39]. Significant increase in Zn in a study by Karlinskiĭ and Bogomolova [40], that there were elevated Zn concentrations in the cases with liver cancer. A result is in accordance with that obtained in the presented study which revealed that excessive Zn administration could lead to liver damage (Zn concentration of G2 was increased in liver tissues compared to control rats).

Fe concentration was increased in liver tissues of the administration of DEN and 2-AAF compared to control rats. Since Fe is a trace element that is physiologically essential, changes in its concentration can lead to oxidative DNA damage, although it is an important nutritional element [41]. A study of Asare et al., [42] have been reported hat over intake of Fe can increase the risk of free radicals to cause further DNA damage, and the present study was in accordance with this report. The Fenton and Haber-Weiss reactions are catalytic amounts of free Fe is enough to generate harmful reactive oxygen species (ROS), disrupting the redox balance of the cell and generating chronic oxidative stress, which damages DNA, lipids, and proteins in hepatocytes and leads to apoptosis of these cells.

Cu concentration was increased in liver tissues compared to control rats. In the previous study, Gel-filtration analyses have shown that the majority of the increased copper exists in the form of Cu-MT (copper-metallothionein), and it is thought that free radicals are generated in the presence of hydrogen peroxide as a result of a Fenton-like reaction and cause hepatitis. Cu is an essential micronutrient involved in fundamental life processes that are conserved throughout all forms of life. The ability of Cu to catalyze oxidation-reduction reactions, which can lead to the production of ROS, and damage of DNA [43]. This was in accordance with my study that explains the effect of an increased Cu concentration in liver tissue.

Mg concentration was increased in liver tissues compared to control rats. Mg is important in stabilizing the structure of DNA and its repair mechanism, act as a cofactor in nucleic acid metabolism, and stability that helps prevent mutations from occurring [44]. In the previous study, Mg deficiency can contribute to the development of an oncogenic milieu by inducing inflammation, oxidative stress, and by inhibiting DNA repair enzymes also linked with increased lipid peroxidation [45, 46]. Mg is necessary for cellular processes of tumor cells; as such, tumor tissue often stores Mg and can lead to low Mg in plasma [45]. The result of this study suggests high intakes of Mg may be associated with an increased risk of the liver disease. Moreover, it showed a significant increase in the level of serum enzyme ALT when compared to control rats, indicating of mediated hepatic damage by increased concentration of Mg, when ALT enzyme leak out from liver into the blood due to tissue damage.

Cr concentration was increased in the liver tissues compared to control rats. Cr is a transition metal; it can be mentioned as the best example when it reacts with DNA causing serious damage to DNA and leads to toxic effects. Cr can generate ROS during its reduction in successive oxidative states. It was found that ROS production has been one of the earliest phenomena for Cr induce cytotoxicity. Therefore, oxidative stress and mitochondrial damage play a role in Cr induced cytotoxicity. These results are supported by previous reports that have shown that Cr induced cytotoxicity in hepatocytes has been mediated through oxidative stress and mitochondrial dysfunction [46-49].

Previous studies have reported that pathogenic mechanism induced by in vivo administration of metal oxide particles is dominated by oxidative stress, cell changes, death, and destruction of DNA [50, 51]. ROS mediated oxidative stress attack DNA and cause DNA damage and, in turn, leads to mutations, genomic instability, and cell death [26-28, 52]. A study showed the damage to hepatocytes caused by changes in concentration of elements, as supported by substantial elevation of serum liver markers in injected rats compared to control rats. Thus, a protective plan geared toward the reduction of the generation of inflammatory, liver damage, and prevent or improve organ dysfunction. In this study, the ingestion of melanin immediately along with DEN and 2-AAF was beneficial in preventing the inflammatory liver injury.

It has shown from this study that Mg is more toxic than all the elements studied, while Cu is considered the least toxic. They are arranged from most toxic to least consecutive: Mg, Fe, Zn, Cr and Cu.

Melanin in G3 and G4 Groups cause a significant decrease in the level of ALT and AST compared to G2, which may be a consequence of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by the concentration of trace elements. Also, melanin induced a significant decrease in trace elements concentrations as Zn, Mg, Cu, Fe and Cr compared to G2. It has also been shown that melanin exhibit significant antioxidant activities [20, 53-55]. Previous studies have shown

that both plant and synthetic melanin can modulate cytokines production and enhance several immune parameters [56] and owing to its antioxidant ability, it reduces DNA damage by creating a cap cover round the nucleus [57,58]. This data was consistent with those published earlier that melanin acts as an antioxidant, confirmed by its ability to stop fatty acid peroxidation [59, 60]. Together with reducing oxidative stress, it also reduces the inflammatory reaction of hepatocytes by preventing the creation of pro-inflammatory cytokines from permeating monocytes, and possibly lymphocytes also. This data might suggest or support the ability to use melanin to significantly reduce the inflammatory liver damage, as observed by the considerable alterations in the liver function markers as well as liver tissue biomarkers observed in this study. This shows that melanin acts as a defense against liver damage and hepatocellular carcinoma HCC.

4. Conclusion

In this study, all measured parameters indicating a remarkable improvement in all liver parameters after melanin treatment. Melanin showed that various sources exhibit significant antioxidant activities, and owing to its antioxidant ability. It has a role in a stabilization of plasma membrane as well as repair of hepatic tissue damage caused by the concentration of trace and heavy elements.

Competing interests

The authors declare that they have no competing interests associated with this manuscript.

Acknowledge

The author acknowledgments the financial support provided by King Abdulaziz City for Science and Technology (General Directorate for Fund and Grants). To King Saud University (KSU) to implement this work through research project No. 1-17-01-001-0067

6. References:

[1] Jemal A, Siegal R, Ward E, Murray T, Xu J, Thun MJ, Cancer statistics. CA Cancer Clin 57 (2007) 43–66.

[2] Ames, B. N., Shigenaga, M. K. & Hagan, T. M, Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Science USA 90 (1993) 7915–7922.

[3] Beyersmann D., A. Hartwig, Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol, 82(8) (2008) 493-512.

[4] Kristina Lossow, Maria Schwarz, Anna Kipp, Are trace element concentrations suitable biomarkers for the diagnosis of cancer? Redox Biology 42(6). (2021): 101900.

[5] Hornik, P., Milde, D., Trenz, Z., Vysloužil, K., & Stužka, V, Colon tissue concentrations of copper, iron, selenium, and zinc in colorectal carcinoma patients. Chemical Papers, 60(4) . (2006) 297-301.

[6] Masoudreza Sohrabi, Ali Gholami, Mohammad Hossein Hosseini Azar, et al, Trace element and heavy metal levels in colorectal cancer: Comparison between cancerous and non-cancerous tissues. Biological trace element research, 183(1) (2018)1-8

[7] C. K. Pusatcioglu, , Nemeth, E., Fantuzzi, G., Llor, X., Freels, S., Tussing-Humphreys, L., and C. Braunschweig, Systemic and tumor level iron regulation in men with colorectal cancer: a case control study. Nutrition & metabolism, 11(1) (2014) 21-.

[8] Drake, E. N., and H. H. Sky-Peck, Discriminant analysis of trace element distribution in normal and malignant human tissues. Cancer research, 49(15), (1989) 4210-4215.

[9] Ganz, T., and E. Nemeth, Hepcidin and iron homeostasis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1823(9) (2012). 1434-1443.

[10] M. A.Mekky, , Hasanain, A. F. A., Abdel-Naiem, M., Orabi, H. H., & A. M. Osman, Trace Elements Status among Patients with Hepatocellular Carcinoma: A Case-Control Study. Ann Digest Liver Dis. 1 (1) (2018) 1004.

[11] Rinaldi, L., Barabino, G., Klein, J. P., Bitounis, D., Pourchez, J., Forest, V., M. Cottier, Metals distribution in colorectal biopsies: New insight on the elemental fingerprint of tumour tissue. Digestive and Liver Disease, 47(7), (2015) 602-607.

[12] Lavilla, I., Costas, M., San Miguel, P., Millos, J., & Bendicho, C. (2009). Elemental fingerprinting of tumorous and adjacent non-tumorous tissues from patients with colorectal cancer using ICP-MS, ICP-OES and chemometric analysis. Biometals, 22(6). (2009), 863-875.

[13] Behzad Kiani1, Fatemeh Hashemi Amin, Nasser Bagheri, Robert Bergquist, Ali Akbar Mohammadi, Mahmood Yousefi, Hossein Faraji, Gholamreza Roshandel, Somayeh Beirami, Hadi Rahimzadeh and Benyamin Hoseini, Association between heavy metals and colon cancer: an ecological study based on geographical information systems in North-Eastern Iran. BMC Cancer (2021) 21:414 – 426.

[14] Goode H.F., Kelleher J., Walker B.E. Relation between zinc status and hepatic functional reserve in patients with liver disease. Gut. 31(1990) 694–697

[15] McClain C., L.C. Su, Zinc deficiency in the alcoholic: A review. Alcohol Clin. Exp. Res. (1983)7:5–10.

[16] N. Cabré, Luciano-Mateo, F., Arenas, M., Nadal, M., Baiges-Gaya, G., Hernández-Aguilera, A., ... & J. Joven, Trace element concentrations in breast cancer patients. The Breast, 42, (2018) 142-149.

[17] David O Olaiya, Olusegun I Alatise, Oyebamiji O Oketayo, Olawale E Abiye, Eusebius I Obianjunwa and Fatai A Balogun, Trace Element Analysis of Cancerous and Non-cancerous Breast Tissues of African Women in Southwest Nigeria Using Particle-Induced X-ray Emission Technique. Breast Cancer: Basic and Clinical Research, 13: 1–6, 2019

[18] Al-Mufarrej SI, Hassib AM, MF Hussein, Effect of Melanin Extract from Black Cumin Seeds (Nigella sativa L.) on Humoral Antibody Response to Sheep Red Blood to Cells in Albino Rats. *J. App. Anim* 29 (2006) 27-41.

[19] A. El-Obeid, Al-Harbi S, Al-Jomah N, A. Hassib, Herbal melanin modulates tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) production. *Phytomedicine* 13 (2016) 324-333.

[20] C.G. Kumar, et al., Physicochemical characterization and antioxidant activity of melanin from a novel strain of Aspergillus bridgeri ICTF-201. *Letters in applied microbiology*, 53(3) (2011) 350–358.

[21] A. S. El-Obeid, Kamal- Eldin, A., Abdelhalim, M. A. K., & A. M. Haseeb, Pharmacological properties of melanin and its function in health. *Basic &* clinical pharmacology & toxicology, *120*(6) (2017) 515-522.

[22] S. Malik, Bhatnagar, S., Chaudhary, N., Katare,D. P., & S. K. Jain, DEN+ 2-AAF-induced multistep hepatotumorigenesis in Wistar rats: supportive evidence and insights. *Protoplasma*, *250*(1) (2013) 175-183.

[23] P. J.Gray, Mindak, W. R., & J. Cheng, Inductively coupled plasma-mass spectrometric determination of arsenic, cadmium, chromium, lead, mercury, and other elements in food using microwave assisted digestion version 1.1. *Elemental Analysis Manual*, (2015). *US Food and Drug Administration*

[24] M.A. Abdelhalim, Atherosclerosis can be strongly influenced by iron and zinc overload or deficiency in the lung and kidney tissues of rabbits. African Journal of Microbiology Research 4(24) (2011) 2748-2753.

[25] C.-C., Huang, Tsai, S.-C. & W.-T. Lin, Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. Experimental gerontology, 43(6) (2008) 571–577.

[26] Deabes MM, Khalil WKB, Attallah AG, El-Desouky TA, Naguib KM. Impact of Silver Nanoparticles on Gene Expression in Aspergillus Flavus Producer Aflatoxin B1. Maced J Med Sci. 6(4) (2018):600-5.

[27] Goda AA, Naguib KM, Mohamed MM, Amra HA, Nada SA, Abdel-Ghaffar AB, Gissendanner CR, El Sayed KA. Astaxanthin and Docosahexaenoic Acid Reverse the Toxicity of the Maxi-K (BK) Channel Antagonist Mycotoxin Penitrem A. Mar Drugs. 9;14 (2008):208.

[28] Gomaa, M.N.E., Ayesh, A.M., Abdel Galil, M.M. et al. Effect of high pressure ammoniation procedure on the detoxification of aflatoxins. Mycotox Res 13 (1997), 23–34.

[29] I. L. C. Chapple, , Brock, G. R., Milward, M. R., Ling, N., & J. B. Matthews, Compromised GCF total antioxidant capacity in periodontitis: cause or effect?. Journal of clinical Periodontology, 34(2) (2007) 103-110.

[31] I. Tamano, S.T. Shirai, A medium-term rat liver bioassay for rapid in vivo detection of carcinogenic potential of chemicals. Cancer Sci 94 (2003) 3–8.

[32] Y.P. Dragan, [30] C. O. Enwonwu, , Phillips, R. S., & J. W. Falkler, Nutrition and oral infectious diseases: state of the science. Compendium of continuing education in dentistry (Jamesburg, NJ: 1995), 23(5) (2002). 431-4.

Campbell HA, Xu XH, C.P. Henry, Quantitative stereological studies of a ‘selection’ protocol of hepatocarcinogenesis following initiation in neonatal male and female rats. Carcinogenesis 18 (1997) 149–158.

[33] T.L. Goldsworthy, Hanigan M.H., H.C. Pitot, Models of hepatocarcinogenesis in the rat contrasts and comparisons. Crit Rev Toxicol 17(1986) 61–89.

[34] D.B. Solt, Medline A, E. Farber, Rapid emergence of carcinogeninduced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. Am J Pathol 88 (1977) 595–618.

[35] R. Simonsen, M.A. Uirji, Interpreting the profile of liver-function tests in pediatric liver transplants. Clin Chem 30 (1984) 1607–1610.

[36] T. Wu, , Sempos, C. T., Freudenheim, J. L., Muti, P., & E. Smit, Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. Annals of epidemiology, 14(3) (2004) 195-201.

[37] C. Pramoolsinsap, Promvanit N, Komindr S, Lerdverasirikul P, S. Srianujata, Serum trace metals in chronic viral hepatitis and hepatocellular carcinoma in Thailand. J Gastroenterol. 29(5) (1994) 610-5.

[38] P. I. Oteiza, , & G. G. Mackenzie, Zinc, oxidant-triggered cell signaling, and human health. *Molecular aspects of medicine*, *26*(4-5), 245-255.*Phytomedicine*, 13(5) (2005) 324–333.

[39] M. K.Mohammad, , Zhou, Z., Cave, M., Barve, A., & C. J. McClain, Zinc and liver disease. *Nutrition in Clinical Practice*, *27*(1) (2012) 8-20.

[40] Karlinskiĭ, V. M., & G. G. Bogomolova, Change in zinc metabolism in malignant neoplasms. Voprosy onkologii, 31(2) (1985) 25-29.

[41] B. N. Ames, DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 475(1-2) (2001) 7-20.

[42] G. A. Asare, Mossanda, K. S., Kew, M. C., Paterson, A. C., Kahler-Venter, C. P., & K. Siziba, Hepatocellular carcinoma caused by iron overload: a possible mechanism of direct hepatocarcinogenicity. *Toxicology*, *219*(1-3) (2006) 41-52.

[43] D. Denoyer, Masaldan, S., La Fontaine, S., & M. A. Cater, Targeting copper in cancer therapy: Copper That Cancer'. Metallomics. 7(2015) 1459–1476.

[44] J. Anastassopoulou, and T. Theophanides, Magnesium–DNA interactions and the possible relation of magnesium to carcinogenesis. Irradiation and free radicals. *Critical reviews in oncology/hematology*, *42*(1) (2002) 79-91.

[45] Y. Song, , Ridker, P. M., Manson, J. E., Cook, N. R., Buring, J. E., & S. Liu, Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older US women. *Diabetes care*, *28*(6) (2005) 1438-1444.

[46] D. E. King, Mainous III, A. G., Geesey, M. E., & R. F. Woolson, Dietary magnesium and C-reactive protein levels. Journal of the American College of Nutrition, 24(3) (2005) 166-171.

[47] N. Soudani, Amara, I. B., Sefi, M., Boudawara, T., & N. Zeghal, Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats. Experimental and toxicologic pathology, 63(6) (2011) 541-548.

[48] A. Patlolla, Barnes, C., Hackett, D., & P. Tchounwou, Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. International Journal of Environmental Research and Public Health, 6(2) (2009) 643-653.

[49] K. J. Liu, & X. Shi, In vivo reduction of chromium (VI) and its related free radical generation. In Molecular Mechanisms of Metal Toxicity and Carcinogenesis, Springer, Boston, MA. (2001) 41-47.

[50] P. Ghosh, Han, G., De, M., Kim, C. K., & V. M. Rotello, Gold nanoparticles in delivery applications. Advanced drug delivery reviews, 60(11) (2008) 1307-1315.

[51] P. J. Borm, Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., K. Donaldson, and J. Krutmann, The potential risks of nanomaterials: a review carried out for ECETOC. Particle and fibre toxicology. 3(1) (2006) 11- .

[52] Yang Xiao zeng , L. Lei, miRDeep-P: a computational tool for analyzing the microRNA transcriptome in plants. Bioinformatics, 27(18) (2011) 2614–2615,

[53] C.-C. Huang, S.-C. Tsai, and W.-T. Lin, Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. *Experimental gerontology*, 43(6) (2008) 571–577.

[54] T.Wu, Sempos, C. T., Freudenheim, J. L., Muti, P., and E. Smit, Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. Annals of epidemiology, 14(3) (2004) 195-201.

[55] V.M. Sava, et al., Isolation and characterization of melanic pigments derived from tea and tea polyphenols. *Food chemistry*, 73(2) (2001)177–184.

[56] A . El-Obeid, Al-Harbi S, Al-Jomah N, A. Hassib, Herbal melanin modulates tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) production. *Phytomedicine*. 13(5) (2006) 324–333.

[57] N. Kobayashi, Nakagawa A, T. Muramatsu, et al. Supranuclear melanin caps reduce ultraviolet induced DNA photoproducts in human epidermis. *J Invest Dermatol*. 110(5) (1998) 806–810.

[58] Montagna W, Carlisle K. The architecture of black and white facial skin. *J Am Acad Dermatol*. 24(6) (1991)929–937.
[59] E. S. Krol, and D. C. Liebler, Photoprotective actions of natural and synthetic melanins. Chemical research in toxicology, 11(12) (1998) 1434-1440.
[60] A. Ezzahir, The influence of melanins on the photoperoxidation of lipids. Journal of Photochemistry and Photobiology B: Biology, 3(3) (1989) 341-349.