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DETECTION AND DETERMINATION OF 4-METHYLIMIDAZOLE IN FOOD SAMPLES CONTAINING CARAMEL PIGMENT

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Abstract

This study was conducted to demonstrate the negative effect of 4-methylimidazole on some health parameters that included liver and kidney enzymes in rats. Forty male rats, aged between 3-4 months and weighing 25 ±, were used in this experiment, divided into 4 groups as follows: Which included (10) animals in each group, the control group was left without any treatment. They were given tap water and diet, and group G1 was dosed with the substance at a concentration of 20 mg/kg, and group G2 was dosed with the substance at a concentration of 40 mg/kg. Group G3 was dosed with the substance at a concentration of 80 mg/kg, for a period of (35) days. The results showed that there was a significant increase in the level of creatinine in the group treated with a concentration of 80 mg / kg of body weight compared with the control group, while the results did not show a significant difference between the control group and the two groups treated with a concentration of 20 and 40 mg / kg. The results also showed a significant increase in the blood urea level in the groups treated with concentrations (20, 40 and 80) mg/kg, and the results also showed a significant increase in (ALP, ALT, AST) enzymes in the groups treated with concentrations (20, 40, 80) mg. /kg,The results showed that there was a significant decrease in the final body weight in the groups treated with concentrations of (40, 80) mg / kg, compared with the initial weight of both concentrations, respectively. While the results showed a significant increase in the final body weight of the control group compared with the initial weight. While the results did not show a significant difference in the initial and final body weight of the treated group with a concentration of (20) mg / kg of body weight. In the histological study of the histological sections, there were changes in hepatitis with infiltration and focal necrosis in the groups (G1, G2, G3) compared with the control group.

Key words: caramel pigment, 4-methylimidazole, creatinine.

Introduction:

Imidazole is an organic compound with the formula C3H4N2. It is a heterocyclic aromatic compound. Its structure consists of an unsaturated pentacyclic ring containing two nitrogen atoms. The imidazole ring is part of the histidine amino acid structure. It is a small heterocyclic compound containing two nitrogen atoms in a pentacyclic ring, and it possesses chemical groups that enable it to act as a general acid or a general base during catalysis.The amino acid histidine contains an imidazole group in its side chain, and also forms part of the theophylline molecule found in tea

leaves and coffee. It has a stimulating effect on the central nervous system, and a histamine inhibitor. Anti-fungal infection compounds are derived from it, the most important of which are ketoconazole, miconazole, clotriazole, It is included in the manufacture of some anti-cancer drugs, especially leukemia, such as mercaptopurine used in the treatment of leukemia, through its interference in the activities of deoxyribonucleic acid (DNA). The potential dangers of 4-Methylimidazol include acute toxicity if swallowed orally, toxic in contact with skin and causing severe skin burns and eye damage. It leads to irritation of the respiratory system, causes drowsiness and dizziness, and leads to cancer (Hamid, 2017). One of the issues facing man today is the health risks due to the use of synthetic materials, proteins, and chemicals in the assembly of food, medicine, beauty care products, and perfumes, without following logical methods and taking into account the permitted quantities, according to international standards (Tripathi, 2007). These chemical compounds may find their direction to the human body directly by adding them to food for the motives behind further developing their quality and expanding the buyer's demand for them.As food is the source of these food additives (Rana et al, 2019). Food additives are characterized as any substance or group of substances, and other basic food components, that are added to food in limited quantities and within the limits permitted globally. These additives are generally used for various purposes
(Al-Awadi et al, 2013). Including preservation, coloring and sweetening, like other food additives, has its advantages and disadvantages. Some examinations showed that many medical problems are related to food coloring (Kasim et al, 2020). Especially those associated with respiratory problems, sleep disorders and memory loss, especially in children (Bateman et al, 2004).

2. Materials and Methods:

2-1 Extraction and detection of methlimidazole using high-performance liquid chromatography (HPLC).

The extraction method was followed for the compound 4-Methlimidozole according to the method described by the scientist (Tae Rang Kim); A volume of (10ml) was taken from food samples and (50ml) of acetonitrile solvent was added to it, and it was placed in a separating funnel and the sample was shaken for 30 seconds, after which the organic layer of acetonitrile was removed and stored in the refrigerator until the analysis was carried out. 4-Methlimidazole was detected in foodstuffs .(Tae Rang Kim others,2013)
The test was conducted in the laboratories of the Ministry of Science and Technology to detect 4-Methlimidazole using high-performance chromatography (HPLC) model SECAM of German origin, where the carrier phase consisting of (methanol, astronitrile, distilled water at a ratio of 70-25-5) was used, at a flow rate of 1 ml / minute ; A C18-ODS column (25 cm * 406 mm) was used to filter the compound and a UV detector with a wavelength of 220 nm was used to detect the substance, and the retention time was relied upon to diagnose the presence of the substance in the samples, and the concentration was calculated by means of the equation

Material

$$\text{concentration} = \frac{\textit{area of the sample x concentration of the standard material} = (\textit{area of the standard material}) \textit{ material concentration of the substance x dilution}}{\textit{sample weight}}$$

Experimental animals:

In this experiment, 40 adult male albino mice of impure strain, aged between 3-4 months and weighing between 23-30 g, were purchased from the Biotechnology Research Center/Al-Nahrain University; They were left to adapt for one week to conduct feeding experiments, then placed in cages with dimensions (25.5 x 19 x 21) cm made of Stainless Steel, then divided into 4 groups (10 per group) as follows:

1- The first group (G): control animals were considered and they were given tap water and standard diet.

2- The second group (G1): was given substance 4 as imidazole at a concentration of 20 mg.

3- The third group (G2): was given substance 4 as imidazole at a concentration of 40 mg.

4- The fourth group (G3): was given substance 4 as imidazole at a concentration of 80 mg.

The duration of the experiment lasted 35 days, from 15/7/2022 to 20/8/2023, and the temperature during the experiment was 20-25 C˚, and the lighting period was not less than 12/day. Free water was used during the experiment period; and the dose was oral by the specialist in the animal house of the Biotechnology Center / Al-Nahrain University

Blood tests:

At the end of the experiment, the animals were fasted for a whole night, after which the mice were anesthetized by chloroform, then their abdominal cavity was opened, and then blood was withdrawn from the heart directly by the method of cardiac puncture using a medical syringe of a size of 3 ml. Then the blood was placed in dry and sterile test tubes, and the tubes containing the blood were left at room temperature for 30 minutes, then the serum was separated using a centrifuge at a speed of 3500 rpm for 15 minutes. The serum was placed in Abendrov tubes and preserved until use, in order to protect it from bacterial and fungal contamination (Gaafar et al, 2010).

Laboratory Kit: Laboratory kits

origin	Kit
Switzerland	**(IL-1B) Elisa Kit**

Sweden	**TNF-a(Tumor Necrosis Factor Alpha)**
USA	**6(IL6) Elisa kit**
Sweden	**Malondialdehyde (MDA)**
USA	**Superooxide Dismutase{Cu-Zn}(SOD1)**
Sweden	**Catalase (CAT)**
USA	**IL,1 my Biosource**
USA	**IL,6 my Biosource**
USA	**TNF my Biosource**
USA	**My Biosource**
USA	**BioVision**

Prepare the standard material:

100 microliters of the highly pure substance were taken and placed in a volumetric vial of 5 ml, and the volume was supplemented with acetonitrile solvent. Where the concentration became 20 ppm and using the law of dilution, 10 ppm of the standard substance was prepared and injected into the HPLC device.

3. Results and discussion:

The standard substance, Methlimidazole, was injected according to the wellestablished conditions to determine its retention time, where the results showed a peak with a retention time of (2.89 min), which refers to the standard compound, Methlimidazole, as in Figure (1).

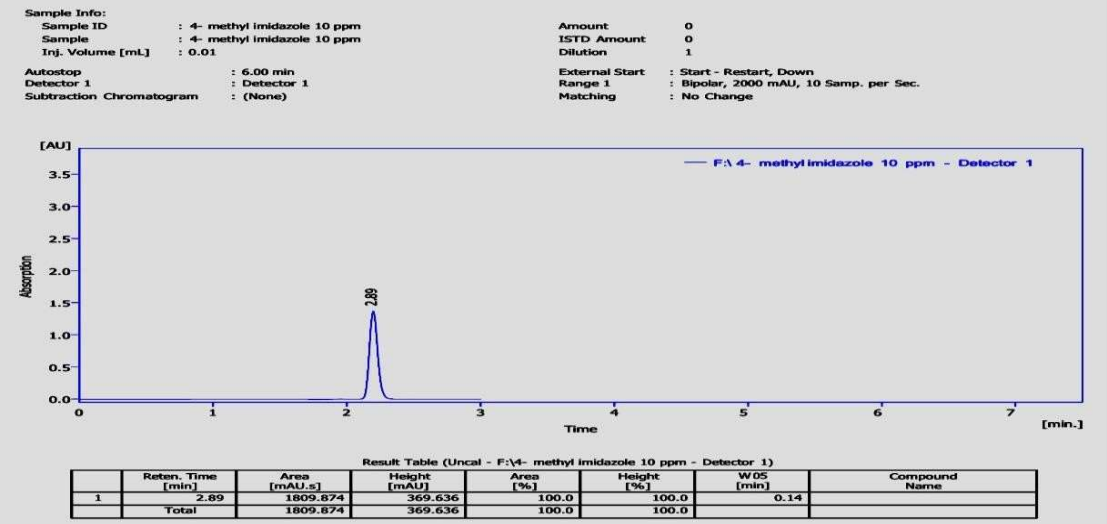

Figure (1) Chromatographic analysis of the standard compound Methlimidazole using HPLC

After collecting samples from the markets and conducting the extraction process according to the scientific literature, the extract of the food samples was injected using HPLC technology.

The results showed that the food samples contained several peaks, and after comparing the retention time of the standard substance with the peaks that appeared in the samples; The results showed that the food models selected in this research contained Methlimidazole-4, as shown in the following figures:

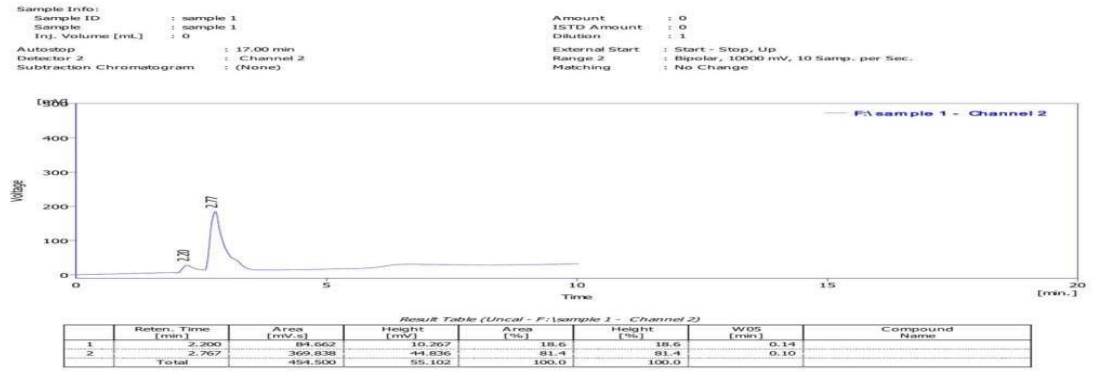

Figure 2: Chromatography of Chipotle Mayonnaise by HPLC

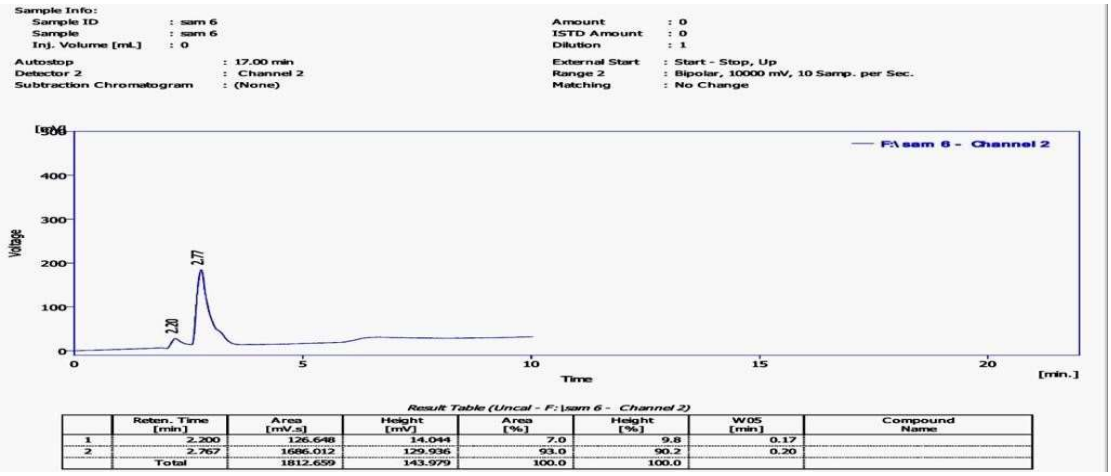

Figure 3: Chromatography in the model (Pepsi Coca Cola) by HPLC

Table (2) Comparison between different treatments in the concentrations of liver enzymes

Mean ± standard error			Treatments
)ALP (IU /L	)ALT (IU /L	)AST (IU /L	
c 8.81± 93.33	c 2.64± 30.00	c 1.73± 22.00	Control
b 10.31± 270.80	b 9.05± 53.80	b 3.61± 60.20	20 mg/ml
ab 13.26± 354.00	a 4.67± 94.40	a 6.69± 107.60	40 mg/ml
a 45.66± 382.00	a 4.49± 91.20	a 6.39± 108.80	80 mg/ml
** 85.62	** 19.668	** 17.489	LSD
0.0001	0.0001	0.0001	P-value
Averages with different letters within the same column are significantly different among themselves. ** (P<0.01).			

The results in Table 2 showed a significant increase in the level of aspartate aminotransferase in the groups treated with a concentration of 20.40 and 80 mg/kg of body weight (60.20 ± 3.61, 107.60 ± 6.69, 108.80 ± 6.39) IU/L compared with the control group (22.00 ±1.73) IU/L on the other hand.The results showed that there was a significant increase in the level of alanine

aminotransferase in the groups treated with a concentration of 20.40 and 80 mg/kg of body weight (53.80 ±9.05, 94.40 ±4.67, 91.20 ±4.49) IU/L compared with the control group (30.00 ±2.64).) IU/L. As for the level of basal phosphatase, the results showed that there was a significant increase in the level of basal phosphatase in groups treated with a
concentration of 20.40 and 80 mg/kg of body weight (270.80 ±10.31, 354.00 ±13.26, 382.00 ±45.66) IU/L.

-2methylimidazole and 4-methylimidazole have been associated with acute toxicity to animals fed commercial grasses or grains; Animals exposed to 2- or 4methylimidazole showed convulsive activity including restlessness, bellowing, foaming at the mouth, and paralysis (Khalid and Mohammad 2007(The ewes fed on hay mixed with ammonia showed facial twitches and general body tremors at first, followed by convulsions; It may lead to death.Neurological signs and convulsive activity have been observed in molasses-fed cattle; Calves suckling from cows fed ammonia-saturated hay ran in circles and could be easily stimulated by noise and touch (Pascal and Other 2017)4 methylimidazole was injected intravenously to goats and calves 20 mg/kg 4-methylimidazole, which resulted in coughing, salivation, urination or defecation within 30 minutes ;Doses 40 to 60 mg/kg caused convulsions or seizures; Oral administration of 4-methylimidazole at doses of 200 mg/kg or more led to the death of calves (Mahmoud, 2018) Absorption was rapid (0.7 - 1 hour) after oral administration of Selesyn (imidazole salicylic acid salt) in tablet and drop form; The proportion of unchanged imidazole detected in the urine was 10-15%, similar to that observed in mice; The major metabolites hyantioic acid and hyandauric acid were below the level of detection in plasma and urine; Therefore, although the metabolic profile of imidazole may be the same as that in rats, it cannot be confirmed; The plasma half-life was less than 3 hours, indicating that the excretion is rapid (Michael F, 2011)Maida, 2018) noted that no imidazole was detected in plasma 4 hours after oral administration of up to 32.7 mg imidazole/kg body weight (administered alone or as the salicylic acid imidazole salt)and 8 hours after ingestion of 66.6 mg imidazole/kg body weight, indicating the rate of excretion is dosedependent and relatively rapid; After systemic absorption ,The results of the study showed that the body initially eliminated the imidazole by renal excretion; A similar elimination pattern can also be expected for orally administered imidazole; Distribution was examined twenty-four hours after intravenous injection of 2-14Cimidazole .Radioactivity was detected in the liver, kidneys, and aorta (associated with elastin) and residual amounts were detected in a number of other tissues (plasma, blood, heart, lung, brain, muscle, skin, and cartilage(These results support the prediction that the imidazole will be well distributed and are consistent with the results of a 90-day repeated-dose toxicity study, in which the liver and kidneys were identified as target organs; No specific information is available on metabolism after oral administration of imidazole.However, within 24 hours of injection of 0.204 mg/kg imidazole body weight, 14% of the radioactivity of the administered dose is excreted in the urine as unchanged product, 39% as hyanttoin, 31% as hydantoic acid and 4% structurally.
In ewes, absorption and elimination of a single oral dose of 4-methylimidazole follows first-order pharmacokinetics; Half an oral dose (20 mg/kg body weight) of 4methylimidazole was absorbed within about 27 minutes, and the maximum plasma level was reached 5 hours after administration

(Karangwa et al., 1990). The bioavailability calculated from plasma data from three ewes was 69%, and the biological half-life was 9.37 hours. Only 0.07 mg/kg dose was recovered in the urine as the major unchanged compound. No metabolites of 4-methylimidazole were detected by high-performance liquid chromatography. In goats and calves, the average residence time for oral or intravenous 4-methylimidazole was approximately 5 hours, and the volume of distribution was 0.9 L/kg body weight. 4-methylimidazole and its metabolites are mainly excreted in the urine, but also in milk and feces, and at the administered dose are distributed mainly in the liver, kidneys and lung. 4methylimidazole was found in milk after oral administration to pregnant and postpartum cows (Moon, 2011). The US Food and Drug Administration (FDA) has conducted carcinogenicity studies in rodents at levels of 4-MI that far exceed current estimates of human exposure to 4-MI from consumption of Class III and Class IV caramel coloring in food products such as cola. In 2012 , The FRA has reassessed consumer exposure to 4-MI from the use of caramel colours, and restated its 2011 conclusion.The EFSA has also noted that 4-MI does not appear to cause DNA mutations (genotoxicity) and that the type of tumors observed in mice from the NTP study can occur spontaneously in these animals (Kumar N and Other, 2011).

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