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RESEARCH ARTICLE

THE ATTENUATION OF MERCURIC CHLORIDE TOXICITY BY FLAVONOIDS IN MALE ALBINO RATS IS INDEPENDENT ON THE NUMBER OF HYDROXYL GROUPS ON B-RINGS

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ABSTRACT

General exposure to mercury is inevitable since it has been shown to be exacerbated through contaminated water and food. The present study aimed to evaluate three different flavonoids and selenite against mercuric chloride ($HgCl_2$) toxicity in male Wistar rats. The rats were randomly divided into ten groups (n=7) as follows: a control group, groups orally administered with 5 mg/kg body weight of either sodium selenite or $HgCl_2$, groups orally treated with 50 mg/kg of body weight morin, naringin, or hesperetin, and groups that were orally co-administered with $HgCl_2$ and sodium selenite, morin, naringin, or hesperetin. All treatments continued daily for two weeks. The $HgCl_2$ toxicity caused significant elevations in the levels/activities of serum total proteins, globulins, total cholesterol, triacylglycerols, tumor necrosis factor-α, interleukin-6, alanine aminotransferase, and γ-glutamyl transpeptidase, as well as hepatic malondialdehyde and catalase. It also caused significant reductions in the hepatic content of reduced glutathione, as well as hemoglobin content and erythrocytes count. Most of these deleterious effects were ameliorated by the concomitant administration of flavonoids or selenite. There was no structure-activity relationship that could be withdrawn from this study. Naringin with the highest number of hydroxyl groups on B-ring and the highest absolute number of hydroxyl groups in general was, with few exceptions, as efficient as the other flavonoids and selenite as well. Every flavonoid had its own biological signature probably due to its metabolism.

INTRODUCTION

Mercury is a highly toxic metal that results in a variety of disorders. Mercury toxicity is associated with acute tubular necrosis and immunologic glomerulo-nephritis, mental retardation, cerebral palsy, seizures[1], arrhythmias and cardio-myopathy[2], and could ultimately causes death. The wide deleterious effects of mercury on humans and animals can be reviewed elsewhere. Exposure to mercury seems to be inevitable. Humans are usually exposed to this toxic element through contaminated water and fish. In addition, mercury is a component of dental amalgams and vaccines[1].

Mercury is absorbed throughout the intestine, recycles through the entero-hepatic system in adults, and is excreted primarily in the feces[3]. Therefore, the liver is a potential target of mercury toxicity. Mercuric ions have a greater affinity to bind to reduced sulfur especially in the thiol-containing molecules like reduced glutathione (GSH), cysteine, and metallothionein[4]. The decrease in free sulfhydryl groups may lead to oxidative stress resulting in tissue damaging effects. Therefore, mercuric toxicity can be ameliorated by antioxidants.

Many of the pharmacological effects of flavonoids are linked to their known biological functions as anti-oxidants, due to free radical scavenging and metal chelating activities[5]. Depending on their chemical structure, flavonoids could fall into one of the following types; flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones[6]. Morin (a flavonol), hesperetin (a flavanone), naringin (a flavanone glycoside) are natural flavonoids that act as antioxidants. The antioxidant activity of flavonoids is sometimes attributed to their hydroxyl groups especially those on B-ring[7].

Selenium (Se) is an essential dietary micronutrient with significant antioxidant properties. Selenocysteine is an integral part of many essential enzymes in humans. Some of these enzymes are glutathione peroxidase (GPx), thioredoxin reductase, deiodinases, and selenoprotein P. However, many of the organic forms of selenium produced contradictory effects on humans and animals. Selenomethionine and seleno-cysteine were shown to be misincorporated into protein instead of methionine and cysteine making selenium biologically unavailable. Some other organic forms are readily disintegrated and excreted[8]. Therefore, sodium selenite, although toxic in high doses, is considered the most effective selenium source[9].

The present study aimed to evaluate the effect of some flavonoids (morin, naringin and hesperetin) and sodium selenite on the hepato-, nephron-, and hemato-toxicity induced by mercuric chloride ($HgCl_2$) in adult male rats. It also aimed to investigate the relationship between the number of hydroxyl groups and the antioxidant activity of flavonoids.

MATERIAL AND METHODS

Chemicals

Morin, hesperetin, naringin, sodium selenite, and $HgCl_2$ were purchased from Sigma (St. Louis, MO, USA), as pure powder.

Animals

Adult male Wistar albino rats (*Rattus norvegicus*) weighing (150 ± 20 g) were obtained from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt). The animals were housed in plastic cages with well-aerated covers at 25 ± 2°C, as well as natural light/dark cycles. Animals were allowed free access to water and were supplied daily with a standard diet. Throughout the experiment, all the procedures and experimental protocols were approved by the ethical committee of the Zoology Department, Ain Shams University (03-2017), and were carried out according to the Guide for the Care and Use of Experimental Animals.

Experimental design

A total number of 70 adult male albino rats were randomly divided into 10 groups (n=7/group) as follows: a control group (group 1), groups 2 and 6 were orally administered with 5 mg/kg body weight of either sodium selenite or $HgCl_2$, respectively[9,10]. Groups 3, 4, and 5 were orally treated with 50 mg/kg body weight of morin, naringin, or hesperetin, respectively[11-13]. Animals in groups 7, 8, 9, and 10 were orally administered with sodium selenite, morin, naringin, or hesperetin, respectively (using the same doses indicated above), two-hours after the administration of $HgCl_2$. All treatments continued daily for two weeks.

Blood collection and sample analysis

At the end of the experiment, animals were killed by decapitation and the blood samples were collected in dry clean centrifuge tubes and allowed to clot for 30 min on ice then blood was centrifuged at 2300 ×*g* for 10 min at 4ºC. Then the serum was immediately separated and stored at -80°C till the determination of biochemical parameters. Other blood samples were obtained in heparinized tubes for the determination of hematological parameters.

Tissue preparation and sample analysis

The liver was perfused through the hepatic portal vein using isotonic cold saline, excised and blot dried. A half gram of the perfused liver was homogenized in four ml of the appropriate buffer and the supernatant was separated and frozen at −80ºC until assayed.

Determination of hepatic oxidative stress parameters

Determination of hepatic GSH and malon-dialdehyde (MDA) levels, as well as catalase (CAT) and glutathione S-transferase (GST) activities, was carried out using Bio-diagnostic kits (Cairo, Egypt), following the instructions of the manufacturer**.**

Serum biochemical analyses

Total cholesterol (TC), triacylglycerols (TAGs), total protein, albumin, urea and creatinine levels, in addition to alanine aminotransferase (ALT) and γ-glutamyl transferase (γ-GT) activities, were estimated in serum using commercial kits (Spectrum diagnostics, Egypt). Serum levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were quantitatively determined by ELISA using KOMA Rat ELISA Kit (Cairo, Egypt), following the instructions of the manufacturer.

Hematological examinations

Red blood corpuscles (RBCs) count, hemoglobin (Hb) content, hematocrit (HCT) value, white blood cells (WBCs), platelets count, mean platelet volume (MPV) were estimated using the coulter counter (Hemat 8 analyzer, SEAC, Germany). Blood indices: mean corpuscular volume (MCV) and red blood cell distribution width (RDW) were then calculated.

Statistical analysis of data

Results were expressed as mean ± standard error (n=7). The distribution of data was tested by the Kolmogorov-Smirnov test. Statistical analyses were performed using One-Way Analysis of Variance (ANOVA). Different group comparisons were performed using Tukey's post-hoc test. Differences were considered significant at $P<0.05$.

RESULTS

The ALT and γ-GT activities were significantly increased ($P<0.001$) in the group treated with $HgCl_2$, as compared to the control group (Table 1). These elevations of ALT activity were abolished upon concomitant administration of morin ($P<0.05$), naringin ($P<0.05$) or hesperetin ($P<0.001$), as compared to the $HgCl_2$-treated group. Similarly, the elevations in activity of γ-GT were prevented by the concurrent administration of selenite or flavonoids ($P<0.001$, as compared to the $HgCl_2$-treated group).

Administration of $HgCl_2$ caused a significant reduction of GSH level when compared to the control group. Concomitant administration of sodium selenite with $HgCl_2$ caused a significant increase ($P<0.01$) in GSH level when compared to the $HgCl_2$-treated group. Co-administration of all flavonoids with $HgCl_2$ did not cause any significant change in GSH content, as compared to the $HgCl_2$-treated group (Table 1). In the current study, GST activity was not significantly affected by any treatment (Table 1). Treatment of rats with $HgCl_2$ resulted in a significant ($P<0.05$) elevation in the CAT activity compared to the control group. Co-administration of selenite or flavonoids did not cause any significant change in the enzyme activity, as compared to the $HgCl_2$ group (Table 1). Although naringin and hesperetin elevated

significantly the CAT activity in normal animals, yet the elevations in the enzyme activity in intoxicated rats they caused did not achieve a statistical significance compared to the $HgCl_2$-treated group.

Treatment of rats with $HgCl_2$ resulted in a significant (P<0.001) elevation in the MDA level, as compared to the control group. These high levels of hepatic MDA were significantly abolished with the co-administration of selenite (P<0.001), or flavonoids (P<0.01) as compared to the $HgCl_2$-intoxicated group (Table 1).

Table 1: Effect of $HgCl_2$, selenite, and flavonoids on liver functions and hepatic oxidative stress parameters in male albino rats.

	ALT (U/L)	γ-GT (U/L)	GSH (mmol/g tissue)	GST (U/g tissue)	CAT (U/g tissue)	MDA (nmol/g tissue)
Control	112.2±6.3	39.1±1.9	0.27±0.01	15.5±0.3	7.1±1.0	30.5±1.0
Sodium selenite	107.4±1.8	50.5±2.4	0.14±0.03	15.5±0.6	15.5±1.0	33.5±3.1
Morin	113.1±6.4	28.5±3.7	0.17±0.01	15.9±0.3	16.4±1.7	36.1±2.9
Naringin	137.2±8.2	29.2±2.6	0.23±0.06	15.9±0.3	31.7±2.5[a]	37.6±4.7
Hesperetin	111.8±1.9	38.9±3.5	0.34±0.07	14.8±0.4	24.4±2.0[a]	31.2±2.8
$HgCl_2$	185.7±5.6[a]	72.3±6.2[a]	0.04±0.01[a]	18.6±3.1	24.3±1.9[a]	91.7±3.9[a]
$HgCl_2$+ Selenite	178.9±6.1	46.5±4.9[b]	0.46±0.06[b]	15.7±0.1	17.5±2.0	65.9±7.6[b]
$HgCl_2$+Morin	158.2±5.1[b]	43.4±3.4[b]	0.12±0.01	16.1±0.1	18.9±1.4	44.8±2.7[b]
$HgCl_2$+Naringin	159.6±5.8[b]	43.6±2.6[b]	0.17±0.01	16.3±0.3	29.4±5.0	29.8±2.4[b]
$HgCl_2$+Hesperetin	130.1±2.9[b]	42.4±3.9[b]	0.22±0.06	13.8±0.8	30.5±7.4	29.8±1.7[b]

Data were expressed as mean ± standard error (n=7). ALT: alanine aminotransferase, γ-GT: gamma glutamyl transpeptidase, GSH: reduced glutathione, GST: glutathione S-transferase, CAT: catalase, MDA: malondialdehyde. [a]P<0.05: significant difference *versus* the control group, [b]P<0.05: significant difference *versus* the $HgCl_2$-treated group.

Treating animals with $HgCl_2$ resulted in a significant (P<0.001) increase in serum total protein and globulins levels in comparison to control group (Table 2). Co-administration of selenite, or flavonoids with $HgCl_2$ reduced significantly (P<0.001) the serum total protein and globulins levels, as compared with the $HgCl_2$-treated group. Although no treatment affected the albumin level, the albumin/globulins (A/G) ratio was significantly (P<0.001) decreased after intoxication with $HgCl_2$ compared to the control group. This reduction of A/G ratio was significantly (P<0.001) prevented when $HgCl_2$ was co-administered with selenite, or flavonoids when compared with the $HgCl_2$-treated group (Table 2).

The serum TC and TAGs levels were significantly (P<0.001) increased after intoxication with $HgCl_2$, as compared to the control group (Figure 1). The serum TC level was significantly decreased in the $HgCl_2$-intoxicated groups co-administered with selenite or hesperetin (P<0.01), morin (P<0.001), or naringin (P<0.05), compared to the $HgCl_2$-treated group. Besides, co-administration of $HgCl_2$ with selenite, or flavonoids caused significant (P<0.001) reductions in serum TAGs level when compared with the $HgCl_2$-treated group.

Table 2: Effect of $HgCl_2$, selenite, and flavonoids on protein profile in male albino rats.

	Total Protein (g/dL)	Albumin (g/dL)	Globulins (g/dL)	A/G Ratio
Control	8.50±0.80	3.46±0.37	5.03±0.62	0.71±0.09
Sodium selenite	7.57±0.79	3.15±0.19	4.41±0.83	0.67±0.12
Morin	9.27±0.85	3.16±0.30	6.11±0.67	0.53±0.06
Naringin	8.32±0.56	3.16±0.30	4.83±0.42	0.73±0.04
Hesperetin	7.14±0.61	3.64±0.13	3.47±0.54	1.15±0.17[a]
$HgCl_2$	14.07±2.06[a]	2.92±0.22	11.15±1.62[a]	0.26±0.03[a]
$HgCl_2$+Selenite	7.10±1.09[b]	3.23±0.36	4.68±0.66[b]	0.72±0.08[b]
$HgCl_2$+Morin	7.72±1.02[b]	3.37±0.29	4.29±0.97[b]	0.59±0.06[b]
$HgCl_2$+Naringin	9.68±1.07[b]	2.91±0.46	6.76±1.24[b]	0.60±0.09[b]
$HgCl_2$+Hesperetin	8.64±0.78[b]	3.02±0.29	5.62±0.55[b]	0.54±0.04[b]

Data were expressed as mean ± standard error (n=7). A/G: albumin/globulin. [a]P<0.05: significant difference *versus* the control group, [b]P<0.05: significant difference *versus* the $HgCl_2$-treated group.

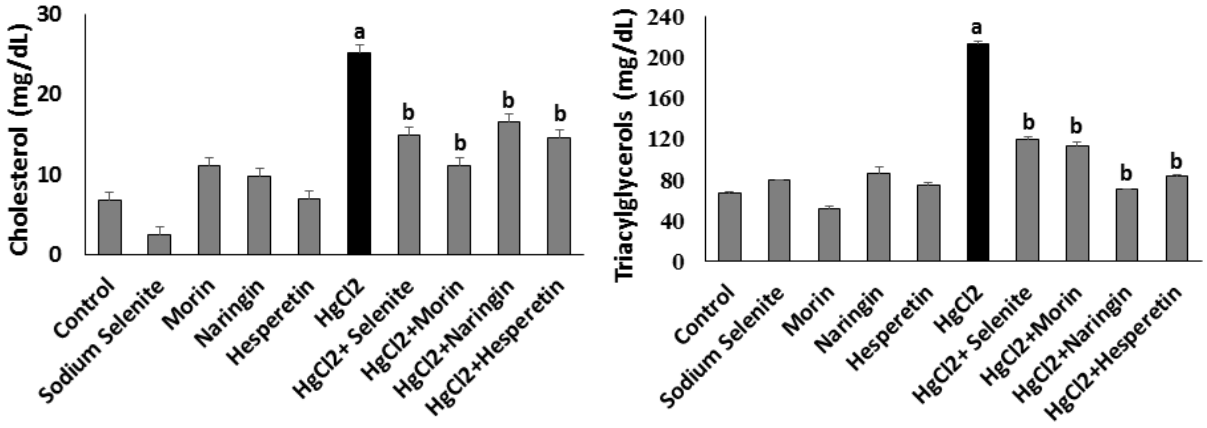


Figure 1: Effect of $HgCl_2$, selenite, and flavonoids on serum total cholesterol (mg/dL) and triacylglycerols (mg/dL) in male albino rats. [a]P<0.05: significant difference *versus* the control group, [b]P<0.05: significant difference *versus* the $HgCl_2$-treated group.

Treating animals with flavonoids induced a significant (P<0.001) increase in urea level when compared to the control group (Figure 2). In addition, results showed that the treatment with the $HgCl_2$ alone or combined with selenite, or any flavonoid under investigation did not result in any significant change in the urea level when compared to the control group. Treating animals with the $HgCl_2$ alone or combined with selenite or flavonoids (except naringin) caused no significant effect on the creatinine level (Figure 2). There was a significant (P<0.05) increase in creatinine level in the groups treated with naringin alone or in combination with $HgCl_2$, as compared with the control or $HgCl_2$-treated group, respectively.

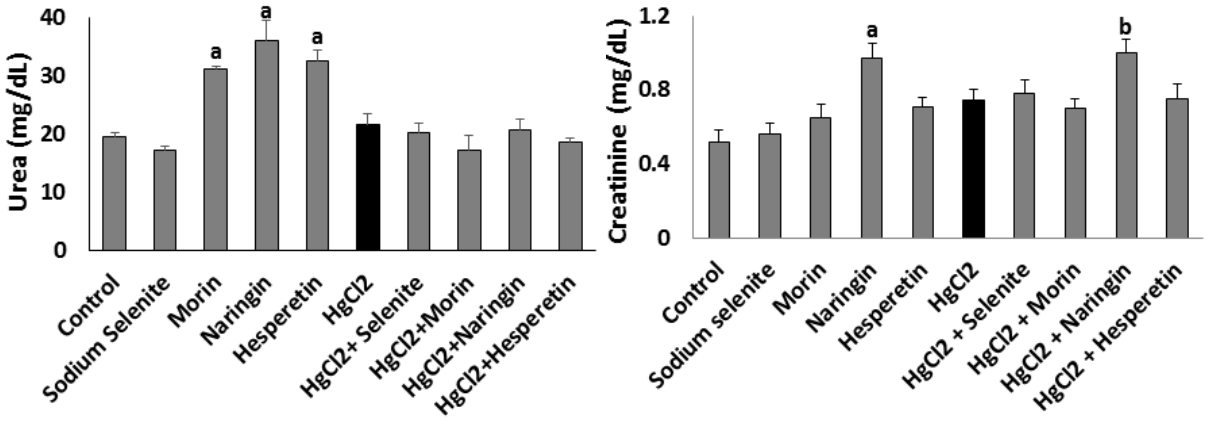


Figure 2: Effect of $HgCl_2$, selenite, and flavonoids on serum urea (mg/dL) and creatinine (mg/dL) in male albino rats. $^{a}P<0.05$: significant difference *versus* the control group, $^{b}P<0.05$: significant difference *versus* the $HgCl_2$-treated group.

The levels IL-6 and TNF-α in the $HgCl_2$-treated rats were significantly ($P<0.001$) increased, as compared to the control group. Co-administration of selenite or flavonoids with $HgCl_2$ resulted in significant ($P<0.001$) reductions in the IL-6 and TNF-α levels, as compared with the $HgCl_2$-treated group (Figure 3).

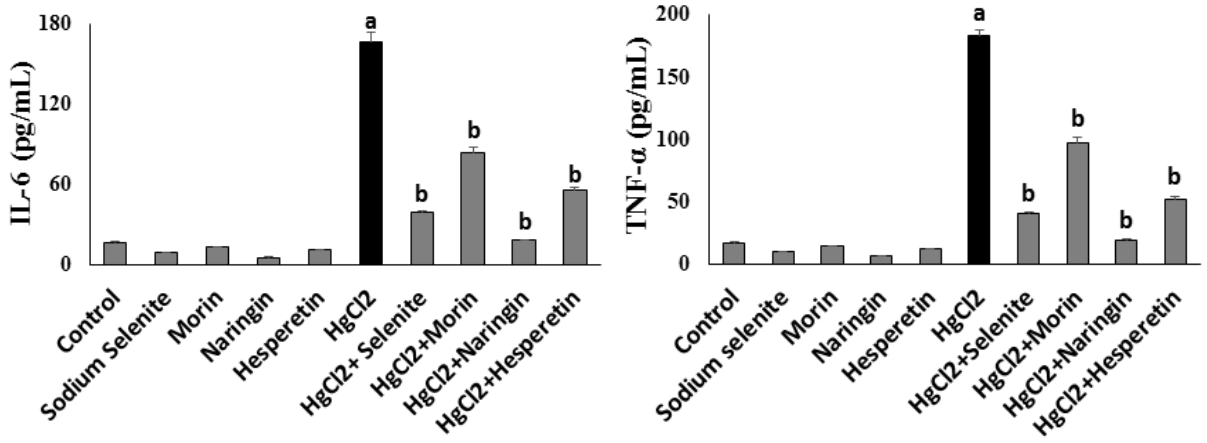


Figure 3: Effect of $HgCl_2$, selenite, and flavonoids on interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels in male albino rats. $^{a}P<0.05$: significant difference *versus* the control group, $^{b}P<0.05$: significant difference *versus* the $HgCl_2$-treated group.

The Hb content and RBCs count were significantly decreased in the groups treated with $HgCl_2$, selenite, morin, or hesperetin ($P<0.01$-0.001), as compared to the control group (Table 3). None of the flavonoids or selenite administered with $HgCl_2$ affected significantly the Hb level or RBCs count, as compared to the $HgCl_2$-intoxicated group. The WBCs increased significantly ($P<0.001$) in the group treated with selenite compared to the control group. There was also a significant ($P<0.05$) increase in the group treated with $HgCl_2$ combined with selenite when compared with the $HgCl_2$-treated group. The results showed non-significant changes in platelets count, MPV and MCV

after all treatments. The RDW% was significantly (P<0.05) increased in the group treated with selenite compared to the control group. There was also a significant (P<0.05) increase in RDW% in the group treated with $HgCl_2$ combined with selenite when compared with the $HgCl_2$-treated group (Table 3).

Table 3: Effect of $HgCl_2$, selenite, and flavonoids on the hematological parameters of male albino rats.

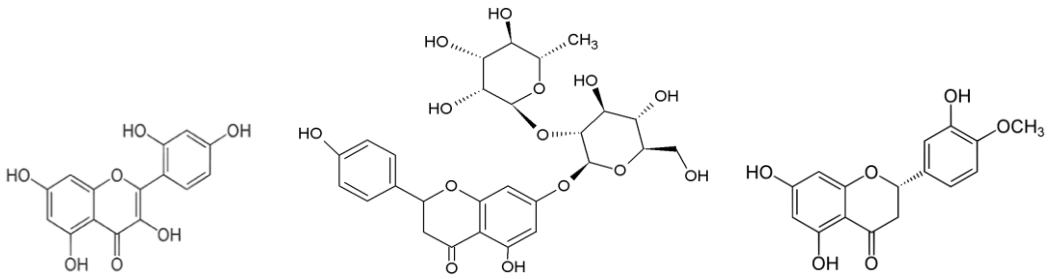
	Hb (g/dL)	RBCs (10^6/mL)	MCV (fL)	RDW (%)	WBCs (10^3/mL)	Platelets (10^3/mL)	MPV (fL)
Control	17.3±0.4	9.4±0.2	50.5±1.0	13.1±0.5	7.4±0.9	552.6±41.6	3.5±0.1
Sodium selenite	12.9±0.6[a]	7.4±0.2[a]	50.1±0.5	15.8±0.5[a]	18.8±2.3[a]	496.6±75.5	4.2±0.3
Morin	14.5±0.3[a]	8.1±0.22[a]	49.7±1.2	13.7±0.8	8.9±1.1	628.3±32.1	3.7±0.1
Naringin	16.6±0.3	9.1±0.3	48.2±0.5	12.9±0.3	8.5±1.0	429.9±29.4	3.6±0.2
Hesperetin	15.1±0.5[a]	8.1±0.2[a]	48.6±0.5	12.3±0.2	8.2±1.4	449.1±43.7	3.7±0.2
$HgCl_2$	14.7±0.4[a]	7.7±0.2[a]	50.7±0.5	12.2±0.2	8.7±1.1	504.9±30.9	3.6±0.2
$HgCl_2$+Selenite	13.6±0.2	7.5±0.2	51.7±1.1	15.8±0.5[b]	15.1±2.1[b]	474.2±36.3	4.3±0.3
$HgCl_2$+Morin	14.2±0.4	7.8±0.2	51.2±0.6	14.1±0.4	10.1±1.6	455.5±10.3	3.5±0.1
$HgCl_2$+Naringin	13.7±0.2	7.4±0.2	51.9±1.5	14.3±0.7	12.1±0.9	502.9±32.7	3.4±0.1
$HgCl_2$+Hesperetin	14.4±0.5	8.0±0.3	54.9±3.4	14.0±0.7	11.0±1.0	496.0±28.9	3.7±0.1

Data were expressed as mean ± standard error (n=7). Hb: hemoglobin, RBCs: red blood cells, WBCs: white blood cells, MPV: mean platelet volume, MCV: mean corpuscular volume, and RDW: red cell distribution width, [a]P<0.05: significant difference *versus* the control group, [b]P<0.05: significant difference *versus* the $HgCl_2$-treated group.

DISCUSSION

Mercury is the most toxic heavy metal and is known to induce toxicity in many organs. Humans are exposed to this metal from numerous sources; contaminated air, water, soil, and food[1]. Selenium and flavonoids have been shown to alleviate heavy metal toxicity[9,14]. Therefore, the present study aimed to evaluate the possible mitigating effect of selenite, and some flavonoids (morin, naringin and hesperetin) against acute $HgCl_2$ toxicity. The common feature of all flavonoids is the presence of A- and B-rings connected by pyran ring (Figure 4). Morin is somewhat similar to hesperetin but morin has one hydroxyl group instead of methoxy group in the B-ring of hesperetin. Naringin has many hydroxyl groups in the B-ring[14]. Therefore, the philosophy behind the choice of these flavonoids was to draw a structure-activity relationship based on their different hydroxyl groups. Hydroxyl groups are known to donate hydrogen to free radicals preventing them from damaging the cells[15].

In the current study, a significant increase in ALT and γ-GT activities in the $HgCl_2$-treated group (as compared to the control group) was observed, which was in agreement with previously reported data[3]. The liver is a major site of metabolism for mercury hence, severely affected by mercury. Previous study also revealed that $HgCl_2$ caused histo-pathological and ultra-structural lesions evidenced by fatty degeneration and



Morin Naringin Hesperetin

Figure 4: The chemical structure of the different flavonoids investigated.

cell necrosis in the liver[16]. The hepatic damage is usually observed as a rise in serum levels of liver enzymes such as ALT. Moreover, γ-GT elevation reveals cholestasis and bile duct necrosis. Therefore, the elevation of these enzymes in serum may be attributed to their liberation from the cells into the circulation, indicating damage of the cell membrane or bile duct problems due to oxidative stress and the resulting lipid and protein oxidation[17]. Since the function of γ-GT is to transfer the γ-glutamyl group from molecules such as GSH to other acceptors. This would expose the cysteine moiety of GSH to neutralize xenobiotics or toxins[18]. Therefore, the elevation in γ-GT activity could be a compensating defense mechanism against the $HgCl_2$ toxicity. The elevations of ALT and γ-GT activities were attenuated or prevented by the co-treatment with selenite, morin, hesperetin or naringin in agreement with previous studies[19,20]. This protective effect could be attributed to the ability of flavonoids to reduce oxidative stress and preserve the structural integrity of hepatocellular membrane by destruction of H_2O_2 and lipid hydroperoxides *via* elevating GSH and other antioxidants or direct scavenging activity against free radicals[21]. However, in the current study and although selenite treatment elevated the GSH level, it was unable to ameliorate the elevation in serum ALT activity after mercuric toxicity but alleviated the increase of γ-GT activity in serum. From the data shown so far, it was essential to investigate the effect of all treatments on antioxidants and lipid peroxidation (LPO), as it seems that these play a major role in the mercuric toxicity and the protection offered by the flavonoids and selenite.

The reactive oxygen species (ROS), free radicals, and oxidants are involved in the hepatic injury induced by $HgCl_2$[22]. The first radicals formed are the superoxide radicals which if left un-neutralized could through several pathways form the most dangerous hydroxyl radicals[22]. The cells are equipped with several mechanisms to fight back against these deleterious radicals. GSH is the most important non-protein antioxidant. It can neutralize many free radicals but unfortunately, it is consumed during the process. When the pro-oxidant burden (mercuric toxicity in the current study) is severe, the cellular defense fails and the resulting oxidative stress damages the cell components or even leads to cell death[23]. GST is another key player of phase II biotransformation enzymes. GST catalyzes the conjugation of electrophiles to GSH[24]. In the current study, GST was not significantly affected by any treatment.

The elevation of LPO in the current study indicates that oxidative insult happened. The GSH would be consumed due to the elevation in γ-GT reported after mercuric toxicity[4]. The CAT activity was significantly elevated after mercuric intoxication, as compared to the control group. Oxidative stress caused by mercury would trigger the induction of antioxidant defense mechanisms as a compensating mechanism[25,26]. The increase in CAT

activity will destroy the H_2O_2, which could otherwise penetrate through the biomembranes and inactivate several enzymes[27]. Co-treatment of mercuric-intoxicated rats with selenite resulted in a significant elevation of GSH that in agreement with previously reported data[10]. Selenium is a trace element that is essential at small amounts for humans and animals for the function of selenium-dependent enzymes[28].

One of the ways that mercury exerts its toxic effects is through the induction of LPO[22]. In the present study, oxidative stress induced by $HgCl_2$ was evidenced in the liver of rats by an increase in LPO end product; MDA in agreement with Deng *et al*.[29]. This elevation in the hepatic MDA level reflects LPO and damage to the plasma membrane as a consequence of ROS formation and oxidative stress[30]. The co-treatment of rats with mercury along with flavonoids or selenite prevented the LPO and reduced significantly the hepatic MDA levels. The selenium has affinity to bind mercury and form the non-toxic Hg–Se–S complex greatly exceeds the binding ability of sulfhydryl compounds. This binding and detoxification capability of selenite could explain its ability to protect the cells from LPO and damage caused by mercury intoxication[30]. The flavonoids also restored the high MDA values towards normal, as compared to the $HgCl_2$-treated group. These results indicated their anti-lipid peroxidative properties and free radicals scavenging capacity[14]. It was found that the antioxidant activity and metal ion chelation capacity of different flavonoids are correlated to the configuration and total number of the hydroxyl groups on the flavonoid rings[15]. In the current study, naringin and hesperetin offered the best anti-LPO activities. Mechanisms of antioxidant action of flavonoids can include suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation, scavenging ROS, and upregulation or protection of antioxidant defenses[31].

Plasma proteins play a prominent role in preventing the increase of systemic metal-related free fractions by delaying metal accumulation and toxicity[32]. In the current study, $HgCl_2$-intoxicated rats showed significant higher serum total protein and globulins levels, as compared to the control group, whereas albumin was lowered though insignificantly. These observations agree well with the findings of Necib *et al*.[10]. Since most plasma proteins are produced by the liver, elevation in total protein level may reflect an increased production of vital proteins to combat mercuric toxicity. The protein synthesis could also be needed to meet out the demand for the repair of damaged tissues[26]. Most elevation in protein level was manifested in elevation in globulins level indicating a boost in the immune response. The A/G ratio is a more sensitive index used to track changes in the composition of serum or plasma proteins and to predict liver and kidney disorders. In the present study, A/G ratio was reduced after mercuric intoxication in comparison to the control group due to the elevation of globulins level rather than a decrease in albumin level.

Lipids are an essential component of cell membranes and also play a significant role as messengers in signal transduction pathways and molecular recognition processes[33]. The present investigation demonstrated that $HgCl_2$ induced a significant elevation in serum levels of TC and TAGs. This reflects abnormalities in lipoprotein metabolism which may result in the development of atherosclerosis[34]. Similar observations have also been made by Samipillai *et al*.[35]. High cholesterol and TAGs in serum could be due to liver dysfunction and disturbance of lipid metabolism and transport. Being an important structural component of cell membrane, therefore, membrane degeneration could be another possible cause of their elevation[36]. $HgCl_2$ was reported to suppress LDL-receptor gene expression, reducing cellular uptake of cholesterol resulting in an increase in serum

cholesterol levels[37]. The present data revealed that TC and TAGs of mercuric-intoxicated groups co-treated with selenite or flavonoids were significantly reduced, as compared with the $HgCl_2$-treated group. This observed anti-lipidemic effect of selenite and flavonoids suggest that they could have a protective effect on the cardiovascular system. The hypo-cholesterolemic effect may result from an increase in the reverse cholesterol transport to the liver for reuse and re-excretion in bile resulting in a decrease of cholesterol level[34]. The reduction in TAGs could be attributed to inhibition of lipolysis in the adipose tissue, reduction in the esterification of TAGs in the liver, and elevation in the activity of lipase[33]. A decline in TAGs could also be correlated to their utilization in membrane biogenesis[38]. These observations are in concert with previous studies that revealed many flavonoids exhibit lipid-lowering properties by reducing significantly the cholesterol and TAGs levels. They found that naringin reduced LDL-cholesterol by 17% and TAGs by 14% in hypercholesterolemic patients[39]. It was reported that morin improved dyslipidemia, likely due to the depletion of circulating non-esterified fatty acids and inhibition of hepatic synthesis of TAGs[40].

Although mercury was previously reported to cause nephrotoxicity[41], it did not significantly affect urea or creatinine levels in the current study. The flavonoids administered to normal animals caused a significant elevation in the urea level. Flavonoids are known to reduce blood ammonia and hence could increase the urea synthesis in addition to their ability to enhance the enzymes involved in urea synthesis such as ornithine transcarb-amylase[42].

The relationship between oxidative stress and inflammation is reciprocal[43]. TNF-α is a potent inflammatory cytokine that is secreted by activated mononuclear leukocytes, and a wide variety of other immune and non-immune cell types[44]. The current data revealed that the concentration of TNF-α and IL-6 were increased in animals intoxicated with $HgCl_2$, which is in harmony with previous data[45]. On the other hand, the co-treatment with selenite, or flavonoids attenuated significantly the $HgCl_2$-induced TNF-α and IL-6 levels in agreement with previous studies[20,40]. They suggested that the attenuation of the inflammatory responses could be attributed to the inhibition of synthesis and secretion of these pro-inflammatory mediators. Thus, we hypothesized that the protective effect of selenite and the selected flavonoids in the present study on acute $HgCl_2$-induced liver injury was, at least in part, mediated by their anti-inflammatory properties. TNF-α is known to be involved in cell apoptosis when the cells are severely damaged by oxidative stress.

The current results of the hematological parameters showed a significant decrease in RBCs count and Hb concentration after $HgCl_2$ that parallel with the findings of Necib *et al.*[10]. Previous studies demonstrated that the decrease in RBCs and Hb after mercuric toxicity can be attributed to the decrease of iron necessary for the synthesis of RBCs[46], or the production of ROS which would increase the destruction of RBCs by liver, spleen, and bone marrow[47]. Administration of morin, hesperetin, or selenite to normal animals also reduced the Hb level and RBCs count. Some flavonoids were reported to reduce the expression of ferroportin and hence reduce the synthesis of Hb and RBCs count[48]. In the current study, all treatments did not effect on platelets, MPV and MCV. Selenite was the only treatment that enhanced the WBCs count and RDW% both in normal, as well as in intoxicated animals. Selenite was found to boost the immune system and increase the WBCs count and activity[49]. RDW and MCV are used to identify the type of anemia. High RDW values along with normal MCV indicate a deficiency of iron, folate, or vitamin B12[50]. Selenite was previously reported to cause anemia[51].

To conclude, mercury caused serious problems in hepatic tissues, induced LPO, disturbed the plasma proteins and caused dyslipidemia, as well as induced inflammation, but did not induce nephrotoxicity. Flavonoids and sodium selenite have prophylactic effects against most deleterious effects caused by mercury intoxication. The effects of mercury along with flavonoids and selenite on hematogram were all sporadic. No structure-activity relationships could be concluded from this study. Naringin with the highest number of hydroxyl groups on B-ring and the highest absolute number of hydroxyl groups in general discerned itself and was the best treatment as manifested only in MDA, TAGs, IL-6, and TNF-α. In all other biochemical parameters investigated, no such significant differentiation among flavonoids was reported. Moreover, every flavonoid has its biological signature probably due to its metabolism. Selenite also offered very much similar alleviation against $HgCl_2$ toxic deteriorations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

WME and EAE planned the study and designed all experiments. MMM carried out the experiments. WME and MMM performed the statistical analysis. WME, EAE, and MMM summarized, discussed, and interpreted the results. WME and EAE drafted the manuscript.

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تخفيف سُمية كلوريد الزئبق باستخدام الفلافونيدات في ذكور الجِرذان المهقاء لا يعتمد على عدد مجموعات الهيدروكسيل في الحلقات "ب"

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إن التعرض للزئبق أمر حتمي وذلك من خلال المياه والأغذية الملوثة. ولذلك هدفت الدراسة الحالية إلى تقييم "3" مركبات فلافونويدية مختلفة وسيلينيت الصوديوم ضد سُمية كلوريد الزئبق في ذكور الجِرذان. تم توزيع الجِرذان بشكل عشوائي إلى "10" مجموعات (ن = 7) على النحو التالي: المجموعة الضابطة، والمجموعات التي تم إعطاؤها "5 ملجم/كجم" من سيلينيت الصوديوم أو كلوريد الزئبق، والمجموعات التي تم إعطاؤها "50 ملجم/كجم" من المورين، نارينجين، أو هسبريتين، والمجموعات التي تم إعطاؤها كلوريد الزئبق مع سيلينيت الصوديوم، المورين، نارينجين أو هسبريتين. استمرت جميع المعالجات عن طريق الفم يوميا لمدة أسبوعين. وقد أظهرت النتائج أن كلوريد الزئبق تسبب في ارتفاعات كبيرة في مستويات البروتينات الكلية والجلوبيولين والكوليسترول الكلي والجلسريدات الثلاثية وعامل ألفا المنكرز للورم والإنترليوكين "6" في المصل، والمالونديالدهيد في أنسجة الكبد، ونشاط كلا من الإنزيم الناقل لمجموعة الأمين للألانين والإنزيم الناقل لمجموعة جاما جلوتاميل في المصل، ونشاط الكاتالاز في أنسجة الكبد. كما تسبب أيضا في انخفاضات كبيرة في محتوى الجلوتاثيون المختزل في الكبد، ومستوى هيموجلوبين الدم، وعدد كريات الدم الحمراء. وتحسنت معظم هذه التأثيرات الضارة عند إعطاء الفلافونويدات أو سيلينيت الصوديوم. ولم تكن هناك علاقة بين نشاط الفلافونيدات وتركيبها يمكن استنتاجها من هذه الدراسة. تشابهت فعالية النارنجين الذي يحتوي على أكبر عدد من مجموعات الهيدروكسيل في الحلقة "ب" وأعلى عدد مطلق من مجموعات الهيدروكسيل بشكل عام، مع استثناءات قليلة، مع باقي مركبات الفلافونويدات الأخرى ومع سيلينيت الصوديوم أيضًا. كان لكل فلافونويد بصمته وتأثيره البيولوجي الخاص به، وربما يرجع ذلك إلى التمثيل الغذائي الخاص بكل منهما.