



## Cardioprotective Effect of Moringa Oil and Okra Oil in Male Rats Exposed to Thioacetamide

Atef M. Al-Attar<sup>1,2</sup>, Isam M. Abu Zeid<sup>2</sup> and Khalid S. Alotaibi<sup>2</sup>

<sup>1</sup>Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia.

<sup>2</sup>Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

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### ABSTRACT

Currently, the rate of pollution, pollutants and toxic chemicals in the environment is increasing, which poses a threat to living organisms. Toxic chemicals affect the functions of the body's organs negatively. Plant extracts and natural products are considered to have effective nutritional and medicinal benefits. Recently, there is an increasing interest in plant extracts and natural products and their effectiveness in treating many diseases. The principal aim of the present study was occurred to evaluate the protective influence of moringa oil and okra oil against thioacetamide (TAA) induced cardiotoxicity in Wistar male rats. Experimental rats were divided into six groups. Rats of group 1 were used as controls. Group 2 was treated with TAA (300 mg/kg body weight). Group 3 was exposed to moringa oil (800 mg/kg body weight) plus TAA. Group 4 was treated with okra oil (800 mg/kg body weight) plus TAA. Groups 5 and 6 were treated with moringa oil and okra oil respectively. TAA treatment caused significant increases of serum creatin kinase (CK), lactate dehydrogenase (LDH) levels in group 2, while the levels of serum reduced glutathione (GSH) and superoxide dismutase (SOD) were statistically decreased. Administration of moringa oil and okra oil reduced the severity alterations of these parameters. These new results indicate that moringa oil and okra oil represent protective effect against cardiotoxicity induced by TAA due to their antioxidant roles.

**Keywords:** Thioacetamide, Cardiotoxicity, Moringa Oil, Okra Oil, Antioxidant, Rats.

### 1. Introduction

Globally, the exposure to pollutants, toxicants and pathogenic factors has been associated with a number of human diseases. Contamination of toxic chemicals in water, air and soil has seriously affected the health of humans and wildlife everywhere. Generally, the effect of toxic chemicals depends on several factors such as the route of exposure, concentration, period and the physiological state of the body. Thioacetamide (TAA) was presented as a fungicide to power carrot rot but was immediately recognized as a hepatotoxic and synthetic carcinogen (Childs and Siegler, 1946). TAA become hepatotoxic model for accounting for liver damage, liver fibrosis, malignancy, and liver redevelopment (Wallace *et al.*, 2015). However, synchronous introduction of TAA leads to liver fibrosis, and continuous presentation can cause liver disease (Keshk *et al.*, 2019). By boosting reactive oxygen species (ROS) production, TAA metabolites covalently bind to liver macromolecules, decrease the antioxidant capability and boost lipid peroxidation (Feillet-Coudray *et al.*, 1999). Experimentally, previous studies showed that TAA can also injure different organs of body besides liver, such as kidneys, testis, intestine, lungs, pancreas, spleen and heart (Al-Bader *et al.*, 2000; Abul *et al.*, 2002; Latha *et al.*, 2003; Yeh *et al.*, 2004; Al-Attar *et al.*, 2017; Mohamed *et al.*, 2018; Ghanim *et al.*, 2021; Gregolin *et al.*, 2021; Türkmen *et al.*, 2022).

**Corresponding Author:** Prof. Atef M. Al-Attar, Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box 139109, Jeddah 21323, Saudi Arabia.  
Email: atef\_a\_2000@yahoo.com

Medicinal plants have become a global concern with ramifications for global health. Herbal medicine has played a critical part in maintaining the global healthcare system for a large population (Sen and Chakraborty, 2017). This is especially true in less-developed or emerging countries, where conventional treatment has been disrupted in the past. Both emerging and developed countries have increased their understanding of the medicinal effects of plants (Alamgir, 2017). *Moringa oleifera* Lam. or Drumstick tree is one of the 13 known species originating from the family *Moringaceae*. Furthermore, *M. oleifera* is a widely-known genus and is used all over the world (Sreeja *et al.*, 2021). The plant is native to India's Northwest Sub-Himalayan areas. It is now found in a variety of African, Arab, South East Asian, and South American countries, as well as Pacific and Caribbean islands. Apart from the 'Drumstick tree,' the *Moringa oleifera* tree is also commonly called as Horseradish tree and Benzolive tree (Fantoukh *et al.*, 2021). The crown of this tree has the shape of an open umbrella. The tree has a short, straight trunk with corky-white bark that hides the spongy, smooth wood beneath it. It stands between 5 and 12 meters tall (Gbenou *et al.*, 2021). The edible seed oil of *Moringa oleifera* is known as "Ben" or "Behen" oil in the commercial world. In Caribbean and Haitian traditions, the pleasant edible oil has a flavor comparable to olive oil and is frequently used as a salad dressing. Moringa seed includes all of the essential fatty acids contained in the oil (Ghazali and Abdulkarim, 2015). The edible seed oil of *Moringa oleifera* is known as "Ben" or "Behen" oil in the commercial world. In Caribbean and Haitian traditions, the pleasant edible oil has a flavor comparable to olive oil and is frequently used as a salad dressing. Moringa seed includes all of the essential fatty acids contained in the oil (Ghazali and Abdulkarim, 2015). Researchers have described it as a plant with various health benefits, including nutritional and medicinal benefits (Ogbe and Affiku, 2011; Chhikara *et al.*, 2020; Padayachee and Bajjnath, 2020).

*Abelmoschus esculentus* L, commonly known as Okra or Lady's finger originates from the family *Malvaceae* of the plant kingdom (Ranga *et al.*, 2019). The Okra is a popular crop of vegetables grown in many places of the globe (Dubey and Mishra, 2017; Kumar *et al.*, 2018; Elkhalfifa *et al.*, 2021). Okra is a dicotyledonous, the Okra plants have trunks, roots, leaves, and pods (Nida'M *et al.*, 2011). The pods contain seeds, pulp, and calyx. Different parts of this plant offer various medical, industrial, and gastronomic benefits. Small seeds with a thick seed coat in okra pods containing a lot of crude (Adelakun *et al.*, 2010; Agbo *et al.*, 2010; Adetuyi and Adelabu, 2011; Dhruve *et al.*, 2015). Depending upon the variety of the Okra plant, the collection of the unripe fruit can be done after two to three months of seed sowing or the first week of flowering (Muhammad *et al.*, 2020). Okra phytochemicals have been investigated for their possible therapeutic effects on a variety of chronic illnesses, such as gastrointestinal disease, diabetes mellitus, and heart ailments. They are also found to be effective for detoxifying the liver, as an antifatigue agent, have antibacterial properties, can regulate the level of cholesterol, and have chemopreventive properties (Prabhune *et al.*, 2017; Elkhalfifa *et al.*, 2021). However, it is worth mention that there are no previous studies on the effect of moringa oil and okra oil against TAA toxicity. The rationale of the present investigation is to explore the cardiotoxicity of TAA and the protective influence of moringa oil and okra oil against cardiotoxicity induced by TAA in male rats.

## 2. Materials and Methods

### 2.1. Experimental design

Sixty Wistar male albino rats, weighing 120-163 g were used as experimental animals in this study. They were kept standard plastic cages under laboratory conditions of  $20 \pm 2$  °C, 55%  $\pm$ 10 humidity and 12:12 h light: dark cycle each day, with free access to food and water. Rats were randomly divided into six experimental groups. Rats of group 1 were untreated and used as controls. Group 2 was given TAA (300 mg/kg body weight) by intraperitoneal injection, twice weekly. Group 3 was orally supplemented with moringa oil (800 mg/ kg body weight)/ day and they were intraperitoneally injected with TAA at the same dose given group 2. Group 4 was orally supplemented with okra oil (800 mg/ kg body weight)/ day and they were intraperitoneally injected with TAA at the same dose given group 2. Groups 5 and 6 were subjected to moringa oil (800 mg/ kg body weight) and okra oil (800 mg/ kg body weight) respectively. At the end of experimental period (6 weeks), all rats were fasted for 10 hours and anaesthetized using diethyl ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 min. and blood sera

were then collected and stored at  $-80^{\circ}\text{C}$  prior immediate determination of creatin kinase (CK), lactate dehydrogenase (LDH), reduced glutathione (GSH) and superoxide dismutase (SOD). The value of CK was estimated according to the method of Horder *et al.* (1991). The method of Weishaar (1975) was used to measure the value of LDH. The methods of Beutler *et al.* (1963) and Nishikimi *et al.* (1972) were used to evaluate the levels of GSH and SOD respectively.

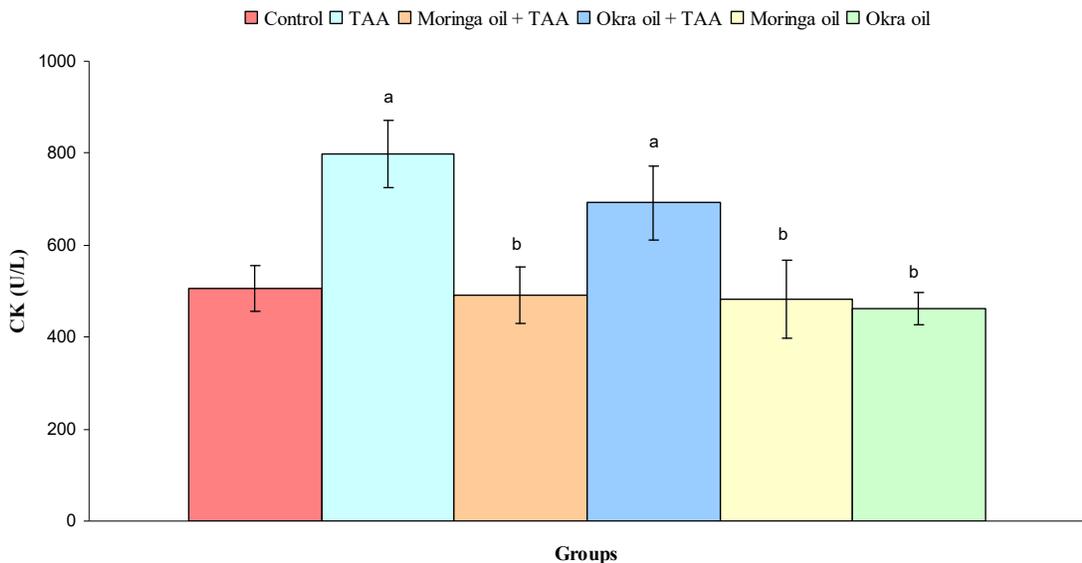
## 2.2. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test. SPSS Windows version 22.0 software was used for statistical analysis. All data were expressed as mean with their standard deviation (S.D.).  $P$  values  $\leq 0.05$  were considered as significant.

## 3. Results

### 3.1. Serum CK

The measured levels of serum CK in all groups are given in Figure 1. Statistically increases in the level of serum CK were detected in rats treated with TAA ( $P \leq 0.002$ ) and okra oil plus TAA ( $P \leq 0.001$ ). This parameter was insignificantly altered in rats treated with moringa oil plus TAA (group 3), moringa oil (group 5) and okra oil (group 6) as compared with control rats of group 1.

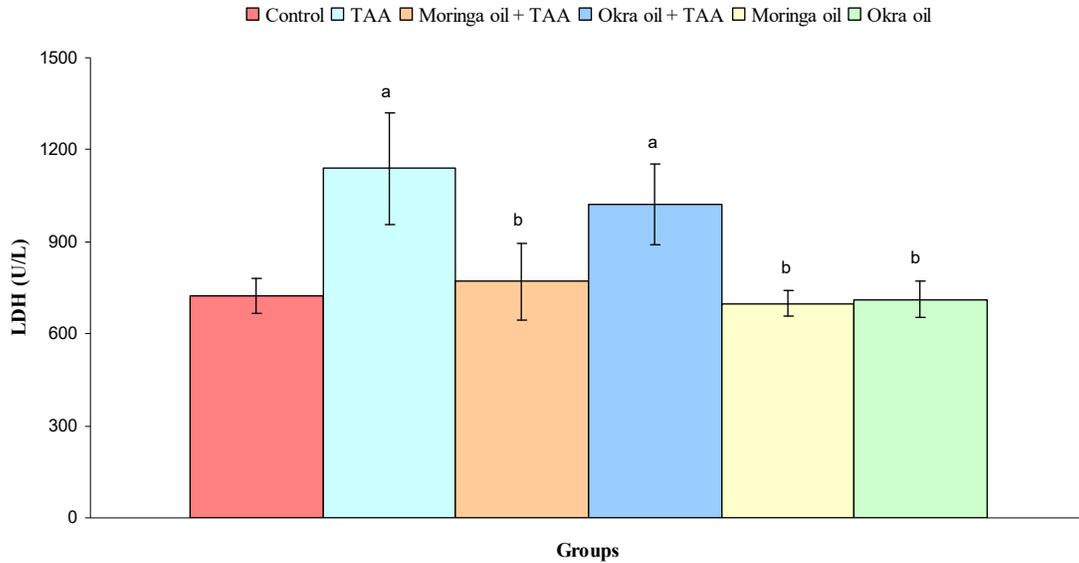


**Fig. 1:** Level of serum CK in control (group 1), TAA (group 2), moringa oil plus TAA (group 3), okra oil plus TAA (group 4), moringa oil (group 5) and okra oil (group 6) treated rats. **a** Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6).

**b** Indicates a significant difference between rats treated with TAA (group 2) and groups 3, 4, 5 and 6.

### 3.2. Serum LDH

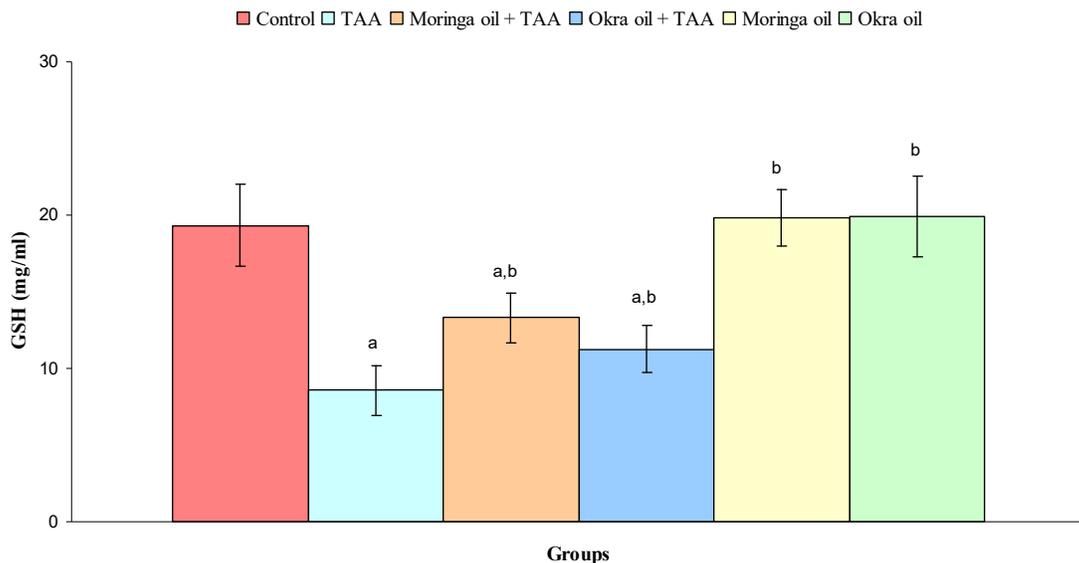
In comparison with control data, the level of serum LDH was markedly enhanced in rats of groups 2 ( $P \leq 0.001$ ) and 4 ( $P \leq 0.001$ ). The levels of serum LDH in rats of groups 3, 5 and 6 were significantly unchanged (Fig. 2).



**Fig. 2:** Level of serum LDH in control (group 1), TAA (group 2), moringa oil plus TAA (group 3), okra oil plus TAA (group 4), moringa oil (group 5) and okra oil (group 6) treated rats. **a** Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6). **b** Indicates a significant difference between rats treated with TAA (group 2) and groups 3, 4, 5 and 6.

### 3.3. Serum GSH

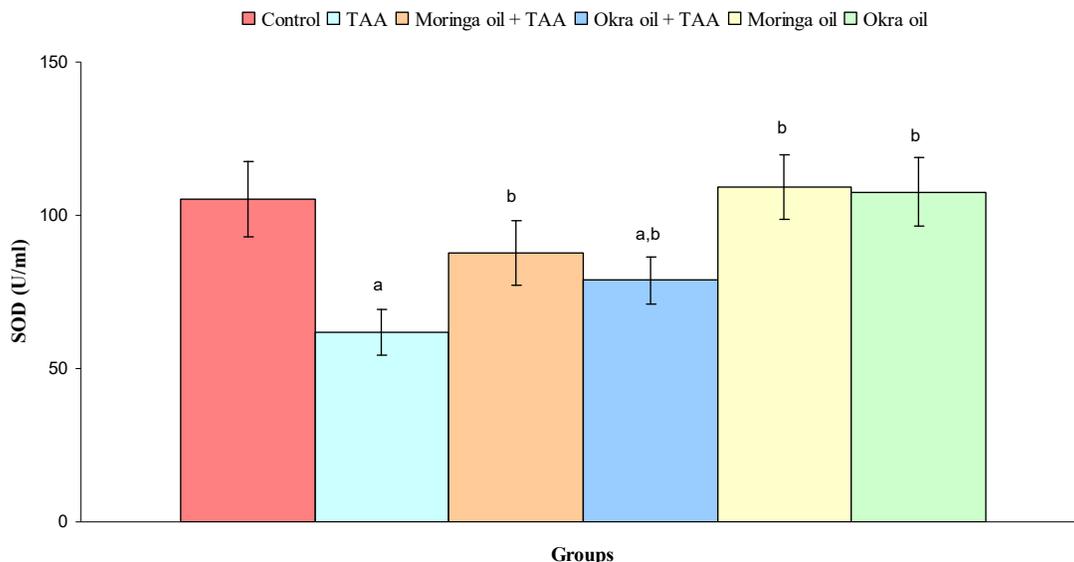
There were significant decreases in the level of serum GSH in rats of groups 2 ( $P \leq 0.000$ ), 3 ( $P \leq 0.02$ ) and 4 ( $P \leq 0.001$ ) compared with control rats. Specifically, there were no significant differences in the levels of serum GSH in rats of groups 5 and 6 (Fig. 3).



**Fig. 3:** Level of serum GSH in control (group 1), TAA (group 2), moringa oil plus TAA (group 3), okra oil plus TAA (group 4), moringa oil (group 5) and okra oil (group 6) treated rats. **a** Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6). **b** Indicates a significant difference between rats treated with TAA (group 2) and groups 3, 4, 5 and 6.

### 3.4. Serum SOD

Treatment with TAA ( $P \leq 0.000$ ) and okra oil plus TAA ( $P \leq 0.01$ ) induced significant decrease in the level of serum SOD. Moreover, there were no significant alterations in the levels of serum SOD in rats of groups 3, 5 and 6 (Fig. 4).



**Fig. 4:** Level of serum SOD in control (group 1), TAA (group 2), moringa oil plus TAA (group 3), okra oil plus TAA (group 4), moringa oil (group 5) and okra oil (group 6) treated rats.

**a** Indicates a significant difference between control (group 1) and treated groups (2, 3, 4, 5, and 6). **b** Indicates a significant difference between rats treated with TAA (group 2) and groups 3, 4, 5 and 6.

### 4. Discussion

In this study, rats subjected to TAA had increased serum levels of CK and LDH. Necrosis raises the blood levels of markers for myocardial infarction, such as CK and LDH, which are diagnostic signs (Al-Attar and Shawush, 2014). One of the utmost accurate plus consistent procedures for determining muscle impairment is to check for elevated CK concentrations in the blood serum. In order for muscles to store high-energy phosphate, creatine must be phosphorylated to creatine phosphate, which is done by CK (Cardinet, 1989; Moss and Henderson, 1994). In contrast, the current increase in serum CK values in rats were exposed to TAA may be due to heart muscle tissue damage. CK is the first cardiac enzyme to enter the bloodstream after a cardiac arrest, and it decreases quickly. It is easier to detect myocardial ischemia in the early stages with elevated levels of serum CK activity than it is with higher peaks of LDH, which is a marker of cardiac tissue damage (Chatterjea and Shinde, 2002). Blood erythrocytes, skeletal muscles and kidneys all have high levels of LDH, as do the liver, heart and blood plasma. There have been several studies that have shown that diseases affecting these organs are associated with substantial increases in average serum LDH activity. A number of cancers have been associated to elevated serum LDH activity (Kanowski and Clague, 1994). Cardiotoxicity was shown in rats when TAA (300 mg/kg bodily size twice a week for 10 weeks) was long-term administered. This is because of the heart's leakage caused by TAA-induced myocardial necrosis (Al-Attar, 2011). TAA-exposed male rats showed elevated levels of CK and LDH in their bloodstreams (Al-Attar and Shawush, 2014). However, myocardial leakage produced by TAA-induced necrosis might explain the present increase of serum CK and LDH levels.

The present study showed that TAA induced oxidative stress which confirmed by the decrease of GSH and SOD levels. Oxidative stress is a typical phenomenon of many cardiovascular abnormalities (Yang *et al.*, 2021). Reactive oxygen species (ROS) and oxidative stress play an important role in pathogenic mechanism of TAA. GSH, a tripeptide present in the bulk of cells, conjugates hydrophilic xenobiotics. In addition to its role in protecting cells against reactive species

(ROS), GSH also plays a role throughout detoxifying foreign toxins and transferring amino acids in the body. In order to protect cells from specific forms of ROS, GSH's sulfhydryl group must be present (Cnubben *et al.*, 2001). Many forms of disease may be traced back to a drop in intracellular GSH concentrations (Kretzschmar *et al.*, 1989). Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. SOD is antioxidant enzyme that considered as the first line defense system against ROS (Ighodaro and Akinloye, 2018). However, previous studies showed that TAA induced oxidative stress which confirmed by significant changes of GSH and SOD (Bashandy *et al.*, 2020; Đurašević *et al.*, 2021; Al-Attar, 2022; Zaghoul *et al.*, 2022).

The present study revealed that moringa oil and okra oil inhibited the toxic effects of TAA. Moringa's therapeutic benefits are attributed to its high concentration of flavonoids as well as other polyphenols in its leaves and seeds. Akinrinde *et al.*, (2020) studied the protective effect of the methanol extract of *M. oleifera* extract in a rat model of renal ischemia-reperfusion injury. The results showed that *M. oleifera* extract effectively attenuated the deleterious effects of renal ischemia-reperfusion via alleviation of tissue oxidative stress. Albasher *et al.*, (2020), and Alqahtani and Albasher (2021) investigated the protective effect of *M. oleifera* extract against lead (Pb)-induced hepatotoxicity and neurotoxicity. They concluded that *M. oleifera* extract attenuated Pb-induced liver and brain damage in rats by restraining oxidative stress, inflammation and apoptosis. Okra is abundant in fiber, vitamin C, folate, calcium, potassium, polysaccharide, polyphenols, and flavonoids (Kumar *et al.*, 2013; Zhou *et al.*, 2013). Okra exhibited anti-fatigue and anti-oxidant properties by decreasing blood lactic acid levels and urea nitrogen levels, boosting hepatic glycogen stores, and decreasing malondialdehyde (MDA) and glutathione peroxide (GPx) levels (Xia *et al.*, 2015). Wahyuningsih *et al.*, (2020) investigated the renoprotective effects of okra pods extract in male mice exposed to Pb. The results revealed that the okra pods extract inhibited the effect of Pb toxicity and this effect was attributed to very strong antioxidant properties of okra pods extract. Collectively, the present results indicate the ability of moringa oil and okra oil to protect against the cardiotoxicity effects of TAA which confirmed by inhibition the change of oxidative stress markers. Further physiological and histopathological studies are required to evaluate the potential protective effects of different doses and concentrations of moringa oil and okra oil against toxic effects of TAA and against other toxicants and pathogenic factors.

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