

T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

**ASSESSMENT OF THE EFFECTIVENESS OF
INTRAVENOUS LIPID EMULSION AS A NEW
TREATMENT METHOD IN GRAYANOTOXIN
(MAD HONEY) INTOXICATION**

THE DOCTOR OF PHILOSOPHY THESIS

EREN GÖKDAĞ, MD

İSTANBUL 2023

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated and numbered

Prof. Dr. Bayram Yılmaz

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

10/07/2023

Eren Gökdağ



DEDICATION



To my family

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SYMBOLS / ABBREVIATIONS

GTX	Grayanotoxin
ILE	Intravenous lipid emulsion
BC	Before Christ
IP	Intraperitoneal
LD	Lethal Dose
Nav	Voltage gated sodium channels
Phe	Phenylalanine
Tyr	Tyrosine
AV	Atrioventricular
ECG	Electrocardiogram
MDA	Malondialdehyde
ACLS	Advanced cardiac life support
NO	Nitric oxide
NADPH	Nicotinamide adenine dinucleotide phosphate
SOD	Superoxide dismutase
ONOO	Peroxynitrite
H ₂ O ₂	Hydrogen peroxide
CAT	Catalase
GSH-Px	Glutathione peroxidase
HNE	4-hydroxynonenal
G6PD	Glucose-6-phosphate dehydrogenase
IV	Intravenous
ET	Endotracheal
FFA	Fatty acids

ARDS	Acute respiratory distress syndrome
MAP	Mean arterial pressure
G	Gauge
KCL	Potassium chloride
ERC	European Resuscitation Council
LAST	Local anesthetic sytemic toxicity



ABSTRACT

Gokdag, E. (2023). Assessment of The Effectiveness of Intravenous Lipid Emulsion as a New Treatment Method in Grayanotoxin (Mad Honey) Intoxication, Yeditepe University, Institute of Health Sciences, Department of Pharmaceutical Toxicology, PhD thesis, İstanbul.

Mad honey poisoning, resulting from the consumption of honey containing grayanotoxin (GTX) from *Rhododendron* species plants, has been reported in several countries. Current treatment approaches involve using atropine, but it can have life-threatening side effects. Intravenous lipid emulsion (ILE) therapy, known for treating lipid-soluble substance poisoning, has been used in poisoning cases involving various drugs. This study aimed to evaluate the efficacy of ILE treatment as an alternative to atropine in grayanotoxin intoxication, focusing on its haemodynamic effects in Wistar albino rats and its potential to increase survival. Haemodynamic parameters, oxidative stress markers, and biochemical levels were monitored and analyzed. The results indicated that ILE treatment improved survival rates of rats, and reduced some oxidative stress parameters in the heart and liver tissues of rats intoxicated with grayanotoxin. However, no significant differences were observed in cardiac malondialdehyde and catalase levels. Additionally, liver malondialdehyde levels were significantly lower in the lipid group compared to the toxin and treatment groups. Liver catalase levels were higher in the treatment group compared to the lipid group. The findings suggest that ILE treatment shows promise as an alternative therapeutic approach for grayanotoxin intoxication, with potential benefits in improving survival and reducing oxidative stress. Further research is warranted to fully understand the mechanism of action and optimize the treatment protocol.

Keywords: Grayanotoxin, Intravenous Lipid, Survival, Rat model

ÖZET

Gokdag, E. (2023). Grayanotoksin (Deli Bal) İntoksikasyonunda Yeni Bir Tedavi Yöntemi Olarak İntravenöz Lipid'in Tedavi Etkinliğinin Değerlendirilmesi, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmasötik Toksikoloji Anabilim Dalı, Doktora Tezi, İstanbul.

Rhododendron türü bitkilerden elde edilen grayanotoksin (GTX) içeren bal tüketiminden kaynaklanan deli bal zehirlenmesi birçok ülkede bildirilmiştir. Mevcut tedavi yaklaşımları atropin kullanımını içermektedir, ancak bunun yaşamı tehdit eden yan etkileri olabilir. Lipitte çözünen madde zehirlenmelerinin tedavisinde kullanıldığı bilinen intravenöz lipid emülsiyonu (ILE) tedavisi, çeşitli ilaçları içeren zehirlenme vakalarında kullanılmıştır. Bu çalışmanın amacı, grayanotoksin zehirlenmesinde atropine alternatif olarak ILE tedavisinin etkinliğini, Wistar albino sıçanlarda hemodinamik etkilerine ve sağkalımı artırma potansiyeline odaklanarak değerlendirmektir. Çalışmada, hemodinamik parametreler, oksidatif stres belirteçleri ve biyokimyasal seviyeler izlenmiş ve analiz edilmiştir. Sonuçlar, ILE tedavisinin sıçanların hayatta kalma oranlarını iyileştirdiğini ve grayanotoksin ile zehirlenen sıçanların kalp ve karaciğer dokularındaki bazı oksidatif stres parametrelerini azalttığını göstermiştir. Bununla birlikte, kardiyak malondialdehit ve katalaz seviyelerinde anlamlı bir farklılık gözlenmemiştir. Ek olarak, karaciğer malondialdehit seviyeleri sadece ILE verilen grupta, toksin ve tedavi gruplarına kıyasla önemli ölçüde daha düşüktü. Karaciğer katalaz seviyeleri tedavi grubunda lipid grubuna kıyasla daha yüksekti. Bulgular, ILE tedavisinin grayanotoksin zehirlenmesi için alternatif bir terapötik yaklaşım olarak umut vaat ettiğini, sağkalımı iyileştirme ve oksidatif stresi azaltma konusunda potansiyel faydaları olduğunu göstermektedir. Etki mekanizmasını tam olarak anlamak ve tedavi protokolünü optimize etmek için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Grayanotoksin, İntravenöz Lipid, Sağkalım, Sıçan modeli

1. INTRODUCTION

Plants often contain components that can be beneficial and sometimes harmful to humans. Secondary products such as food, honey, marmalade, etc. obtained from these plants also carry components and can cause poisoning.

Folk use of honey as a medicine dates back to 2100 B.C. and has been used for colds, wound healing, diabetes, sexual stimulation, hypertension, diabetes, ulcers and gastritis. However, consumption of honey containing toxins can lead to poisoning. Consumption of honey containing grayanotoxin (from *Rhododendron* species plants) is called mad honey poisoning and mad honey poisoning has been reported in many countries around the world, including Turkey, China, Philippines, Korea, Indonesia, Japan, Nepal, Austria, Germany, Brazil and some countries in North America (1-11).

The toxic effects of grayanotoxin on the cell occur through sodium channels. The effect of grayanotoxin on voltage-dependent sodium channels has 3 stages. Firstly, grayanotoxin binds to these channels in the opening phase of voltage-dependent channels. Then the channels are modified. Finally, it causes activation potential hyperpolarisation of modified Na channels. This causes voltage-dependent activation or inactivation of the cell membrane (12). Symptoms include burning sensation in the mouth and pharynx, anoxia, salivation, vomiting, muscle weakness, blurred vision and bradycardia followed by severe hypotension, respiratory paralysis, coma and death (13).

Typically, symptoms manifest within a timeframe of 20 minutes to 3 hours following ingestion and endure for a period of 1-2 days. In severe instances where individuals seek medical attention at the emergency department, the typical treatment approach involves administering atropine alongside saline (14-15).

However, it is known that life-threatening side effects due to atropine can be observed, albeit rarely (16-18).

Intravenous lipid emulsion (ILE) therapy is used to treat patients poisoned with lipid-soluble substances, especially local anaesthetics. And GTX has been reported as both highly water soluble and lipid soluble (19,20). Although its efficacy and mechanism of action have not been established, it has also been used many times in the treatment of poisoning by antiarrhythmic, antipsychotic and antimalarial drugs (21,22).

The nectar, pollen and leaf parts of some plants of the heather family (Ericaceae) such as Rhododendron, Pieris, Agarista and Kalmia may contain diterpene grayanotoxins (GTxs). Poisoning due to grayanotoxin can also be seen in animals. A case series published in 2018, three goats which showing severe clinical signs due to poisoning with grayanotoxin after eating *Pieris japonica* leaves treated with ILE and 2 of them survived (23).

In this study, the efficacy of ILE treatment as an alternative treatment method to Atropine in Grayatoxin intoxication was evaluated haemodynamically in Wistar albino rats and it was observed that it increased survival. Acute oxidative stress parameters in heart and liver tissues were also evaluated.



2. GENERAL DESCRIPTION

2.1. Mad honey

Mad honey is distinct from commercially available honey because it contains grayanotoxin, which is responsible for its intoxicating and poisonous effects. The name "mad honey" is given to it precisely because of these effects it produces (24). Grayanotoxins are a type of diterpenes, which are cyclic hydrocarbons with multiple hydroxyl groups, and they do not contain any nitrogen atoms. (25). Intoxication is caused by grayanotoxins, which are obtained by honey bees from the nectar and pollen of various plants within the *Rhododendron* genus (Ericaceae family). The primary sources of grayanotoxins include *R. luteum*, *R. flavum*, *R. simsii*, and *R. ponticum*. For this reason, grayanotoxins are often referred to as rhodotoxins. (26) Grayanotoxins are also referred to by alternative names such as acetylandromedols or andromedotoxins (27). The toxins found in *Tripterygium hypoglaucum*, a plant belonging to the Celastraceae family, are known to possess a high level of toxicity. In southwestern China, the consumption of honey contaminated with pollen from *T. hypoglaucum* resulted in the death of 25.8% (8 out of 31) of patients between the years 2007 and 2012 (28). Cases of mad honey intoxication have been documented in numerous countries worldwide, such as Turkey, China, Philippines, Korea, Indonesia, Japan, Nepal, Austria, Germany, Brazil, and certain countries in North America. However, a significant portion of the reported cases, particularly the majority, originate from Turkey (1-11). Salici and Atayoglu (29) have reported that Turkish researchers contributed to the majority of articles published on mad honey intoxication/poisoning. Out of the 84 articles examined, 69 were authored by Turkish researchers, encompassing a total of 1199 cases (29). Honey that contains grayanotoxin exhibits higher toxicity levels during the spring season compared to mad honey produced in other seasons. Mad honey is characterized by its bitter and sharp taste, which can cause throat irritation. For this reason, it is also referred to as bitter honey (1).

Honey poisoning occurs when honey that is derived from bees feeding on the flowers of *Rhododendron* plants is consumed. The poisoning resulting from *Rhododendron* plants is commonly referred to as mad honey poisoning or grayanotoxin poisoning.

In Turkey, the *Rhododendron* species that are frequently found and recognized as toxic include *Rhododendron luteum* and *R. Ponticum* (Figure 1-2).



Figure 1. *Rhododendron luteum*

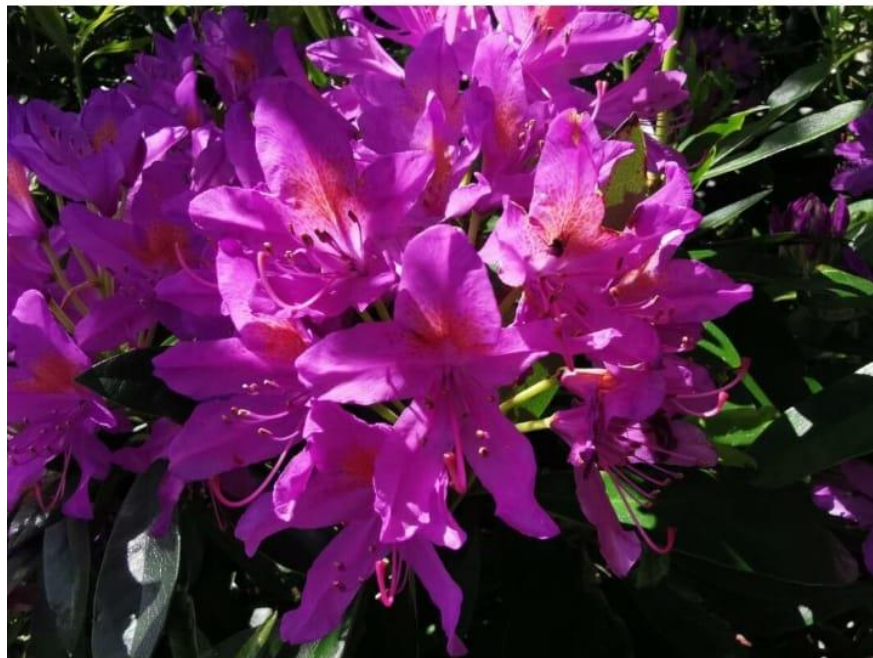


Figure 2. *Rhododendron ponticum*

Mad honey poisoning was first described by the Athenian writer and military commander Xenophon in 401 BC as follows; “... *but the swarms of bees in the*

neighborhood were numerous, and the soldiers who ate of the honey all went of their heads, and suffered from vomiting and diarrhea, and not one of them could stand up, but those who had eaten a little were like people exceedingly drunk, while those who had eaten a great deal seemed like crazy, or even, in some cases, dying men. So they lay there in great numbers as though the army had suffered a defeat, and great despondency prevailed. On the next day, however, no one had died, and at approximately the same hour as they had eaten the honey they began to come to their senses; and on the third or fourth day they got up, as if from a drugging". It was even used as a biological weapon in the Third Mithridatis War between the Pontic Kingdom and the Roman Empire in northern Anatolia in the first century BC. (30,31)

Instances of poisoning caused by mad honey were documented in Europe and North America during the 18th century. During the annual meeting of the American Pharmacists Union in 1896, Kebler reported cases of mad honey poisoning in the United States, contributing to the existing literature. The report detailed eight cases of poisoning that occurred in Princeton, New Jersey. Kebler not only discussed his own cases but also referenced previous research. According to Kebler, Barton was the first American to report the effects of mad honey poisoning. Barton presented his findings at a gathering of American philosophers in 1794 and later published them in 1802. One of the cases involved a 54-year-old woman who had ingested mad honey. She exhibited symptoms such as a burning forehead, pale complexion, shortness of breath, and an irregular pulse when she arrived at the hospital. The patient experienced visual hallucinations and subsequently lost consciousness, experiencing a brief generalized tonic-clonic seizure. After vomiting, the patient regained consciousness, but she still had numbness in her tongue and dilated pupils (mydriasis) (26). And since it was first reported, poisoning cases due to mad honey have continued to be seen especially in our country.

Mad honey is also used as an alternative medicine for high blood pressure, diabetes, gastrointestinal disorders, abdominal pain, arthritis, impotence, improving sexual performance, various viral infections, skin disorders, pain and colds (1). Its use as a sexual enhancer is the biggest reason for the ongoing demand. Only South Korea imported over 8000 kg of mad honey from Nepal between 2003-2004 and the South Korean government banned its import in 2005. However, despite the ban, poisoning cases still have been continued to occur (9-18, 21-30). Unfortunately, mad honey cases will

unfortunately continue to occur all over the world due to the traditional use among the people and the easy transport of honey.

2.2 Geographical Distribution of Mad Honey Disease

Poisoning due to mad honey is common in our country, especially in the Black Sea region. The intensive growth of *Rhododendron* species in this region is the main reason for this frequency. The main *Rhododendron* species containing grayanotoxin are found in the northern regions of our country (Figure 3) (33).

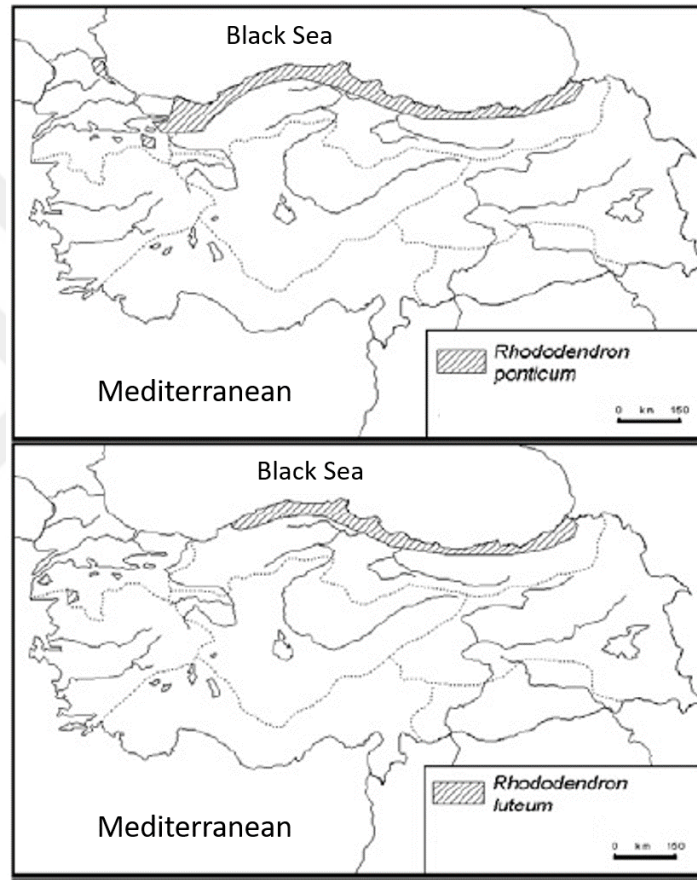


Figure 3. Geographical distribution of toxic rhodanderson species in Turkey

While the majority of cases have historically been reported in the Black Sea region, there is a potential for the disease to occur in other regions due to various factors.

The transport and export of locally produced honey from Turkey or other affected areas can lead to cases of mad honey disease in different parts of the world. As the Turkish population spreads geographically, it increases the likelihood of encountering honey contaminated with grayanotoxins in regions where it was previously uncommon.

For instance, cases of mad honey disease have been reported in other parts of Turkey itself, as well as in countries such as Germany, Austria, Switzerland, and Korea. In the case of Korea, significant quantities of toxic honey were imported until 2005 when its importation was prohibited.

Furthermore, there have been instances of grayanotoxin intoxication from honey produced outside of Turkey. Some cases in Nepal and Reunion Island highlight the occurrence of local wild honey poisoning caused by grayanotoxins.

Overall, while the known cases of mad honey disease are primarily concentrated in the Black Sea region, the transport, export, and consumption of contaminated honey from Turkey or other affected areas, as well as the global movement of populations, can potentially result in cases occurring in other regions. It is essential to be aware of the risks associated with consuming honey from known high-risk areas and to follow appropriate safety measures (34).

2.3. Grayanotoxins

Grayanotoxins (andromedotoxin; rhodotoxin; dodecahydro-1,1,4a, 8a-tetramethyl-7,9aa H-cyclopenta[b]heptalene-2,4,8,11,11-aa,12(1H)- hexol-12-acetate (GTX-I); GTX-II; GTX-III) are found in the leaves and flowers of plants of the Ericaceae family, such as rhododendron (*Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*) (Figure 4). Three toxic components exist: GTX-I, GTX-II, and GTX-III. As early as the 1950s, grayanotoxins I, II and III were isolated from the leaves of *L. Grayana* (33,34) GTX-I and GTXIII are most potent with intraperitoneal LD50 in male mice of 1.28 and 0.91 mg/kg, respectively. The complex actions arise from a reversible increase in membrane permeability to Na^+ , resulting in depolarization, which can be blocked by tetrodotoxin. This mechanism is the basis of the membrane depolarization in squid axon, the positive inotropic effect in mammalian myocardium and may involve the $\text{Na}^+/\text{Ca}^{2+}$ exchange system. Grayanotoxins affect both cardiovascular and respiratory systems. Symptoms include a burning sensation in the mouth and pharynx, anoxia, salivation, emesis, muscular weakness, dimness of vision and bradycardia followed by severe hypotension, respiratory paralysis, coma, and death (13).

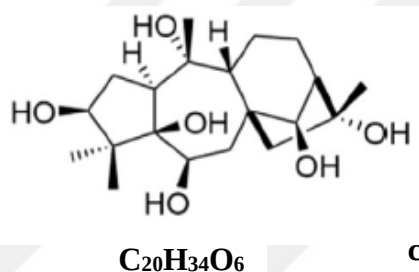
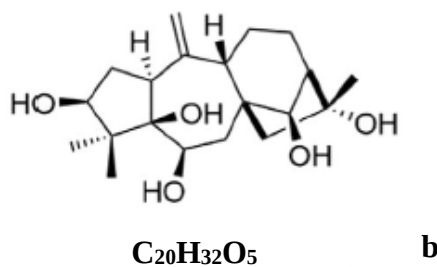
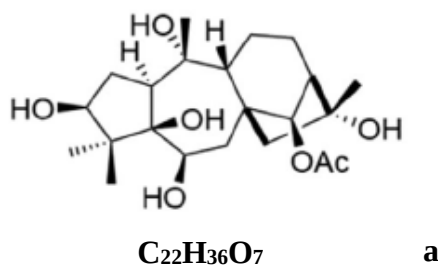


Figure 4. Structures of grayanotoxin- I(a) and grayanotoxin-II (b) and grayanotoxin-III(c)

2.4. Molecular effects of Grayanotoxin

Grayanotoxin's toxicity stems from its ability to attach itself to the group II receptor site located within the cell's voltage-gated sodium channels (Nav1x). Typically, the sodium channel alpha subunit protein consists of 24 interconnected membrane-spanning alpha-helices arranged in four sets of six alpha-helices each (S1-S6). These helices align the pore region in the resulting channel. Experiments conducted with a hydrophilic grayanotoxin analog, desacyl asebotoxin VII, using a squid axonal membrane, indicated that the probable binding site for grayanotoxin is located on the internal surface of the membrane. This hydrophilic analog showed activity only when applied from the cytoplasmic side. In a mutation analysis of Nav1.4, Maejima et al. demonstrated that all four S6 domains play a significant role in the interaction with

grayanotoxin. Binding of grayanotoxin alters the configuration of the channels to the extent that it prevents sodium channel inactivation, resulting in the cell remaining in a depolarized and activated state (12). Grayanotoxins only bind to the channel when it is open, and subsequently, the modified sodium channel's activation potential shifts towards hyperpolarization. Whether grayanotoxins interact with other members of the sodium channel family still requires further clarification (37).

Toxins can exert their effects on sodium ion channels at the cellular level by interfering with the transmission of action potentials and blocking these channels. This interference can lead to alterations in the function of sodium channels, resulting in changes in the excitability of excitable cells, such as muscles and nerves (37).

The binding and access of grayanotoxin to sodium channels are regulated by specific amino acid residues, namely phenylalanine (Phe) and tyrosine (Tyr). These residues facilitate the affinity of grayanotoxin to sodium channels, enabling the toxin to interact with these channels effectively (12).

Once bound, grayanotoxin exerts several effects on sodium channels. It prevents their inactivation, leading to sustained channel activity. This results in elevated membrane permeability, inhibits repolarization, and shifts the membrane potential towards hyperpolarization. As a consequence, depolarization is prolonged, resembling the effects of a cholinergic agent. These alterations in membrane potential and ion conductance contribute to the manifestation of symptoms such as bradycardia (slow heart rate), hypotension (low blood pressure), and depression of respiratory rate.

Notably, the sodium channels in skeletal muscles are more sensitive to grayanotoxin compared to those in cardiac muscles. Therefore, the observed responses in nerves, skeletal muscles, cardiac muscles, and the central nervous system are all interconnected and influenced by the membrane effects induced by grayanotoxin. It is important to understand that the specific molecular interactions and mechanisms by which grayanotoxin affects sodium channels and cellular functions may still require further investigation to fully elucidate the complex processes involved (38).

Specifically, grayanotoxin is known to bind to voltage-dependent sodium channels while they are in their open state. By attaching to these channels, grayanotoxin modifies their activity, potentially leading to disruptions in the normal functioning of

excitable cells. This can have implications for various physiological processes, including inotropy (changes in cardiac muscle contraction) and the transmission of nerve impulses.

The exact mechanisms by which toxins interact with sodium channels and modulate their function can vary depending on the specific toxin involved. Further research is needed to fully understand the intricacies of these interactions and their physiological consequences.

Grayanotoxins are likely to primarily target the voltage-gated sodium channels found in neurons. In animal studies conducted by Onat et al., injecting a small dose equivalent to 50 mg of honey directly into the brain's ventricles of anesthetized albino rats resulted in significant bradycardia (slow heart rate) and respiratory depression (39). However, a much larger quantity of honey extract, equivalent to 1 and 5 g/kg, was required when administered intraperitoneally to produce the same effects. This suggests that the central nervous system may play a more important role in the pathophysiology of grayanotoxins compared to the peripheral nervous system. Another possibility is that intraperitoneal injection may lead to higher elimination rates (39,40).

Nevertheless, in rats that underwent bilateral vagotomy (surgical removal of the vagus nerve), bradycardia did not occur following grayanotoxin administration. This indicates that peripheral vagal stimulation, which involves the vagus nerve, plays a role in grayanotoxin-induced bradycardia. As muscarinic receptors of the M2 subtype mediate vagal stimulation in the heart muscle, the effects of atropine, a non-specific anti-muscarinic agent, and AF-DX 116, a selective M2-receptor antagonist, were examined in another rat study. Rats were intraperitoneally injected with honey extract equivalent to 1 or 5 g/kg, and when significant bradycardia (defined as approximately 75% of the control heart rate) occurred, either 2 mg/kg of atropine sulfate or 20 mg/kg of AF-DX 116 was administered. The results showed that atropine completely eliminated both the grayanotoxin-induced bradycardia and respiratory depression, while AF-DX 116 only counteracted the bradycardic effect. Based on these findings, it was concluded that M2 receptor subtypes are involved in grayanotoxin-induced cardiotoxicity but not respiratory toxicity (12,34,40).

2.5. Clinical signs and symptoms

A number of clinical signs have been associated with gyanatoxin. Most often hypotension, cardiac rhythm disorders (first, second and third degree atrioventricular (AV) block, asystole, and sinus bradycardia), nausea and/or vomiting, sweating, dizziness and impaired consciousness have been observed. More rarely, syncope, blurred vision or diplopia and salivation have been described. In some rare cases, convulsion, atrial fibrillation, asystole and myocardial infarction were observed (34).

Several cases of mad honey intoxication resulting in hospitalization have been documented, with cardiac manifestations of grayanotoxin being the primary cause. The most common physical findings in these cases are bradycardia and hypotension, and other symptoms and findings are generally associated with these two primary symptoms. These two symptoms are present in up to 90% of the reported cases. Stimulation of the afferent cardiac branches in the vagus nerves leads to tonic inhibition of the vasomotor centers, which subsequently reduces sympathetic output. This process ultimately results in bradycardia, hypotension, and peripheral vasodilation. Patients may experience sensations of chest heaviness or tightness and exhibit ST changes on electrocardiograms (ECGs), resembling those seen in acute coronary syndromes. These manifestations are caused by the combination of bradycardia and hypotension, which reduce blood supply to the coronary arteries and subsequently decrease oxygen supply to the heart muscle. Cases of mad honey-induced myocardial infarction/ischemia have also been reported (41-43).

Sinus bradycardia, nodal rhythms, and varying degrees of atrioventricular block are the most commonly observed cardiac rhythm disorders in cases of mad honey intoxication or poisoning. Other symptoms that have been reported include hypothermia, second-degree heart block, extreme QT prolongation leading to blocked left bundle branch, atrial fibrillation, asystole, and Wolff-Parkinson-White syndrome. It is worth noting that there has been only one documented case of mad honey intoxication in a pediatric patient, who experienced sinus bradycardia (1).

In addition to the cardiovascular effects and rhythm disorders mentioned earlier, patients affected by mad honey intoxication may exhibit symptoms related to the gastrointestinal, respiratory, and neurological systems. These symptoms include dizziness, excessive sweating, feeling lightheaded, confusion or impaired consciousness,

tingling or numbness around the mouth, blurred vision, double vision, vomiting, nausea, chest pain, fatigue, and overall weakness. Some individuals may also experience increased saliva production and convulsions, which can be attributed to the depolarization of neurons in the central nervous system.

Animal studies have provided insights into the effects of grayanotoxin, the main toxic component of mad honey. These studies have revealed various effects, including respiratory depression, decreased blood lipid and sugar levels, renal toxicity (evidenced by proteinuria and haematuria), alterations in the antioxidant enzyme system, induction of stress (noted by increased levels of lipid peroxidation marker, MDA), liver toxicity (characterized by focal necrosis, congestion, dilation of hepatic central vein, transaminitis, infiltration of inflammatory cells in the liver tissue, and involvement of the hepatic portal triad), as well as reduced amplitude and frequency of epileptiform spike, convulsions, and seizures (1).

It is important to note that while these effects have been observed in animal studies, there is limited information on the specific effects in humans. However, a study by Turkmen et al. demonstrated significant blood pressure lowering effects in rats injected with grayanotoxin III (a component of mad honey) at doses of 200 mcg/kg, 400 mcg/kg, and 800 mcg/kg. The study further observed a dose-dependent decrease in blood pressure and heart rate in the rats. These findings provide insight into the mechanisms underlying the hypotension and bradycardia frequently observed in humans intoxicated or poisoned by mad honey (44).

It is crucial to exercise caution when interpreting the results of animal studies and consider that the effects observed in animals may not directly translate to humans. Further research, including human studies, is needed to fully understand the physiological effects and potential toxicity of mad honey in humans.

When people ingested grayanotoxin, Symptoms usually appear within 20 minutes to 3 hours after ingestion and persist for 1 to 2 days. In severe cases presenting to the emergency department, treatment usually consists of atropine (0.5 mg once, or twice if there is no response in terms of blood pressure and heart rate; 2 mg once) and saline (100 mL/h) (14). Usually, the intoxication could be cured by atropine and normal saline infusion. In some severe cases when atropine and saline are not adequate, advanced cardiac life support (ACLS) bradyarrhythmia protocols could be helpful. These effective

protocols dramatically reduced the mortality rate. In fact, there has been no modern report of a fatality although it may appear that fatal complications may develop in some cases (35).

Also, there is a case report on goats that ILE treatment can be useful in grayatoxin poisonings (23).

2.6. Grayanotoxin and oxidative stress

Oxidative stress refers to an increase in the production of free radicals within cells or tissues. It occurs when there is an imbalance between the body's defense system and the production of oxidants. Oxidative stress commonly results in the degradation of membrane lipids. Malondialdehyde (MDA) is a byproduct that forms as a result of the oxidative breakdown of certain natural macromolecules, and its presence can be either free or bound to specific biological structures. Additionally, nitric oxide (NO) is produced at the cellular level in the biological system during the process of oxidative stress. (45)

Superoxide anion is produced in living cells during mitochondrial respiration or when the enzymatic systems like nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase are abnormally activated. Superoxide dismutase (SOD) interacts with both nitric oxide (NO) and superoxide anion, effectively inhibiting the formation of peroxynitrate and peroxynitrite (ONOO). SOD converts superoxide anions into hydrogen peroxide (H_2O_2) using metal ions like copper and iron present in the physiological environment. Catalase (CAT) converts two molecules of H_2O_2 into two molecules of water (H_2O) and oxygen (O_2). Mitochondria are a significant source of intracellular H_2O_2 , and CAT plays a crucial role in eliminating this accumulating radical. Glutathione peroxidase (GSH-Px), a selenoenzyme, primarily protects cells from the harmful effects of hydroxyperoxides by utilizing glutathione (GSH).(45-47)

The occurrence of oxidative stress involves two main mechanisms. The first mechanism is the increase in the production of free radicals. The second mechanism is the insufficient functioning of the enzymatic and non-enzymatic antioxidant defense systems within the cells. One of the significant mechanisms through which toxic components or diseases exert their toxic effects is by promoting the generation of high levels of free radicals. Commonly employed techniques to evaluate the potential oxidative stress caused by free radicals include measuring the levels of MDA, NO and 4-hydroxynonenal (HNE) in blood and tissues. Additionally, assessing the activity of

antioxidant enzymes is a preferred method to detect changes in the antioxidant defense mechanism. Enzymes such as CAT, SOD, GSH-Px, and glucose-6-phosphate dehydrogenase (G6PD) are among the enzymes that are evaluated based on their activity levels.(45,48,49)

The consumption of mad honey leads to various side effects and toxic reactions related to the presence of GTX. While some of the toxic effects of GTX and their mechanisms have been identified in both humans and animals, there is limited information available regarding the oxidative stress effects caused by mad honey or GTX.

Considering the effects of GTX, it is not unreasonable to think that it may increase oxidative stress. Previous reports have suggested that honey has the potential to induce oxidative stress, and this is attributed to the presence of GTX (50,51).

Eraslan et al. also observed an increase in MDA, NO, HNE levels in acute, subacute and chronic periods and a decrease in SOD, CAT and GSHPx antioxidant enzyme activities in rats given GTX compared with control groups in their study in 2018 (47). In the light of these data, it is clear that GTX creates free oxygen radicals and causes serious variability in oxidative stress parameters. It is also in parallel with the results of previous studies (50,51).

2.7. Atropine

Atropine or atropine sulfate is approved by the FDA for various indications, including anti-sialagogue/anti-vagal effect, treatment of organophosphate/muscarinic poisoning, and management of bradycardia. It functions as a competitive and reversible antagonist of muscarinic receptors, making it an anticholinergic drug. This description encompasses information about its approved uses, mechanism of action, proper administration guidelines, potential side effects, contraindications, toxicology considerations, and recommended monitoring procedures (52).

2.7.1 Mechanism of Action

Atropine functions as an antimuscarinic agent by competitively inhibiting the acetylcholine receptors on postganglionic neurons. It also has a direct vagolytic action, leading to the inhibition of acetylcholine receptors in smooth muscle, thereby reducing parasympathetic activity. As a result, the predominant effect is an increase in sympathetic

stimulation, leading to enhanced cardiac output and other side effects associated with antimuscarinic medications (52).

2.7.2 Atropine Indications

Atropine or atropine sulfate is approved by the FDA for the treatment of several conditions, including anti-sialagogue/anti-vagal effect, organophosphate/muscarinic poisoning, and bradycardia. The drug gets its name from the plant *Atropa belladonna*, from which it was originally synthesized (52).

2.7.3 Atropine Administration

Atropine can be administered through various routes including intravenous (IV), subcutaneous, intramuscular, or endotracheal (ET) methods, with IV administration being preferred. For ET administration, it is recommended to dilute 1 mg to 2 mg of atropine in 10 mL of sterile water or normal saline before administering. In pediatric cases, the usual IV dose should be doubled and diluted in 3 to 5 mL for ET administration.

Here are the recommended dosages for different indications:

- Antisialagogue/Anti-vagal: Administer 0.5 mg to 1 mg every 1 to 2 hours.
- Organophosphate or Muscarinic Poisoning: Administer 2 mg to 3 mg every 20 to 30 minutes, with doses up to 20 mg possible. Titrate the dose to control secretion.
- Bradycardia: Administer 1 mg every 3 to 5 minutes, with a maximum cumulative dose of 3 mg. Repeat as necessary to achieve the desired heart rate. Atropine is most effective for sinus and AV nodal disease.

For Rapid Sequence Intubation Pretreatment:

- Adults: Administer 0.01 mg/kg IV in cases of bradycardia caused by repeated dosing of succinylcholine.
- Pediatric: Administer 0.02 mg/kg IV, with a minimum dose of 0.1 mg.
- Routine use as a pretreatment is not recommended.

In general, the dosing of atropine can be repeated every 5 minutes, up to a maximum of 0.04 mg/kg.

It's important to note that dosing in adults greater than 0.5 mg and slow IV pushes may lead to paradoxical bradycardia (although likely transient) and ventricular fibrillation (52).

2.7.4. Adverse effects

The most frequently observed adverse effects of atropine are associated with its antimuscarinic properties. These include dry mouth (xerostomia), blurred vision, increased sensitivity to light (photophobia), rapid heart rate (tachycardia), flushing, and warm skin. Constipation, difficulties with urination, and reduced sweating (anhidrosis) can also occur, particularly in vulnerable populations such as the elderly. Although rare, cases of delirium or coma have been reported. Hypersensitivity reactions may occur, usually presenting as a skin rash that may progress to skin exfoliation.

However, life-threatening side effects due to atropine have been reported, although rarely (16-18).

2.7.5. Contraindications

Clinicians should exercise caution when administering atropine to patients with certain conditions. These include coronary heart disease, acute myocardial ischemia, congestive heart failure, tachycardia, or hypertension. The increased cardiac demand and potential exacerbation of tachycardia and hypertension can have negative effects on patient outcomes.

Additionally, caution is warranted when using atropine in elderly patients, those with chronic lung disease, acute angle glaucoma, obstructive diseases such as uropathy, toxic megacolon, paralytic ileus, pyloric stenosis, or prostatic hypertrophy. Atropine use should also be approached with caution in patients with myasthenia gravis or in situations involving exposure to high environmental heat (52).

2.7.6. Atropine in rats

Although atropine is the standard treatment approach in grayanotoxin intoxication, we did not use atropine as a control group in our study because it is very difficult to see its anticholinergic effects in rats. Many reports stated that it should be used at doses up to 80 mg/kg for anticholinergic effects and suggested that another drug should be used to create a model (52-55).

2.8. Intravenous lipid emulsion

Intravenous lipid emulsions (ILE) were originally developed to provide essential fatty acids as a nutritional supplement when enteral feeding was not possible or inadequate. Besides serving as a concentrated source of cellular energy, fats also play a crucial role in various cellular functions. They contribute to the formation of cell membranes, participate in signal cascades as second messengers, regulate inflammation and platelet function, and are involved in the biosynthesis of cholesterol and endogenous steroids (56).

In recent years, the potential of intravenous lipid emulsions (ILE) to function as an antidote for drug toxicity, specifically in cases of local anesthetic toxicity, has been acknowledged. This application of ILE involves using lipid emulsions to effectively mitigate the adverse effects of certain drugs, providing a promising approach in the field of toxicology.

The use of intravenous lipid emulsions (ILE) as an antidote in humans has been documented in relatively limited reports. In 2006, Rosenblatt et al. reported the first successful utilization of intravenous lipids in a human case involving bupivacaine systemic toxicity. This case marked an important milestone in demonstrating the potential effectiveness of ILE as a treatment for drug toxicity in clinical settings. Since the initial successful case reported by Rosenblatt et al. in 2006, numerous additional cases have been documented, amounting to hundreds of reported cases. These cases further support the growing body of evidence for the use of intravenous lipid emulsions (ILE) as an effective antidote in various instances of drug toxicity. The accumulation of these cases highlights the increasing recognition and utilization of ILE as a treatment option in clinical practice. The available reports on ILE as an antidote encompass not only local anesthetic toxicity but also involve a broader range of drugs. These reports include cases involving cardiovascular drugs, antipsychotic agents, and herbicides, among others. However, it is important to note that despite the growing number of reported cases, there is a lack of randomized clinical trials with adequate statistical power to definitively assess the effectiveness of ILE rescue therapy. Further research and well-designed clinical trials are needed to establish a clearer understanding of the efficacy of ILE as a therapeutic intervention (56,57).

2.8.1 Mechanisms of action as an antidote

The precise mechanisms of action of parenteral lipid emulsion in toxicology are not yet fully elucidated. However, three proposed mechanisms have been suggested.

2.8.1.1 Modulation of Intracellular Metabolism

It is hypothesized that ILE may influence intracellular metabolism, potentially altering the toxic effects of certain drugs or toxins. The modulation of intracellular metabolism is proposed as one of the mechanisms of action of ILE in the treatment of xenobiotic-induced toxicity. In experimental models, where xenobiotics disrupt intracellular energy metabolism, the administration of ILE has shown effectiveness in reversing toxicity, suggesting a role in repairing or circumventing the dysfunction caused by these xenobiotics. For example, in the case of bupivacaine-induced toxicity, which blocks carnitine-dependent mitochondrial lipid transport and inhibits ATPase synthetase in the electron transport chain, ILE has been shown to reverse cardiac dysfunction even at doses thought to be too low to significantly decrease bupivacaine concentrations. This suggests that ILE's beneficial effects are not solely related to the binding of bupivacaine but may involve a metabolic effect by providing an exogenous source of fatty acids (FFAs) as an energy supply (57).

However, there is limited experimental evidence to fully support the modulation of intracellular energy metabolism as the sole mechanism of action of ILE. Some studies indicate that ILE's effects may involve mechanisms other than mitochondrial energy supply. For instance, in an *in vivo* model of verapamil toxicity, where rodents were pretreated with a fatty acid oxidation inhibitor (oxfenicine) and then resuscitated with ILE, there were no significant differences in survival time and mean arterial pressure compared to the control group. This suggests that ILE's action in verapamil toxicity may not be primarily through mitochondrial energy supply (57).

The understanding of ILE's mechanisms of action is still evolving, and different mechanisms may be at play depending on the specific xenobiotic and its toxic effects. Further research is needed to elucidate the precise mechanisms involved in the beneficial effects of ILE in the treatment of xenobiotic-induced toxicity.

2.8.1.2. Lipid Sink, Sponge, or Conduit Mechanism

This theory proposes that ILE acts as a "sink" or "sponge" for lipophilic substances, drawing them away from target tissues or organs and sequestering them within the lipid emulsion. This mechanism may reduce the concentration and distribution of the toxic agent, thereby mitigating its harmful effects (57).

The lipid sink, conduit, or sponge model proposes that ILE acts by "soaking up" lipid-soluble xenobiotics, removing them from the site of toxicity. This mechanism suggests that ILE sequesters the xenobiotic in a nonaqueous part of the plasma, separate from the aqueous plasma that bathes the tissues. It is believed that ILE alters the distribution of lipid-soluble xenobiotics, redistributing them away from the site of toxicity and creating a "lipid conduit."

Experimental studies have provided support for this mechanism. For example, in studies on bupivacaine toxicity, lipid emulsion administration decreased the distribution of bupivacaine into tissues, resulting in decreased concentrations in the brain and myocardium. Similar effects have been observed with other xenobiotics such as clomipramine and amiodarone, where lipid emulsion altered their pharmacokinetics, leading to increased concentrations and changes in tissue distribution.

Case reports and experimental models also demonstrate that ILE can remove xenobiotics from the central nervous system (CNS), resulting in a reversal of CNS effects. This indicates that the mechanism of action is related to the removal of the xenobiotic from the CNS rather than an alteration in metabolism.

The degree of lipid solubility of a xenobiotic, as measured by partition coefficients ($\log P$ or $\log D$), is also considered clinically important. Lipid solubility affects the amount of xenobiotic that partitions into the serum lipid. *In vitro* models have shown that the distribution of bupivacaine and ropivacaine in lipid emulsion decreases at lower pH levels.

While the lipid sink or sponge model provides a compelling explanation for the effects of ILE on lipid-soluble xenobiotics, it is important to note that the precise mechanisms of action may vary depending on the specific xenobiotic and its mode of toxicity. Further research is needed to fully understand the mechanisms by which ILE acts as a lipid sink or conduit and its role in the treatment of xenobiotic-induced toxicity.

2.8.1.3. Activation of Ion Channels

Another proposed mechanism suggests that ILE may directly interact with ion channels in cell membranes, leading to changes in ion flux and membrane potential. This modulation of ion channels could potentially counteract the toxic effects of certain drugs or toxins.

The activation of calcium (Ca^{2+}) and sodium (Na^+) channels by lipid emulsion has been hypothesized as another potential mechanism of action. In experimental models using human embryonic kidney cells expressing human cardiac Na^+ channels, it was observed that linolenic and stearic acids, present in lipid emulsion, decreased bupivacaine-induced Na^+ channel blockade. This suggests that the fatty acids in lipid emulsion may have a protective effect on Na^+ channels (57).

Additionally, fatty acids have been shown to directly activate myocardial Ca^{2+} channels, leading to an increase in Ca^{2+} current. Oleic, linoleic, and linolenic acids, which are found in lipid emulsion, can act on the Ca^{2+} channel to enhance Ca^{2+} current. This activation of Ca^{2+} channels by fatty acids may contribute to the development of dysrhythmias.

Studies are not representative of the administration of lipid emulsion in humans or animals, and the dosages used in these experiments cannot be directly translated into clinical dosing regimens. Further research is needed to explore the specific effects of lipid emulsion on ion channels and their relevance to the treatment of xenobiotic-induced toxicity (57).

It's important to note that while these mechanisms have been proposed, further research is necessary to fully understand the exact modes of action by which ILE exerts its therapeutic effects in toxicology.

While the mechanisms of action of ILE in toxicology are not yet fully understood, the lipid sink, conduit, and sponge model is considered to be the most compelling explanation. This model suggests that ILE acts as a reservoir for lipid-soluble xenobiotics, regardless of their specific mechanisms of toxicity. By sequestering these lipophilic substances, ILE may help reduce their concentration and distribution in target tissues, thereby exerting a beneficial effect (57).

The effects of ILE may vary depending on the specific xenobiotic involved, and the mechanisms of action are likely multifactorial and can be influenced by various factors. While the lipid sink model provides a plausible explanation for the observed effects of ILE on lipid-soluble xenobiotics, further research is needed to fully elucidate the complete range of mechanisms by which ILE exerts its therapeutic benefits in toxicology.

2.8.2 Adverse effects

Indeed, while ILE has been generally considered safe, there have been reports of potential adverse effects associated with its administration. The systematic review you mentioned evaluated the possible adverse effects of ILE given for parenteral nutrition or poisoning (57).

Some of the reported complications and adverse effects associated with ILE administration include:

- Acute kidney injury: In rare cases, ILE administration has been linked to acute kidney injury, although the exact mechanism is not well understood.
- Cardiac arrest: Although rare, there have been reports of cardiac arrest following ILE administration, particularly in cases of local anesthetic toxicity. However, it's important to note that ILE is also used as a treatment for cardiac arrest caused by local anesthetic toxicity.
- Ventilation-perfusion mismatch and acute respiratory distress syndrome (ARDS): These pulmonary complications have been reported, but their frequency is low.
- Venous thromboembolism: There have been rare cases of venous thromboembolism associated with ILE administration.
- Hypersensitivity reactions: Allergic reactions, including rash, itching, and anaphylaxis, have been reported in rare instances.
- Fat embolism syndrome: Although rare, the potential for fat embolism syndrome exists following ILE administration, particularly in patients with compromised microcirculation or significant trauma.

- Fat overload syndrome: Prolonged or excessive administration of ILE can lead to fat overload syndrome, which is characterized by hypertriglyceridemia, hepatomegaly, splenomegaly, and potential liver dysfunction.
- Pancreatitis: In rare cases, ILE administration has been associated with acute pancreatitis.
- Extracorporeal circulation machine circuit obstruction: In some instances, the lipid emulsion has been reported to cause obstruction or malfunctioning of extracorporeal circulation machine circuits.
- Increased susceptibility to infection: Although not well-documented, there have been reports suggesting an increased risk of infection associated with ILE administration (55).

It's important to note that these adverse effects are relatively rare and the benefits of ILE therapy, particularly in cases of toxicological emergencies, often outweigh the potential risks. The decision to use ILE should be made based on a careful evaluation of the individual patient's condition, potential benefits, and risk factors. Close monitoring during and after ILE administration is crucial to promptly identify and manage any potential complications.

ILE can interfere with laboratory studies, making the results uninterpretable. Hyperlipidemia induced by ILE can affect various laboratory tests and lead to inaccurate measurements or the inability to perform certain tests. The duration of interference can last for several hours and may vary depending on the specific laboratory system used (57).

In vitro studies have shown that colorimetric methods are more susceptible to the effects of ILE compared to potentiometric methods. Glucose measurement by colorimetric methods, for example, may be significantly affected by ILE, leading to erroneous results. Hypoglycemia can be falsely reported as hyperglycemia due to interference by the lipid emulsion. For instance, a true glucose concentration of 48.6 mg/dL (2.7 mmol/l) might be inaccurately reported as 223.2 mg/dl (12.4 mmol/l).

To minimize interference, it is recommended to perform centrifugation at high speeds (e.g., 140,000 g for 10 minutes) to separate the lipid emulsion from the plasma or serum before conducting laboratory tests. This can help reduce the impact of lipid interference on the accuracy of test results (57).

The specific effects of ILE on assays for toxic drug concentrations are not well established. The influence of ILE on these assays can vary depending on the specific drug and assay methodology. Therefore, it is generally recommended to perform analytic testing before administering ILE whenever possible, to obtain reliable baseline measurements.



3. MATERIAL and METHOD

3.1 Materials

3.1.1 Chemicals

Acetic Acid / Sigma Aldrich

Bovine Serum Albumin (BSA) / Sigma Aldrich

Citric Acid / Sigma Aldrich

Copper Sulfate (CuSO_4) / Sigma Aldrich

Disodium phosphate (Na_2HPO_4) / Sigma Aldrich

Ethylenedinitrilotetraacetic acid disodium salt dihydrate (Na_2EDTA) / Sigma Aldrich

Formaldehyde / Sigma Aldrich

Glutathione (GSH) / Sigma Aldrich

Glutathione Reductase (GR) / Sigma Aldrich

Grayanotoxin-III (GTX-III) / Düzce University Medical Faculty, Medical Pharmacology Department

Hydrogen peroxide (H_2O_2) / Sigma Aldrich

Intavenous lipid emulsion (Clinoleic %20) / Baxter

Ketamine (Ketazol %10) / Richter Pharma ag

Monosodium diphosphate (NaH_2PO_4) / Sigma Aldrich

Nicotinamide adenine dinucleotide phosphate (NADPH) / Sigma Aldrich

Potassium Chloride (KCl) / Sigma Aldrich

Sodium Azide / Sigma Aldrich

Sodium Carbonate (Na_2CO_3) / Sigma Aldrich

Sodium dodecyl sulfate ($\text{NaC}_{12}\text{H}_{25}\text{SO}_4$) / Sigma Aldrich

Sodium Potassium Tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6$) / Sigma Aldrich

Thiobarbituric Acid (TBA) / Sigma Aldrich

t-butyl hydroperoxide (TBHP) / Sigma Aldrich

Tetramethoxy propane / Sigma Aldrich

Trichloro acetic acid (TCA) / Sigma Aldrich

tri-Sodium citrate dehydrate / Sigma Aldrich

Xylazine (Rompun (2%) / Bayer

3.1.2. Devices

Spectrophotometer / Thermo Scientific,

Evolution 300 Centrifuge / Sigma 3-16 PK

Tissue Homogeniser / Heidolph, Silent Crusher S

Veterinary patient monitor (Aurora 12) / Konsung Medical Equipments

3.1.3. Animal Material

27 adult (14-18 weeks old) female Wistar albino rats weighted between 235-277gr were obtained with the approval of Yeditepe University Animal Experiments Local Ethics Committee (HADYEK; decision number 2021/08-3). All applicable international, national and/or institutional guidelines for the care and use of animals were followed. Animals were kept in 12-h daylight and 12-h night environment at 24 ± 4 °C and were fed with standard water and food.

3.1.4. Preperation of Grayanatoxin

Grayanatoxin-III to be used was prepared with a honey sample containing 45 µg/ml GTX-III. Samples were obtained from the Pharmacology laboratory of Düzce University. The ratio of GTX-III in honey was determined according to the analysis method of Dönmez and Kaya published in 2020 (58)

Firstly, the LD50 of GTX-III was determined in rats. Honey extract containing 80 $\mu\text{g/kg}$ GTX-III was given intraperitoneally (i.p.) to 2 animals from the same cage. No death was observed in the animals. The dose was doubled (160 $\mu\text{g/kg}$) and again given i.p. to two animals. Decreased movement was observed in the animals, but they returned to normal physical activity after a while and no death was observed in the follow-up. The third and last time, when 240 $\mu\text{g/kg}$ dose was given, death was observed in both animals. In the experiment, 240 $\mu\text{g/kg}$ dose was used to see the effectiveness of ILE on the cardiac effects of the toxin. (Figure 5).

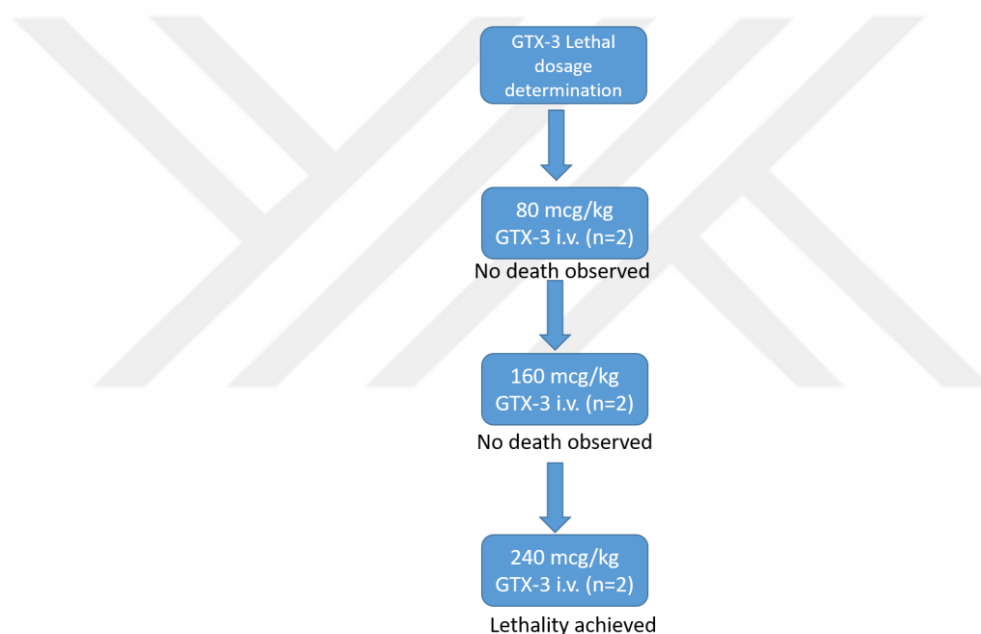


Figure 5. Determination of lethal dosage of GTX-III

3.1.5. Experiment Design

Animals were anaesthetised by i.p. administration of 80 mg/kg Ketamine and 8 mg/kg Xylazine and placed on a thermal barrier to prevent heat loss. After making sure that complete anaesthesia is achieved by looking at skin or finger pinch responses and palpebra or corneal reflex, the left femoral artery was cannulated with a 26 G catheter and monitoring was performed to monitor systolic, diastolic, mean arterial pressure (MAP) and heart rate. Then, rats were treated with 240 $\mu\text{g/kg}$ of GTX-III intraperitoneally. Blood

pressure levels were recorded by the investigator. Cardiac rhythm was monitored with cardiac electrodes. Heart rate was also be recorded by the investigator. All monitoring was done with a veterinary monitor (Figure 6).

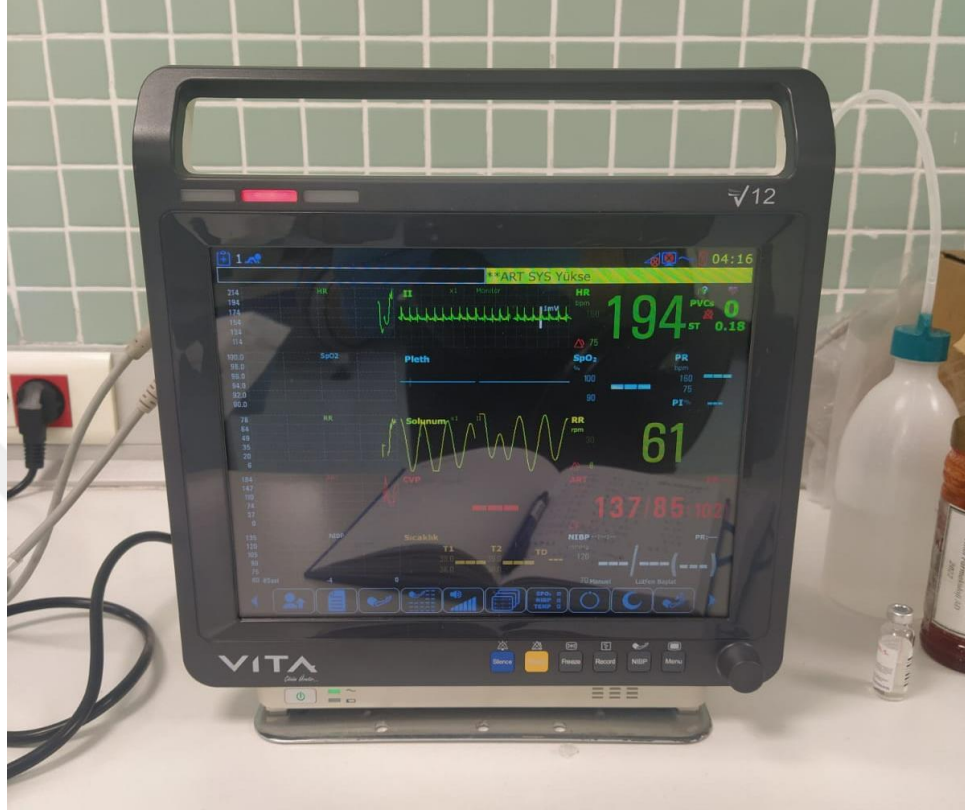


Figure 6. Veterinary monitor used for the experiment (green indicates heart rate, yellow indicates respiratory rate, and red indicates blood pressure)

In order to determine the toxic effects of intraperitoneally administered mad honey and the onset time of its effects, MAP and heart rate was monitored at the beginning of the procedure and every 5 minutes. Bradycardia, hypotension and changes in heart rhythm were observed in rats. In the follow-up, the last QRS wave in the ECG in which QRS is not seen for 5 minutes was considered as the moment of death.

After the toxic dose of grayanotoxin was determined by a preliminary study, the rats were divided into 3 groups of 7 rats in each group:

1. Lipid group (ILE) (also mentioned by control group),
2. Toxin group (GTX-III),
3. Treatment group (GTX-III + ILE)

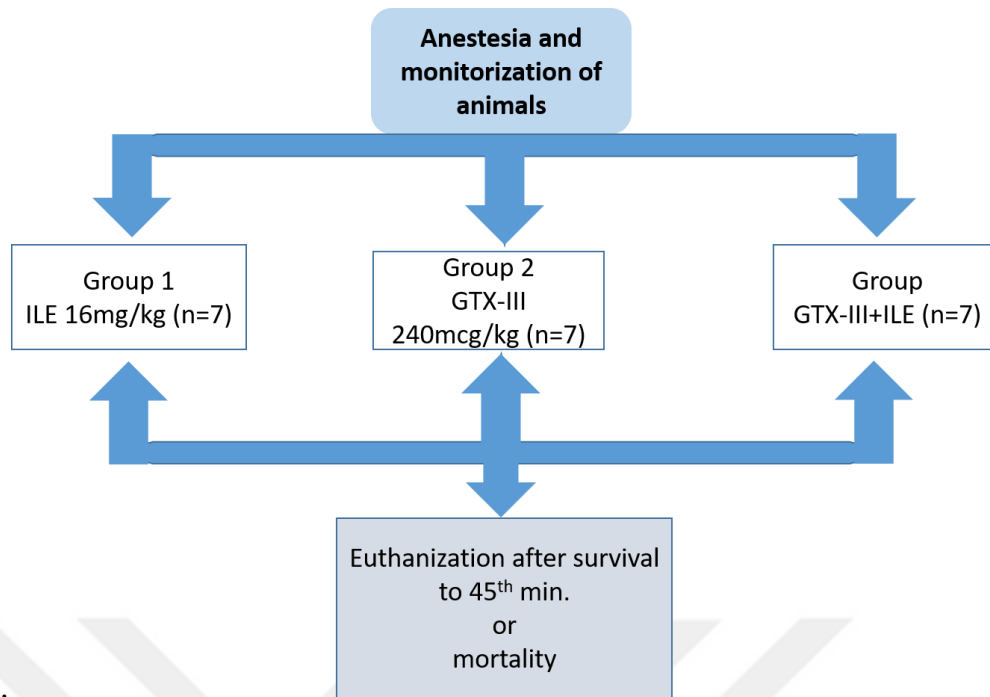


Figure 7. Experimental groups and design of the study

The first group was given ILE (16 ml/kg IV) which its dose was based on the dose in previous studies and the changes observed in vital parameters were recorded. The second group was given GTX-III (240 µg/kg) only at the determined lethal dose, and finally the treatment group was formed by giving GTX-III (240 µg/kg) and ILE (16 ml/kg IV) together (Figure 7). After anaesthesia was achieved with 80 mg/kg ketamine and 8 mg/kg Xylazine by intraperitoneal administration, skin or finger pinch responses and palpebra or corneal reflex were checked and rats were placed on a thermal barrier to prevent heat loss. Then, the femoral artery was identified after excision in the left inguinal region, cannulated with a 26 gauge (G) catheter, and a pressure transducer was connected for mean arterial pressure (MAP) and heart rate monitoring. A 26 G catheter was then be inserted into the right femoral vein, again for delivery of the treatments to be administered. Blood pressure levels and heart rate was recorded by the investigator. After the rats are anaesthetised, ECG monitoring was performed immediately and heart rates and rhythms was monitored (Figure 8). ILE infusion was started at 5 minutes after toxin administration and then heart rhythm, heart rate and blood pressure levels was recorded, MAP was measured and respiratory rate was checked every 5 minutes. The treatment was continue until death occurs and the first QRS without a QRS wave for 5 minutes was considered as the moment of death. After death, the blood samople was collected from

the heart and biochemical levels of Sodium (Na^+), Potassium (K^+), Hydrogen (pH), Bicarbonate (HCO_3^-), glucose, lactate and troponin were measured. Heart and liver tissues were taken and stored at -80°C for the evaluation of oxidative stress parameters. The data obtained from all groups were statistically interpreted and the efficacy of ILE treatment in Grayanotoxin intoxication was measured and evaluated. The experiment was terminated at 45 minutes considering the duration of anaesthesia efficacy of the animals in the Lipid group.



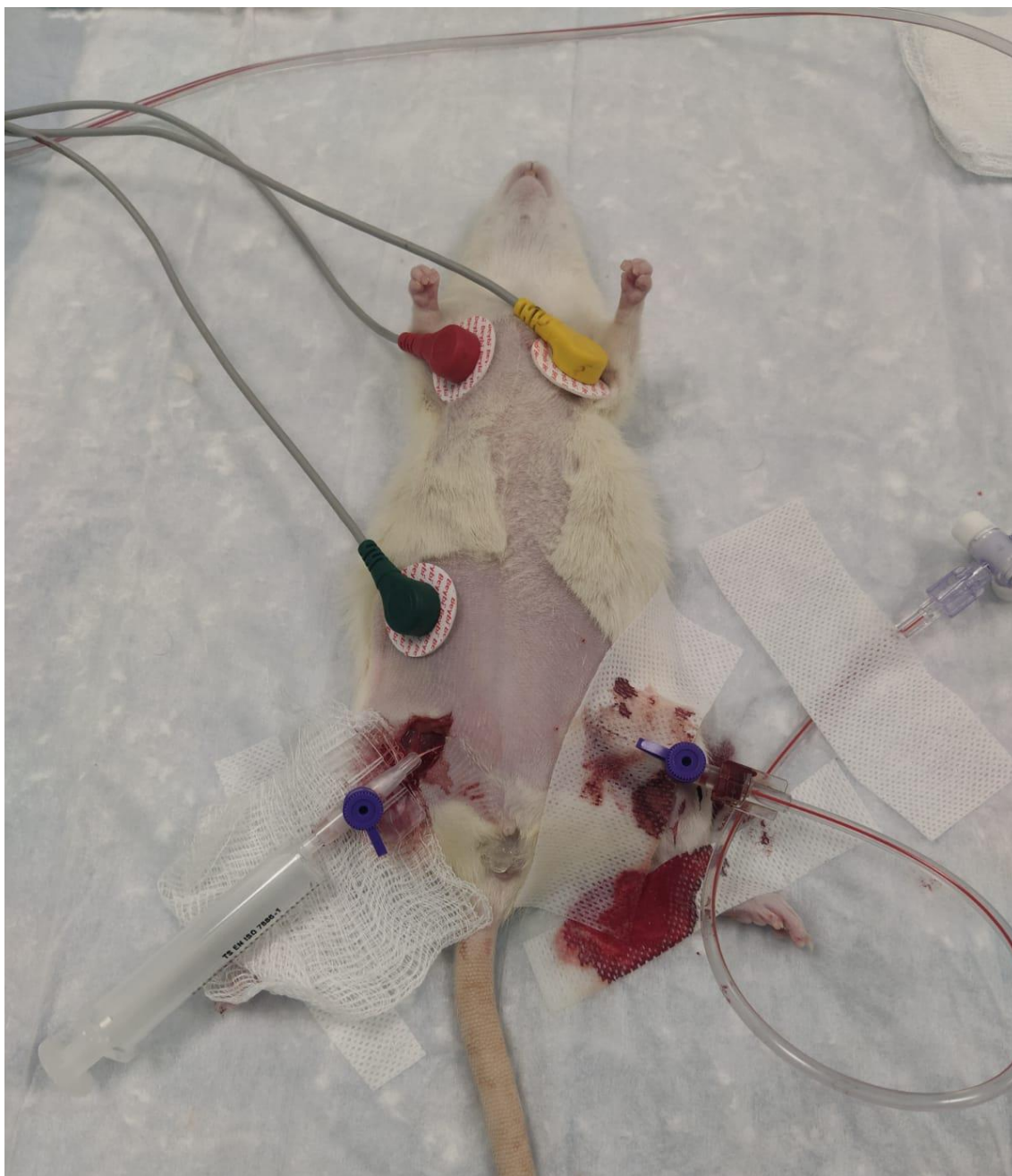


Figure 8. An image of a experiment subject (Left femoral intra artery access, right femoral intravenous access and heart monitorization electrodes placed)

3.1.6. Biochemical Analysis

At the end of the study, heart and liver MDA level, antioxidant enzyme activity were measured by spectrophotometric methods. In addition, activities of enzymatic antioxidants such as CAT, and GPx were determined.

3.1.6.1. Tissue Homogenization

1 g of the liver and heart tissue samples obtained separately from each rat were homogenized by the addition of 1.15% KCl solution and homogenized at 75 x 1000 rpm using a tissue homogenizer. For each sample, 1 ml of liver and heart homogenate was collected and placed in a deep freezer until used for protein assay.

3.1.6.2. Lowry Protein Assay

The Lowry protein assay is a biochemical method used to determine the total concentration of proteins. The presence of proteins in a sample solution is indicated by a color change that is dependent on the protein concentration. This color change can be measured using colorimetric techniques. In the assay, 250 mL of a 2% solution of sodium carbonate (Na_2CO_3), 2.5 mL of a 0.5% solution of copper sulfate (CuSO_4), and 5 mL of a 1% solution of sodium potassium tartarate (NaKTar) are mixed together to create the Lowry Reagent. The Folin solution is diluted with distilled water at a ratio of 1:2 (v/v). Six standard solutions with known protein concentrations were prepared from a 2% stock solution of bovine serum albumin (BSA). The absorbance values of the proteins were then measured at 640 nm using a spectrophotometer (59).

3.1.6.3. Measurement of Oxidative Damage Biomarkers - MDA Analysis

To create 0.2 mL of 10% samples and standard solutions, we utilized an 8.1% solution of sodium dodecyl sulfate (SDS), a 20% solution of acetic acid, and a 0.8% solution of thiobarbituric acid (TBA). The total volume of all samples was adjusted to 4 mL. Six standard solutions were prepared from a stock solution of tetramethoxy propane with a concentration of 0.05%. After adding distilled water and trichloroacetic acid (TCA) solution to the samples, we conducted spectrophotometric measurements to determine the absorbance values at 532 nm, representing the amount in nanomoles per gram of protein (59).

3.1.6.4. Liver and heart tissue GPx Analysis

GPx activities were assessed in homogenized samples of liver and heart. The reaction mixture consisted of 50 mL, containing 0.303 grams of Trizma base, 0.0186 grams of Na_2EDTA , 0.00834 grams of NADPH, 0.0130 grams of sodium azide, 0.0307 grams of GSH, and 50 units of GR. To initiate the reaction, 10 μL of the homogenate was combined with 990 μL of the reaction mixture, and then 10 μL of t-butyl hydroperoxide

(TBH) was added. The reduction in absorbance at 340 nm was monitored for a duration of 3 minutes, indicating the GPx activity in units per gram of protein (59).

3.1.6.5 Liver and heart tissue CAT Analysis

To create the phosphate buffer solution, 1.29 grams of monosodium diphosphate and 2.57 grams of disodium phosphate were dissolved in 500 mL of distilled water. The pH was adjusted to 7.0 by utilizing a solution of citric acid. For the hydrogen peroxide solution, 200 μ L of a 30% H₂O₂ solution was added to 50 mL of the phosphate buffer solution. In order to prepare eight standard solutions of CAT, they were diluted in the phosphate buffer. To assess the CAT enzyme activity in relation to the increasing concentration of H₂O₂, spectrophotometric measurements were conducted at 240 nm, representing the activity in kilounits per gram of protein (59).

3.1.7. Statistical Analysis

For statistical analyses, R version 2.15.3 programme (R Core Team, 2013) was used. Median, first quartile, and third quartile were used for reporting the study data. Kruskal-Wallis test and Dunn-Bonferroni test were used for intergroup analyses. Statistical significance was accepted as $p < 0.05$.

4. RESULTS

Survival times between the experimental groups and weight comparisons of the groups are given in Table 1. The survival times of the control (lipid) group and the treatment group were significantly higher than the survival times of the toxin group. In the lipid group, the duration of the experiment was limited to 45 minutes based on the duration of anaesthetic activity. There was a significant difference between the group weights, but all substances given were given as dose/kilogram.

Table 1. Comparison of experiment groups for weight and survival. (n:sample size, Q1:lower quartile, Q3:upper quartile)

	Lipid (n=7)	Toxin (n=7)	Treatment (n=7)	p value
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)	
Survival (min.)	45 (45, 45)	18 (17, 21)	35 (33, 45)	<0.001*
Weight (gr.)	267 (258, 274)	238 (235, 244)	258 (254, 265)	0.007*

Kruskal-Wallis test

*p<0.05

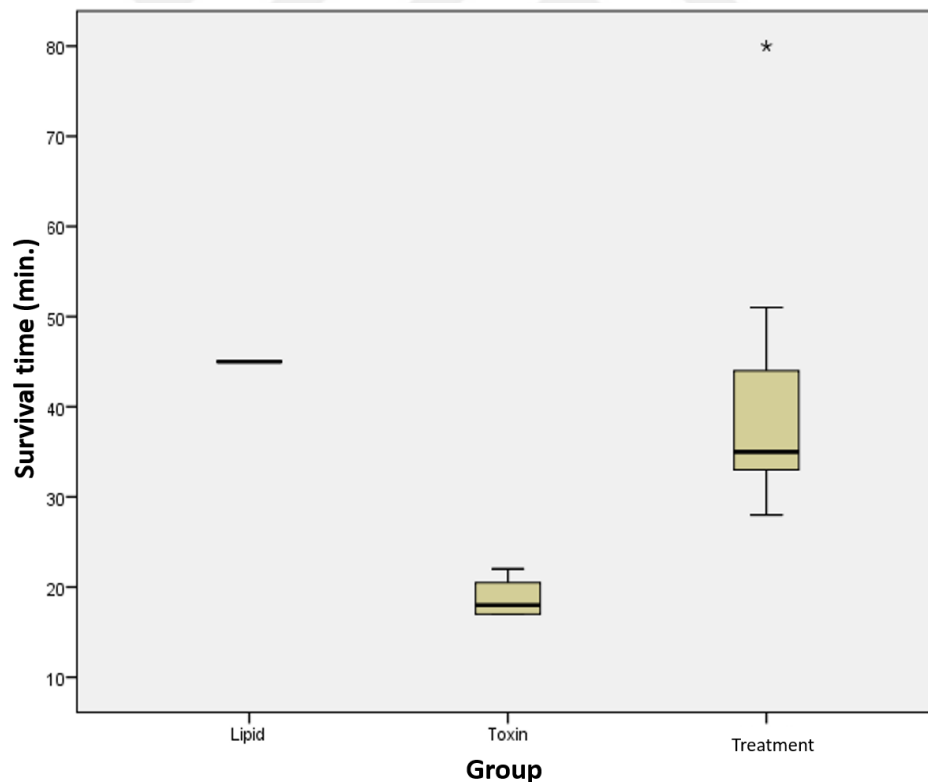


Figure 9. Survival time comparison of experiment groups (* represents one subject that survives to 80th minute. For statistical analysis survival time limited with lipid group as 45 minutes)

There was a statistically significant difference between the groups in terms of survival time ($p < 0.001$). As a result of the evaluations performed using Dunn-Bonferroni test, it was found that the duration of survival in the toxin group was shorter than that in the lipid and treatment groups ($p < 0.001$, $p = 0.036$, respectively). No significant difference was found between the other groups ($p > 0.05$) (Figure 9).

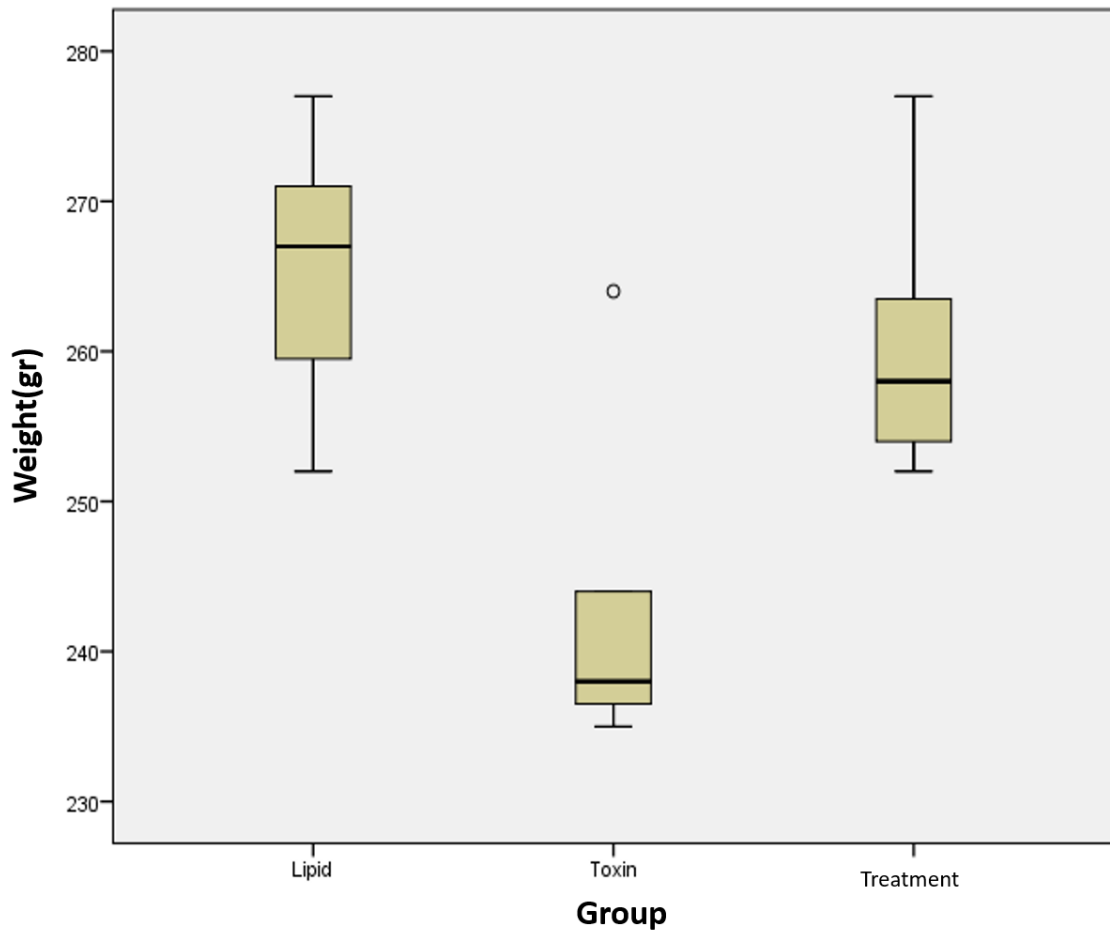


Figure 10. Weight comparison of experiment groups

A statistically significant difference was found between the groups in terms of weight ($p = 0.007$). As a result of the evaluations performed using Dunn-Bonferroni test, it was found that toxin cases were lighter than lipid group cases ($p = 0.007$). No significant difference was found between the other groups ($p > 0.05$) (Figure 10).

Systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP) and heart rate of all subjects were monitored continuously. Baseline values were recorded at 5 minutes after anaesthesia and the agents (ILE and/or GTX) were administered at 10 minutes after anaesthesia and this moment was called minute 0 of medication. Vital parameters were recorded every 5 minutes. Since the survival of the subjects in the toxin group was short, the time for statistical comparison ended at the 15th minute. Comparisons of systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP) and heart rate are shown in Table 2, Table 3, Table 4 and Table 5, respectively.

Table 2. Systolic blood pressure over time comparison data of experiment groups (n:sample size, Q1:lower quartile, Q3:upper quartile)

Systolic blood pressure	Lipid (n=7)	Toxin (n=7)	Treatment (n=7)	p value
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)	
Anesthesia 5. min.	138 (113, 156)	136 (118, 173)	150 (117, 156)	0.618
Medication 0. min.	137 (116, 142)	144 (122, 167)	156 (118, 184)	0.325
Medication 5. min.	165 (143, 194)	126 (113, 137)	176 (150, 180)	0.006*
Medication 10. min.	168 (115, 186)	115 (98, 123)	140 (130, 158)	0.016*
Medication 15. min.	134 (103, 145)	73 (46, 110)	127 (125, 146)	0.005*

Kruskal-Wallis test

*p<0.05

There was no statistically significant difference between the groups in terms of systolic blood pressure values at 5.min after anaesthesia and at the time of drug administration (p>0.05).

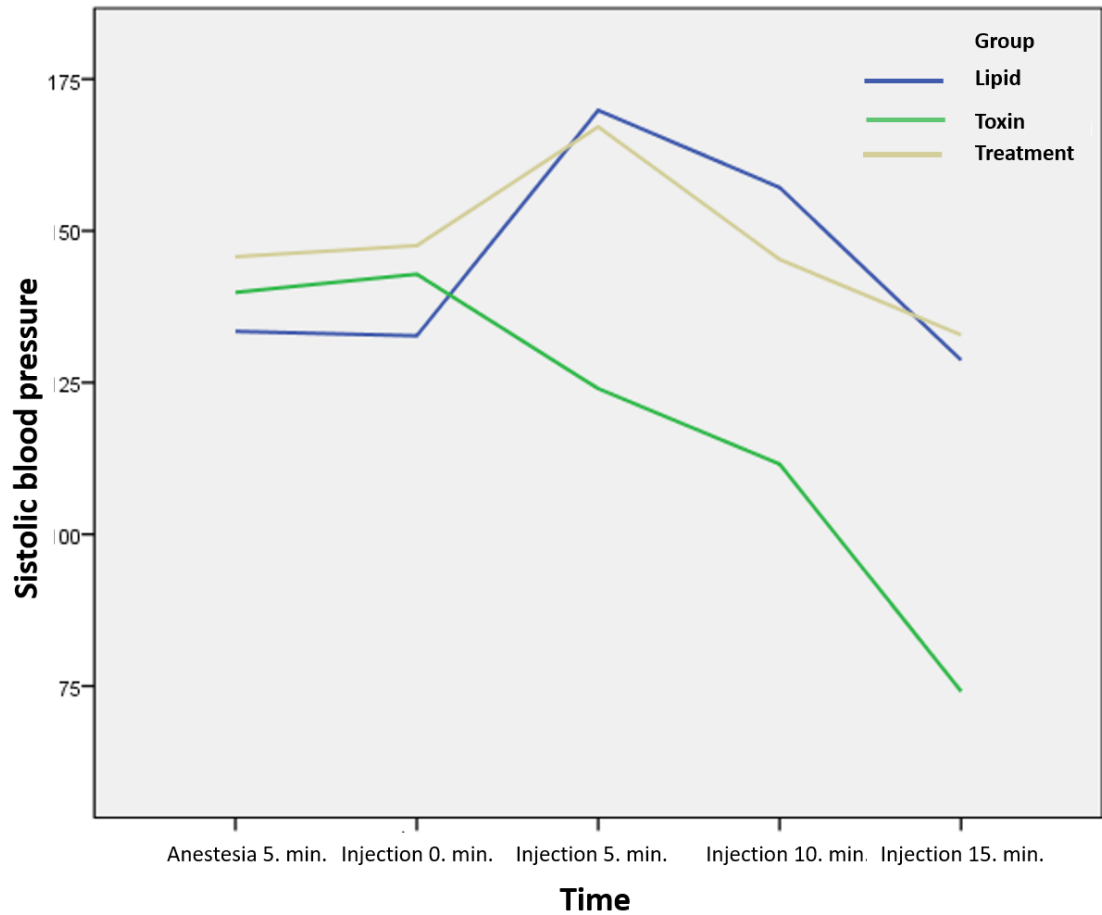


Figure 11. Systolic blood pressure over time comparison of experiment groups

There was a statistically significant difference between the groups in terms of systolic blood pressure values at 5.min after anaesthesia ($p=0.006$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than those of the lipid and treatment groups ($p=0.015$, $p=0.017$, respectively). No significant difference was found between the other groups ($p>0.05$) (Figure 11).

A statistically significant difference was found between the groups in terms of systolic blood pressure values at the 10th minute after the medication ($p=0.016$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than the values of the lipid and treatment groups

($p=0.040$, $p=0.037$, respectively). No significant difference was found between the other groups ($p>0.05$).

A statistically significant difference was found between the groups in terms of systolic blood pressure values at 15 min after the medication ($p=0.005$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than the values of the lipid and treatment groups ($p=0.037$, $p=0.007$, respectively). No significant difference was found between the other groups ($p>0.05$).

Table 3. Diastolic blood pressure over time comparison data of experiment groups
(n:sample size, Q1:lower quartile, Q3:upper quartile)

Diastolic blood pressure	Lipid	Toxin	Treatment	p value
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)	
Anesthesia 5. min.	91 (75, 104)	98 (90, 116)	110 (90, 112)	0.454
Medication 0. min.	98 (79, 102)	94 (85, 113)	108 (70, 119)	0.483
Medication 5. min.	108 (94, 116)	90 (80, 97)	115 (96, 120)	0.038*
Medication 10. min.	104 (76, 119)	82 (76, 88)	95 (77, 104)	0.183
Medication 15. min.	92 (70, 95)	40 (25, 64)	89 (84, 95)	0.006*

Kruskal-Wallis test

* $p<0.05$

There was no statistically significant difference between the groups in terms of diastolic blood pressure values at 5.min after anaesthesia, time of drug administration and 10.min after drug administration ($p>0.05$).

There was a statistically significant difference between the groups in terms of diastolic blood pressure values at 5.min after anaesthesia ($p=0.038$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group subjects were lower than the values of the treatment group subjects ($p=0.044$). No significant difference was found between the other groups ($p>0.05$).

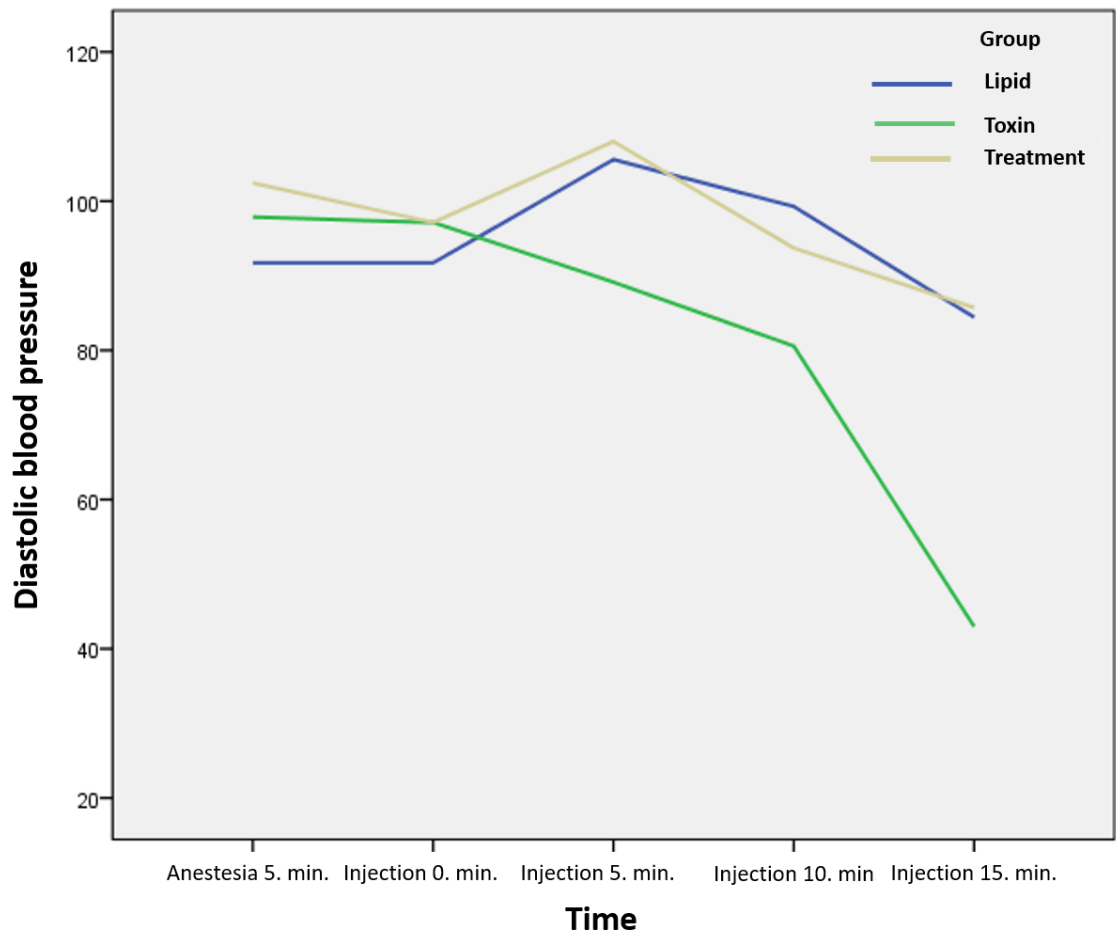


Figure 12. Diastolic blood pressure over time comparison of experiment groups

It was found that there was a statistically significant difference between the groups in terms of diastolic blood pressure values at the 15th minute after the drug ($p=0.006$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than those of the lipid and treatment groups ($p=0.019$, $p=0.014$, respectively). No significant difference was found between the other groups ($p>0.05$) (Figure 12).

Table 4. Mean Arterial Pressure (MAP) over time comparison data of experiment groups (n:sample size, Q1:lower quartile, Q3:upper quartile)

Mean Arterial Pressure (MAP)	Lipid	Toxin	Treatment	p value
	Median (Q1, Q3)	Median (Q1, Q3)	Medyan (Q1, Q3)	
Anesthesia 5. min.	105.33 (89, 121.33)	110.66 (99.33, 131.66)	123.33 (99, 126.66)	0.444
Medication 0. min.	112.66 (91.33, 113.33)	108 (98.66, 132.33)	116.66 (86, 141)	0.558
Medication 5. min.	127 (114.33, 142)	106.66 (91, 108.66)	136.66 (114, 140)	0.009*
Medication 10. min.	125.33 (87, 139.66)	93 (80.66, 98.66)	110 (92.66, 122)	0.088
Medication 15. min.	108 (80.66, 109.66)	53.33 (31.66, 79.33)	101.66 (98.33, 105)	0.005*

Kruskal-Wallis test

*p<0.05

There was no statistically significant difference between the groups in terms of MAP values at 5.min after anaesthesia, time of drug administration and 10.min after drug administration ($p>0.05$).

A statistically significant difference was found between the groups in terms of MAP values at 5.min after anaesthesia ($p=0.009$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than the values of the lipid and treatment groups ($p=0.026$, $p=0.023$, respectively). No significant difference was found between the other groups ($p>0.05$).

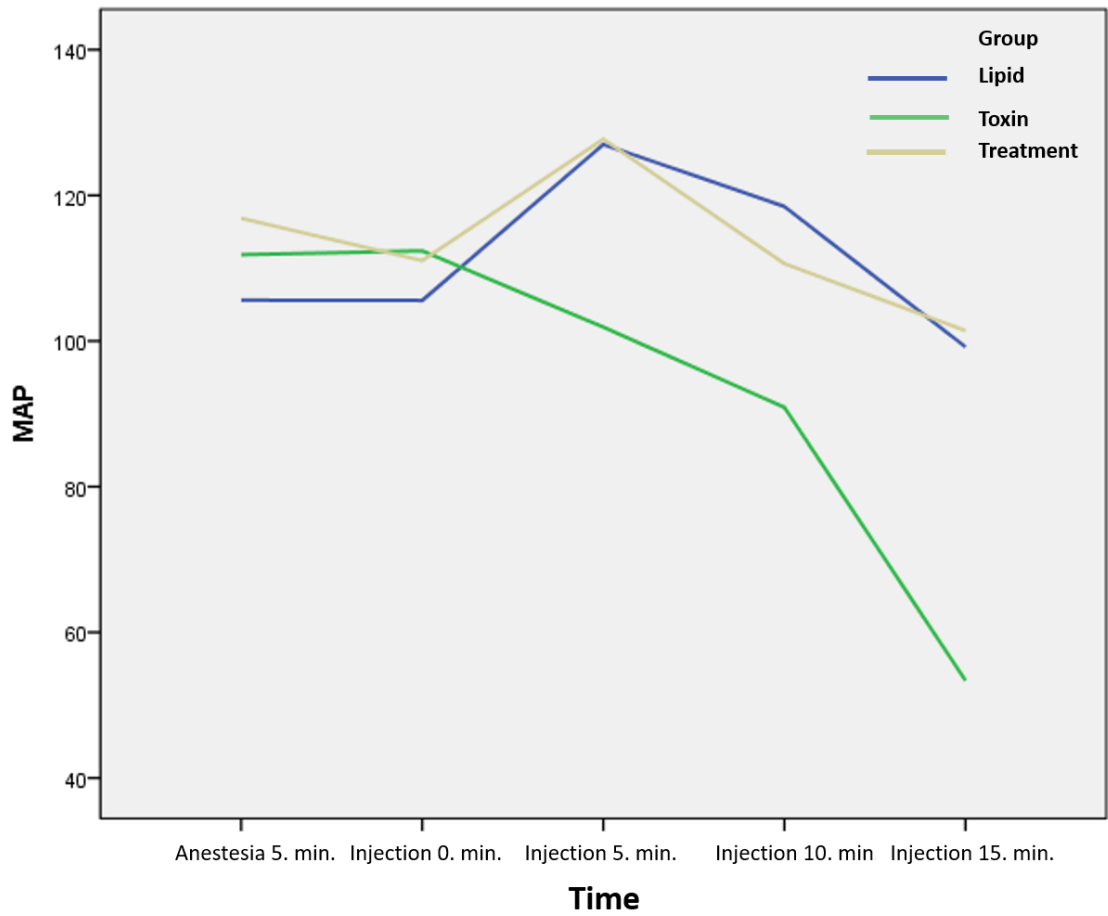


Figure 13. Mean arterial pressure over time comparison of experiment groups

It was found that there was a statistically significant difference between the groups in terms of MAP values at 15.min after medication ($p=0.005$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the toxin group were lower than the values of the lipid and treatment groups ($p=0.015$, $p=0.012$, respectively). No significant difference was found between the other groups ($p>0.05$) (Figure 13).

Table 5. Heart rate over time comparison data of experiment groups (n:sample size, Q1:lower quartile, Q3:upper quartile)

Heart Rate	Lipid	Toxin	Treatment	P
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)	
Anesthesia 5. min.	203 (195, 229)	231 (214, 245)	243 (240, 265)	0.009*
Medication 0. min.	188 (177, 205)	221 (215, 235)	230 (172, 232)	0.322
Medication 5. min.	176 (164, 206)	172 (154, 209)	211 (116, 217)	0.769
Medication 10. min.	175 (166, 202)	169 (150, 190)	187 (129, 201)	0.818
Medication 15. min.	169 (155, 206)	112 (80, 183)	175 (122, 183)	0.213

Kruskal-Wallis test

*p<0.05

No statistically significant difference was found between the groups in terms of time of drug administration, pulse rate values at 5.min, 10.min and 15.min after anaesthesia (p>0.05).

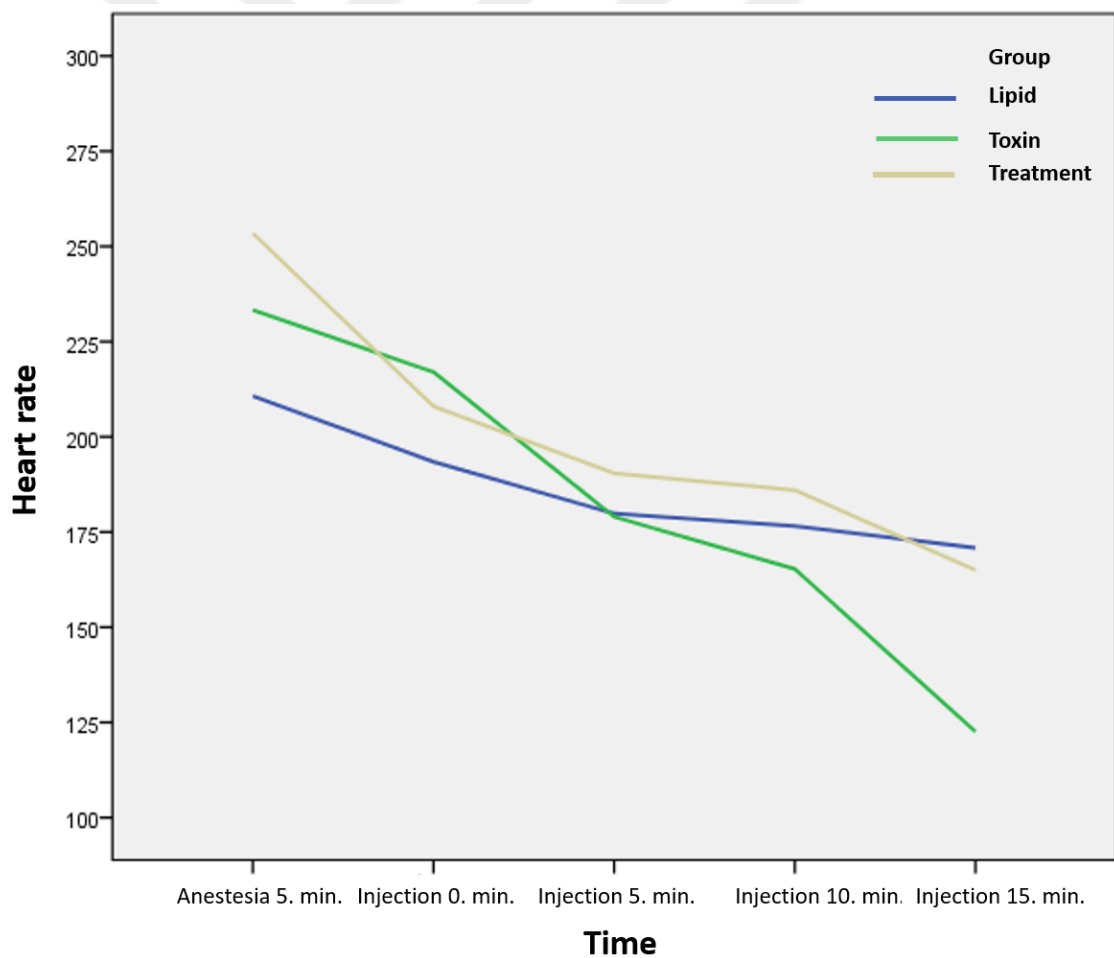


Figure 14. Heart rate over time comparison of experiment groups

There was a statistically significant difference between the groups in terms of pulse rate values at 5.min after anaesthesia ($p=0.009$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the lipid group subjects were lower than the values of the treatment group subjects ($p=0.007$). No significant difference was found between the other groups ($p>0.05$) (Figure 14)

Oxidative stress parameters (MDA, CAT and GPx) were studied in heart and liver tissue. The evaluation between the groups is shown in Table 6.

Table 6. Comparison of experiment groups for heart and liver MDA levels, and antioxidant enzyme activities (GPx, CAT). . (n:sample size, Q1:lower quartile, Q3:upper quartile)

	Lipid (n=7)	Toxin (n=7)	Treatment (n=7)	p value
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)	
Heart MDA	0.61 (0.53, 0.66)	0.69 (0.64, 0.76)	0.75 (0.56, 1.07)	0.094
Heart CAT	0.04 (0.03, 0.04)	0.04 (0.03, 0.04)	0.04 (0.04, 0.05)	0.576
Heart GPx	0.10 (0.09, 0.11)	0.09 (0.07, 0.11)	0.07 (0.06, 0.08)	0.016*
Liver MDA	0.51 (0.41, 0.59)	0.90 (0.77, 1.08)	1.24 (0.62, 1.39)	0.002*
Liver CAT	1.52 (1.01, 1.62)	1.86 (1.55, 2.16)	2.32 (1.88, 2.64)	0.006*
Liver GPx	0.14 (0.07, 0.22)	0.18 (0.12, 0.31)	0.09 (0.06, 0.14)	0.072

Kruskal-Wallis test

* $p<0.05$

No statistically significant difference was found between the groups in terms of cardiac MDA and CAT values ($p>0.05$).

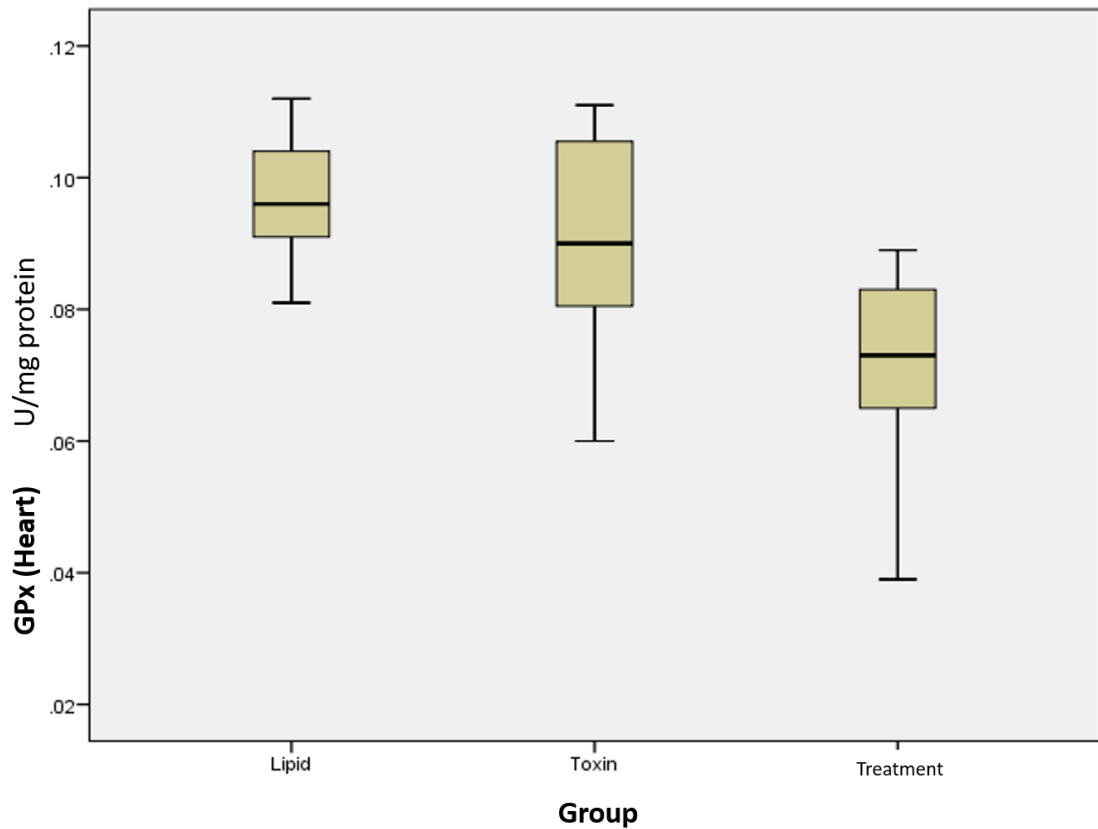


Figure 15. GPx enzyme activity measurements in heart tissue

A statistically significant difference was found between the groups in terms of cardiac GPx values ($p=0.016$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the treatment group subjects were lower than the values of the lipid group subjects ($p=0.015$). No significant difference was found between the other groups ($p>0.05$) (Figure 15).

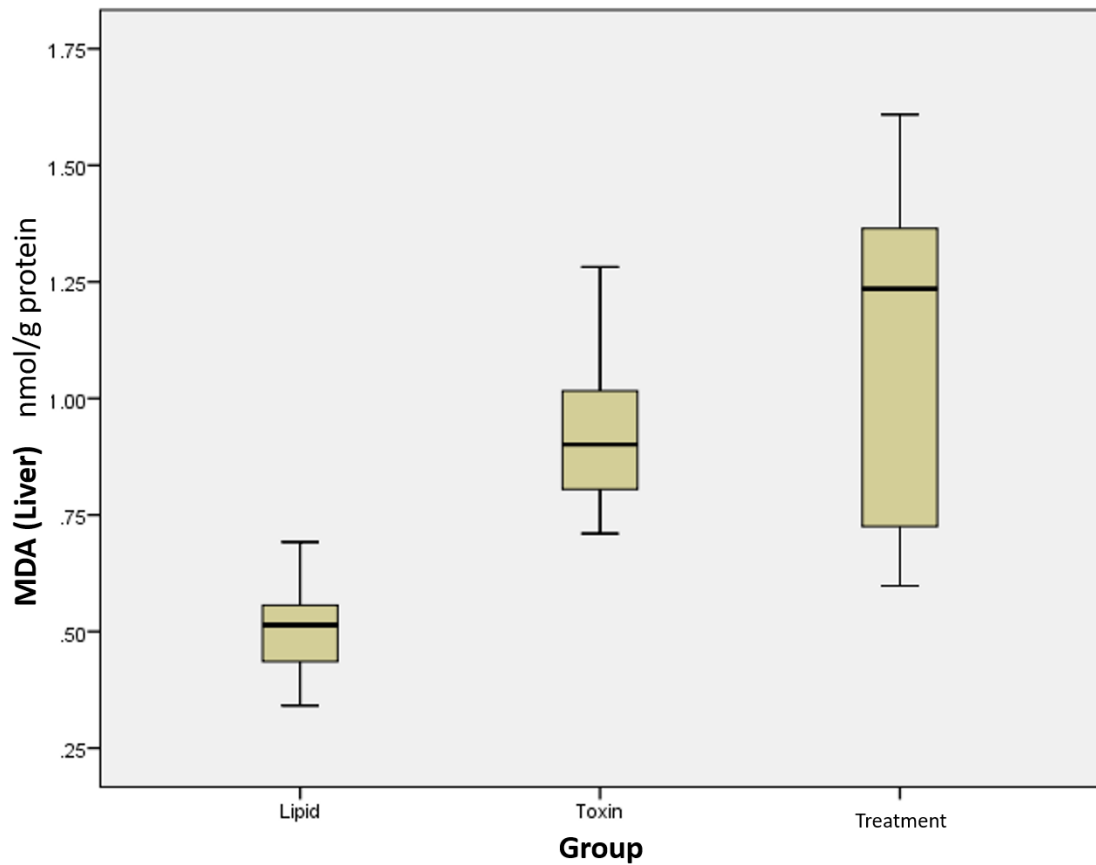


Figure 16. MDA level measurements in liver tissue

A statistically significant difference was found between the groups in terms of liver MDA value ($p=0.002$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the lipid group were lower than those of the toxin and treatment groups ($p=0.012$, $p=0.004$, respectively). No significant difference was found between the other groups ($p>0.05$) (Figure 16).

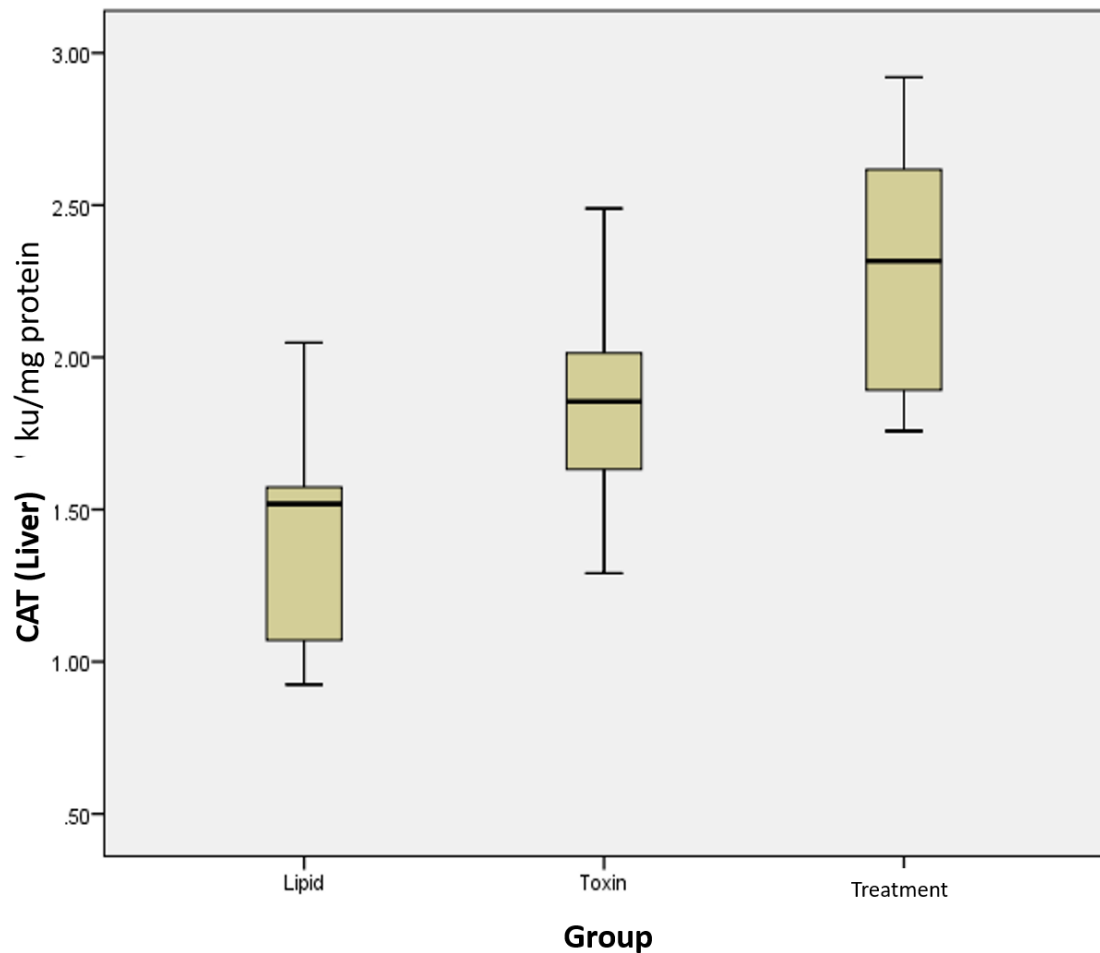


Figure 17. CAT enzyme activity measurements in liver tissue

There was a statistically significant difference in liver CAT values between the groups ($p=0.006$). As a result of the evaluations performed using the Dunn-Bonferroni test, it was found that the values of the treatment group were higher than the values of the lipid group ($p=0.004$). No significant difference was found between the other groups ($p>0.05$) (Figure 17.)

There was no statistically significant difference between the groups in terms of liver GPx values ($p>0.05$).

Unfortunately, blood biochemistry parameters (Sodium [Na⁺], Potassium [K⁺], Hydrogen [pH], Bicarbonate [HCO₃⁻], Glucose, Lactate and Troponin) were inconclusive due to the effects of intravenous lipid emulsion on blood analysis.

5. DISCUSSION and CONCLUSION

There are many case reports and studies showing the efficacy of intravenous lipid emulsion therapy, especially in poisonings with cardiac-acting substances. Although it seems promising, unfortunately, the lack of high-quality controlled human studies and important publications prevents the use of lipid emulsion therapy as a first-line agent for indications other than systemic toxicity of local anaesthetics. For a lipid-soluble xenobiotic, lipid emulsion therapy may be considered as an adjunctive treatment in the absence of response to first-line treatment, but it is not currently accepted as standard of care.

The aim of our study was to investigate the effectiveness of ILE treatment as an alternative treatment modality in mad honey intoxication. In this study, we investigated whether ILE treatment is an effective method in reversing the cardiotoxicity of GTX-III toxin. LLE treatment effects on GTX-III toxin induced oxidative stress were also evaluated in the acute period.

The efficacy of ILE treatment in local anaesthetic toxicity was first reported by Weinberg et al. in 1998 in a study on rats (62). Subsequently, the efficacy of ILE treatment in systemic toxicity of local anaesthetics was demonstrated in studies conducted in the same laboratory and since then, the efficacy of ILE treatment has been supported by studies conducted in other laboratories and systematic analyses conducted in the clinical setting. Rosenblatt et al. 2008 reported the first clinical application of ILE therapy in systemic toxicity due to local anaesthetics and the patient was successfully resuscitated (57). After the laboratory experiments demonstrated successful resuscitation from bupivacaine toxicity, the use of lipid emulsion infusion was examined in animal studies involving different types of overdoses, particularly those commonly encountered in emergency medical situations. These models highlighted the potential advantages of using lipid emulsion in the treatment of overdoses caused by tricyclic antidepressants, beta-blockers, and calcium channel blockers. However, the first use of ILE therapy for drug-induced toxicity other than local anaesthetic toxicity was described by Sirianni et al. who reported the successful rescue of a young man on the brink of suicide who had a witnessed cardiac arrest hours after being hospitalised for an overdose of bupropion and lamotrigine (63). Since then, many published case reports and studies have continued to demonstrate the efficacy of ILE treatment.

Our study was also revealed that ILE treatment significantly increased survival in GTX-III treated animals. Mean arterial, systolic and diastolic blood pressures of ILE-treated animals were significantly different from those of toxin-treated animals. The European Resuscitation Council (ERC) recommends ILE treatment for intoxication with cardiovascular drugs such as beta-blockers, calcium channel blockers and tricyclic antidepressants in its latest guidelines (59). Again, in an animal study on digitalis glycosides, Turan et al. (2018) observed that ILE treatment increased survival rate in experimental animals (60).

In a previous study, Eraslan et al. (2017) reported that mad honey induced oxidative stress following acute, subacute and chronic exposures (50). In the acute phase group of this study, significant increases were found in MDA levels in liver (48%), kidney (26.78%), brain (56.46%), testis (72.41%), heart (20.21%) compared to the control group values. Again, significant decreases were observed in CAT activities of liver (26.78%), kidney (26.64%), brain (40.72%), testis (28.52%), heart (22.31%) compared to the control group. In addition, when the GPx activities in the tissues were evaluated, the activity in the liver tissue (37.55%) was significantly increased compared to the control group, while a decrease in the activity in other tissues was observed. Consistent with our study, mean cardiac MDA levels increased by 19.80 % in the toxin group and 27.45% in the treatment group compared to the control (lipid) group. Again in our study, mean liver MDA levels were found to be 85.31% higher in the toxin group and 116.07% higher in the treatment group compared to the control (lipid) group. This increase in MDA levels indicates that GTX triggers free radical formation and this formation cannot be compensated by the cell. When the tissue CAT activities were evaluated in our study, no difference was observed in the heart tissue, while in the liver tissue, mean CAT values were 32.21% higher in the toxin group and 63.77% higher in the treatment group than the control (lipid) group. The increase in CAT enzyme activity, which is an antioxidant enzyme, shows that GTX increases the free radical level and the antioxidant enzyme system is activated. When we look at the mean GPx activity in our study, a decrease of 6.18% in the toxin group and 26.80% in the treatment group is observed in the heart tissue compared to the control (lipid) group. Again, a 30.06% decrease was detected in the treatment group compared to the control (lipid) group in liver tissue. This decrease in GPx activity in the GTX treated groups indicates that these antioxidant enzymes are used in very large amounts during the detoxification of free radicals. In the study of Eraslan et al. an increase

in liver GPx activity was observed, while a significant decrease was found in other tissues, which is similar to the results in heart tissue in our study (50).

In our study, no significant change was observed between the groups in cardiac MDA levels and cardiac CAT activities. Although there was a 28.57% increase in liver GPx activity levels in the toxin group and a 35.71% decrease in the treatment group compared to the control (lipid) group, no statistically significant difference was found.

In that study the acute effects were observed for a period of 1 day, while this period was 15 minutes in present study. The short duration of our experiment may explain the difference results on oxidative stress parameters. Although a clinical response was obtained, the differences in oxidative stress parameters may be attributed to the fact that the experiment lasted for a maximum of 45 minutes. The experimental period was terminated at 45 minutes considering the effectiveness of anaesthesia. Since none of the animals in the toxin group survived at 22 minutes, time comparisons were limited to 15 minutes.

The short duration of the experiment in our study may have caused variability in the evaluation of oxidative stress parameters. In addition, biochemical blood parameters could not be measured as a side effect of ILE treatment. Again, since a very high dose should be used for atropine to be effective in rats and due to the negative effects of such a high volume dose in animals; the absence of atropine group, which is the standard treatment method in GTX poisoning can be considered as limitations of the study.

In conclusion, it was observed that ILE was effective in reversing the negative effects of GTX-III on haemodynamics and increasing survival in rats. However, more comprehensive studies are needed for the use of ILE as a treatment method in humans in delibal poisoning. Because of ethical reasons, the experimental groups are quite small and death was observed in a very short time in animals given toxin.

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7. CURRICULUM VITAE

Personal Informations

Name	Eren	Surname	Gökdağ
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Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate	Emergency Medicine	Ministry of Health University	2017
Master			
University	Cerrahpaşa Faculty of Medicine	İstanbul University	2010
High school	Scince	Denizli Erbakır Science High	2003

All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs),

Languages	Grades (#)
English	97.5 (YÖKDİL)

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
Emergency Medicine Specialist	Istanbul City Hospital	2023-Present
Emergency Medicine Specialist	Memorial Sisli Hospital	2021-2023

Computer Skills

Program	Level
MS Office	Average

*Excellent , good, average or basic

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

American Journal of Emergency Medicine: F. Sarı Doğan, Ö. Güneysel, E. Gökdağ, et al., Demographic characteristics and delayed neurological sequelae risk factors in carbon monoxide poisoning, https://doi.org/10.1016/j.ajem.2019.12.037
Hong Kong Journal of Emergency Medicine 21 Haziran 2018, Research article; Modification of Glasgow-Blatchford scoring with lactate in predicting the mortality of patients with upper gastrointestinal bleeding in emergency department. Zeynep Konyar, Ozlem Guneyzel, Fatma Sari Dogan and Eren Gokdag, DOI: 10.1177/1024907918783159

Articles published in other journals

Ayvaci BM, Gökdağ E. A rare case: Descending necrotizing mediastinitis. Turk J Emerg Med. 2022;22(4):230-232. Published 2022 Sep 30. doi:10.4103/2452-2473.357345
Visual Journal of Emergency Medicine Volume 11, April 2018, p51-52. Visual Case Discussion; Asymptomatic widespread pneumocephalus. Eren Gökdağ, Ersin Tunca, Özlem Güneysel. doi: 10.1016/j.visj.2018.02.009

Proceedings presented in international scientific meetings and published in proceedings book.

7th Eurasian Congress on Emergency Medicine & 17th Turkish Congress on Emergency Medicine, Nov 25-28 2021, Presentation: Covid-19 Hastalarında Pulmoner Bt Skorunun Hastalığın Şiddeti İle Korelasyonunun İncelenmesi. Merve Ekşioğlu, Barış Murat Ayvaci, Ayşegül Görmez, Eren Gökdağ
7th Eurasian Congress on Emergency Medicine & 17th Turkish Congress on Emergency Medicine, Nov 25-28 2021, Presentation: Covid-19 Hastalarında Nötrofil/Lenfosit Oranının Hasta Sonlanımları Üzerine Etkisi. Barış Murat Ayvaci, Merve Ekşioğlu, Eren Gökdağ

Journals in the proceedings book of the refereed conference / symposium

10th International Congress of the Turkish Society of Toxicology, October 16-19, 2019, Antalya. Oral Presentation; Chlorine Gas Inhalation: A Case Report. Eren Gökdağ
--

I. Ulusal Acil Tıpta Toksikoloji Sempozyumu 14-15 Aralık 2018, İstanbul, Sözlü Sunum; Datura Stramonium Intoxication: A Family Case Report; Eren Gökdağ, Fatma Sarı Doğan, Ömerul Faruk Aydın, Kerem Dost Bilmez.

Others (Projects / Certificates / Rewards)

E3-nurse project; The Scientific and Technological Research Council of Turkey (TÜBİTAK) and EUREKA Cluster, Celtic-Plus / Sosoft Information Technologies; Medical Advisor.
MENATOX 2020 Annual SAID ALMAZROUI MEMORIAL AWARD, 19 Feb 2020, İstanbul

