Pharmacognostic Screening of *Elaeis guineensis* (Aracaceae) Jacq. Oil and its Effect as an Antidote on Cyanide Poisoning

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**ABSTRACT**

**Introduction:** *Elaeis guineensis* is a perennial monocot belonging to the family Arecaceae. It is the source of the oil commonly called African palm oil or macaw fat which in traditional medicine has many uses.

**Aim:** This study is focused on the pharmacognostic screening of *Elaeis guineensis*, and its antidotal effect on cyanide poisoning.

**Method:** The extracted oil was subjected to various screening techniques in order to determine its quality, purity and chemical constituents. The oil was macroscopically examined; acute toxicity test of *Elaeis guineensis* oil was carried out on rats. The oil was subjected to heating to determine the moisture content. Physicochemical analysis was also carried out on the palm oil extract. The physico-chemical analysis was carried out to determine the acid value, saponification value, ester value, hydroxyl value and iodine value. The LD₅₀ for the pure cyanide was carried out on the rats using “Up and Down” method. The antidotal study of *Elaeis guineensis* oil was carried out on the rats.

**Result:** Macroscopic evaluation showed, the oil was in fresh condition, smooth texture, bright red colour, characteristic taste, oily appearance and a characteristic smell. The Phytochemical analysis showed the presence of flavonoids, phenols, tannins, saponins, alkaloids, steroids and terpenoids. The physico-chemical analysis showed that the oil has an acid value of 31.2, Saponification value of 194.8, Ester value 163.54, Peroxide value of 18.0, Hydroxyl Percentage of 2.07% and Free Fatty Acid of 3.65. The moisture content was calculated to be 0.2%. For the acute toxicity test on the oil using Lorkes method no death was recorded. The LD₅₀ of the cyanide carried out on the rats showed that the lethal dose of cyanide is 5 mg/kg. The antidotal effect of *Elaeis guineensis* oil showed the absence of death on the group given oil extract alone and the groups that were poisoned and given the oil (antidote) within 4 minutes. Deaths were recorded for the groups that were administered antidotes after 8 minutes.

**Conclusion:** *Elaeis guineensis* oil has counteracting effect on cyanide poisoning if administered within four minutes of cyanide ingestion.

**Keywords:** *Elaeis guineensis*, phytochemical analysis, physicochemical analysis, macroscopy, antidotal effect.

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**INTRODUCTION**

It is hard to find any indigenous food prepared in Nigeria without the use of palm oil and this explains the vital role it plays in daily diets. Currently, palm oil is the world largest edible oil and is the main source of domestic or edible oil in Africa¹. Fresh palm oil is the most common Antidote for ingested poisons amongst indigenous people of south-south Nigeria. In traditional medicine, palm oil is a very popular antidote.

Poison can be defined as a substance that can cause animals to die or become very sick when taken in a very small quantity into their bodies.²

Antidotes are substances when taken that can stop the harmful effect of a poison or correct and improve the bad effects²

In Medieval Europe, there was increased availability of poisons; shops known as apothecaries, selling various medicinal wares, were open to the public and from there, substances that were traditionally used for curative...
purposes were employed for their negative uses. At the same
time, in the Middle East, Arabs developed a form of arsenic
that is odorless and transparent, making the poison difficult
to detect. This unhealthy "poison epidemic" was also
prevalent in parts of Asia at this time, as well. In the modern
world, intentional poisoning is less common than in the
middle ages; rather, the more common concern is the risk of
accidental poisoning from everyday substances and
products. Cyanide is a singularly charged anion consisting of
one carbon atom and one nitrogen atom joined with a triple
bond, CN-. The most toxic form of cyanide is free cyanide,
which includes the cyanide anion itself and hydrogen
cyanide, HCN, either in a gaseous or aqueous state. HCN is
highly soluble in water. HCN gas and liquid are colorless and
have the odor of bitter almonds. Plant materials containing
≥200 ppm of cyanogenic glycosides are dangerous. Hydrogen
cyanide was first isolated from Prussian blue dye in 1786
and cyanide first extracted from almonds around 1800. It can
exist as a gas, hydrogen cyanide, a salt, potassium cyanide.
Natural substances in some foods such as lima beans,
almonds can release cyanide. Cyanide is also found in
manufacturing and industrial sources such as insecticides,
photographic solutions, and jewelry cleaner. It has been used
as a poison in mass homicides and suicides. During World
War II, the Nazis used cyanide as an agent of genocide in gas
chambers34 Cyanide's main effect is that it inhibits oxidative
phosphorylation, a process where oxygen is utilized for the
production of essential cellular energy sources in the form of
ATP. It does so by binding to the enzyme cytochrome
C oxidase and blocks the mitochondrial transport chain. After
that, cellular hypoxia and the depletion of ATP occurs; leading
to metabolic acidosis. The utilization of oxygen by the tissue
occurs and is followed by the impairment of vital functions5
Signs generally occur within 15–20 min to a few hours after
animals are exposed to cyanide poisoning, and today after onset
clinical signs is rarely ≥2 hr. Excitement can be displayed
initially, accompanied by rapid respiration rate. Dyspnea
follows shortly, with tachycardia. The classic "bitter almond"
breath smell may be present. Animals may stagger and
struggle before collapse. In other cases, sudden unexpected
death may ensue. Mucous membranes are bright red but may
become cyanotic terminally. Venous blood is classically
described as "cherry red" because of the presence of high
venous blood pO2; however, this color rapidly changes after
death. Serum ammonia and neutral and aromatic amino
acids are typically increased. Cardiac arrhythmias are
common due to myocardial histotoxic hypoxia. Death occurs
during the first several asphyxial convulsions. The heart may
continue to beat for several minutes after struggling, and
breathing stops. Normally expected cyanide concentrations
in blood of most animal species are usually <0.5 mcg/ml
Cyanide concentrations in muscle are similar to those in
blood, but concentrations in liver are generally lower than
those in blood. Differential diagnoses include poisonings by
nitrate or nitrite, urea, organophosphate, carbamate,
chlorinated hydrocarbon pesticides, and toxic gases (carbon
monoxide and hydrogen sulfide), as well as infectious or
noninfectious diseases and other toxidromes that cause
sudden death.8

*Elaeis guineensis* is a species of palm commonly
called African oil palm. It is the principal source of red palm oil
or macaw-fat and palm kernel oil. It is native to west and
southwest Africa, specifically the area between Angola and
the Gambia. The species name *guineensis* refers to the name
for the area, and not necessarily the modern country which
now bears that name. The pericarp consists of the exocarp
(the outer layer also referred to as the skin), the mesocarp
(the layer containing the red palm oil), and the endocarp
(consisting of a hard shell enclosing the kernel or
endosperm, which contains the kernel oil) (Palm oil is
extracted from fleshy mesocarp of the fruit either by milling
mechanically or by the traditional manual method,7 which
contains 45 - 55% oil, but varies from light yellow to orange-
red in color, and melts at 25°C. The oil color is determined
by its carotenoids. The major carotenoids found in palm oil
are the beta-carotenes. Palm kernel oil is obtained from the
kernels enclosed in the endocarp. Palm oil contains saturated
palmitic acid, oleic and linoleic acid, giving it a higher
unsaturated acid content than palm kernel or coconut oils.
Palm oil also has some minor constituents including
phospholipids. Folk remedies of oil palm also include
treatment for cancer and use as liniment5 It has shown to
prevent premature aging, protection of the liver, and
reduction of risk of cancer.10,11 Fresh palm oil is the most
common antidote for ingested poisons in South-South
Nigeria. It is also used as a cough remedy. Palm oil has wound
healing properties hence it is implored in the treatment of
wounds and cuts as well as in treatment of burns. In Igbo
land, palm oil and raw egg white (albumin) is formed into a
homogenous mixture. The mixture is used to promote the
healing and enhance the regeneration of tissue. Eight leaves
of *Vernonia amygdalis* fried in little quantity of pure palm oil
is used in treatment of burns on the skin. Skin rashes, moles,
boils and itches are some of the skin infections that are
reated with red palm oil. Studies carried out at the
Michael Okpara University of Agriculture, Umudike, Abia
State, Nigeria12 using five microorganisms which includes;
*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas
areuginosa*, *Candida albicans*, and *Aspergillus niger* which
are causative agents of some infections on the skin can
be treated with palm oil and palm kernel oil. In the past, some
health researchers thought palm oil could increase the risk of
heart disease because of its high content of saturated fatty
acids, but toxic further research by scientist has proved that
palm oil contains no cholesterol and that it has lots of the
short-chain fatty acids which are helpful to the humans. Palm
oil provides a rich source of beta-carotene and vitamin E,
namely tocopherols and tocotrienols which are recognized
nutritional anti-oxidants that act as scavengers of the oxygen
atom or free radicals. Research has shown that consumption
of red palm oil enhances vitamin A levels in humans, and it
is beneficial in preventing vitamin A deficiency.13 Vitamin A
deficiency may lead to blindness, skin disease and weakened
immune function. The vitamin A content in red palm oil plays
important roles in growth, development and in visual
process. Studies have indicated that the potential mechanism
of action for the improvement in glucose metabolism
inhibitory polypeptide, which itself stimulates insulin
secretion and slows gastric emptying. Studies have indicated
that the potential mechanism of action for the improvement in glucose
metabolism involves inhibition of the enzyme dipeptidyl
peptidase-4, the effect of which is to protect gastric
hormone inhibitory polypeptide, which itself stimulates insulin
secretion, and in the other hand suppresses ghcagon
secretion and slows gastric emptying. *E. guineensis* oil is rich
in catechins and polyphenols. In a human clinical trial, the
patients who were given supplements 'palm tocotrienol
complex' for two months showed significant reduction in
aortic systolic blood pressure. Tocotrienol-rich Fraction
(TRF) of palm oil exhibited cardio-protective ability in the
experimental animals15 Palm oil has anti-dotting effect and it
helps in prevention of thrombus in the blood vessels. A
human study showed that tocotrienol (from palm oil) supplementation can reduce stenosis of the carotid
diseases and atherosclerosis.16 Other reports showed that palm oil diets increases the production of prostacyclin or thromboxane.17
Thus scientific evidence indicates that the palm oil diet is
anti-thrombotic. Studies in animals confirmed that palm oil
prevents the formation of plaques in the arteries. The anti-
bacterial activity of this plant extract against different micro-
organisms and anti-oxidant activity have been reported.
Moreover, Chong together with Sasiharan and some others 20 described the potential of *E. guineensis* leaf methanol extract as an infected wound healing agents. Studies have shown that tocotrienols fractions of palm oil were able to induce an inhibitory action on the human breast cancer cells, whereas the alpha-tocopherols were not able 21. Palm oil is composed of fatty acids esterified with glycerol. Palm oil has an especially high concentration of saturated fat, specifically, of the 16-carbon saturated fatty acid palmitic acid, to which it gives its name. Monounsaturated oleic acid is also a major constituent of palm oil. Unrefined palm oil is a large natural source of tocotrienol 22 part of the vitamin E family.

**MATERIALS AND METHODS**

**Collection and preparation of plant materials**

A freshly harvested palm fruit was collected from Owerri in Imo state at The sample parts were identified by a taxonomist Mr. Ozioko, in the Department of Botany Nnamdi Azikwe University Awka. After collection of the palm fruit, it was washed to remove impurities and boiled in 5 L of water to enhance the softening of the mesocarp. The fruits were sieved out from the water and were then pounded. The nuts were removed i.e. removal of the palm kernel. The fiber obtained from the pounded fruit was placed in a clean sieve/sac, and the oil was pressed out. This process was carried out while the fiber is hot. The crude extract obtained was boiled and final separation of the pure oil took place.

**Animals**

A total of 30 adult rats weighing between (81-100) g of both sexes were obtained from Animal House of the Department of Pharmacology, Madonna University, Elele Campus, Rivers State. They were allowed to acclimatize for a period of 2 weeks as they had free access to food and water and were maintained under the standard conditions while in the house.

**Macroscopic examination**

The macroscopic examination was carried out. This includes organoleptic evaluation (taste, texture and smell).

**Determination of Moisture Content**

The moisture content was determined by finding the difference between the weight of the sample before drying and the weight of the sample after drying.

**Phytochemical Analysis**

Phytochemical screening was carried out to detect the presence for Flavonoids, Phenols, Tannins, Saponins, Alkaloids, Steroids, Terpenoids following standard methods

**Physicochemical analysis**

The physicochemical analyses were determined following the standard methods

**Acid Value**

To a mixture of 20 ml ethanol and 20 ml diethyl ether in a 250 ml Erlenmeyer flask was added approximately 1g *Elaeis guineensis* oil and shaken for about 20 seconds, 5 drops of phenolphthalein was added as an indicator; the mixture was titrated against the standardized 0.1 M potassium hydroxide (KOH) solution until a pink colour, which persisted for at least 15 seconds was observed. Burette reading x factor of alkali/ weight of oil.

**Saponification Value**

A 2 g of the sample was weighed and transferred into the distillation flask, fitted with a reflux condenser, then, 20 ml of 0.5 M of potassium hydroxide (KOH) was added. The flask was heated in boiling water under reflux for 1 hour and then cooled by placing in ice water. The mixture was titrated with 0.5 M hydrochloride (HCL). A blank titration was also carried out.

Saponification value = F x (b-a) x 28.05/ weight of oil

\[ F = \text{Factor of 0.5 M HCL} \]

\[ b = \text{Volume of 0.5 M KOH} \]

\[ a = \text{volume of 0.5 M HCL} \]

**Ester Value**

Ester value = saponification value – acid value

**Free Fatty Acid**

A 2.0 g of the extract (*Elaeis guineensis*) oil was transferred into 250 ml Erlenmeyer flask followed by the addition of 50 ml of n-hexane and 1 ml of phenolphthalein indicator. The flask was shaken vigorously and titrated against 0.04 M NaOH. The shaking continued until the observation of a slight pink color which was steady for about 15 seconds which signified the end point. The expression for free fatty acid (FFA) is as follows:

\[ \%\text{FFA} = \left( \frac{V \times M \times 282}{W} \right) \times 100 \]

Where, \%FFA = Percentage free fatty acid (oleic acid)

\[ V = \text{Average volume of NaOH used (ml)} \]

\[ M = \text{Molarity of NaOH} \]

\[ 282 \text{g/mol} = \text{Molecular weight of oleic acid} \]

\[ W = \text{Weight of oil} \]

**Peroxide Value**

A 2.0 g of the *Elaeis guineensis* oil was weighed and dissolved in a mixture of glacial acetic acid and chloroform, at ratio (3:2), then, a 0.5 ml saturated potassium iodide solution was added to the mixture in the flask and covered. The mixture was shaken for a minute then a 30 ml of distilled water was added and titrated against 0.1M sodium thiosulphate solution (Na2S2O3) shaking vigorous, using starch mucilage as indicator. A sudden disappearance of the blue color signifies the end point. Blank determination was also carried out.

**Hydroxyl Value**

A 2.0 grams of the *Elaeis guineensis* oil was transferred into distillation flask fitted with a reflux condenser and a 1ml of acetic anhydride was added. The flask was boiled under reflux for 5 minutes and then was cooled by placing in ice water. The mixture was titrated with 0.5 M ethanolic potassium hydroxide. Phenolphthalein was used as an indicator. A blank determination was also carried out.

**Iodine Value**

A 0.4 g of the extract was weighed into conical flask and then dissolved by the addition of 20 ml of carbon tetrachloride. After which, a 25 ml of wij’s reagent was added with the help of a safety pipette in a flame chamber. A stopper was inserted and the mixture was swirled vigorously before it was then placed in a dark room for 2.5 hours. At the end of this period, a 20ml of potassium iodide and a 125 ml of water was added with the use of a measuring cylinder. The resulting solution was then titrated with 0.1 M sodium thiosulphate solution (Na2S2O3) until the initial yellow color almost disappeared. Few drops of 1% starch indicator was then added with few drops of thiosulphate added

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meticulously as the titration continued shaking vigorous until the blue coloration disappears.

**Pharmacological Evaluation**

**Acute Toxicity**

The acute toxicity of *Elaeis guineensis* was carried out using the 23 The test was carried out in two phases. A total of 13 rats were employed. In phase one, a total of 9 rats were used, which were then grouped into 3 groups of 3 rats. Group 1, 2 and 3 received 10, 100 and 1000 mg/kg of the extracted oil orally respectively. The animals in the three groups were monitored for at interval of three hours until a period of 24 hours for mortality, the result observed was documented appropriately. In phase 2, the animals were grouped into four groups containing one rat each. Group 1, 2, 3 and 4 received 2000, 3000, 4000 and 5000 mg/kg of the oil respectively. The animals were also monitored for 24 hours for mortality, and the result was documented.

**Evaluation of the Antidotal effect of the Elaeis guineensis oil**

The antitodal activity of *Elaeis guineensis* oil was carried out using animal model. A total of 30 rats were used, they were grouped into 6 groups of 5 rats each, and each group of 5 rats had similar body weight. The animals received treatments as follows

Group 1(control 1): received a dose of 5 mg/kg of potassium cyanide only.

Group 2: received 3 ml of Palm oil each at 0 minutes after administration of KCN at a dose of 5 mg/kg.

Group 3: received 3 ml of palm oil each at 4 minutes after administration of KCN at a dose of 5mg/kg.

Group 4: received 3 ml of palm oil each at 8 minutes after administration of KCN at a dose of 5 mg/kg.

Group 5: received 3 ml of palm oil each at 12 minutes after administration of KCN at a dose 5 mg/kg.

Group 6; (control 2): Each rat received 3 ml of palm oil only.

All the administrations were through oral routes and the numbers of deaths were properly documented.

**Statistical Analysis**

Statistical analysis of one way anova was carried out using a software Graph pad Prism5 version.

**RESULTS**

**Macroscopic Analysis**

<table>
<thead>
<tr>
<th>Table1: Macroscopic Description of Elaeis guineensis oil</th>
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<tbody>
<tr>
<td>Characteristics</td>
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<tr>
<td>Condition</td>
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<tr>
<td>Texture</td>
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<tr>
<td>Colour</td>
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<td>Taste</td>
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<tr>
<td>Appearance</td>
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<td>Odour</td>
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</table>

**Toxicity Test**

There was no death recorded at the dose of 5000 mg/kg body weight of oil. This indicates that the oil is very safe.

**Physicochemical Properties**

<table>
<thead>
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<th>Table 2: Physicochemical properties of Elaeis guineensis Oil</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>Acide Value</td>
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<tr>
<td>Saponification Value</td>
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<tr>
<td>Ester Value</td>
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<tr>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>Peroxide Value</td>
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<tr>
<td>Iodine Value</td>
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<td>Hydroxyl Group %</td>
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</table>

**Qualitative Phytochemical Composition of Elaeis guineensis Oil**

<table>
<thead>
<tr>
<th>Table 3: Phytochemical Screening of the Oil Sample</th>
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<tbody>
<tr>
<td>Phytochemical</td>
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<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Phenols</td>
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<tr>
<td>Tannins</td>
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<tr>
<td>Saponins</td>
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<td>Alkaloids</td>
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<td>Steroids</td>
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<td>Terpenoids</td>
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</table>

**Result from the Biological Activity for Antidotal Effect**

<table>
<thead>
<tr>
<th>Table 4: The Biological Activity for the Antidotal Effect of the Extracted Oil</th>
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<tr>
<td>Group</td>
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<td>1</td>
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<td>5</td>
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<td>6</td>
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</table>

Moisture content and volatile content = 0.2 %

**DISCUSSION AND CONCLUSION**

The phytochemical screening of Elaeis guineensis oil revealed the presence of several metabolites. In table 5, it is shown that *Elaeis guineensis* oil contains Tannins, Terpenoids, Alkaloids, phenols, Flavonoids. The presence of some of these metabolites may contribute to the effect of this plant as
remedy for various diseases which includes, its antimicrobial effect, hepatoprotective effect, cardiovascular effect, treatments of wounds and even as antidotes for poison. Table 2 identifies the various physicochemical properties of Elaeis guineensis oil. From the values obtained for the Acid value, Saponification value, Ester value, peroxide value and Hydroxyl value, which is, 31.26, 137.3, 107.57, 18, and 83 respectively, the oil can be said to be of good quality, and safe for consumption. The moisture content of the oil indicates the presence of little or insignificant moisture content.

Poisoning is common in humans and to treat the poisoning antidotes are used. In the treatment of cyanide poisoning, antidotes are classified as those needed immediately (WHO). The average fatal concentration for humans was estimated at 5.46 ppm for 10 min. This data point is based on the work of those who considered the resistance of man to HCN to be similar to that of the goat and monkey and four times that of the mouse. With these, the rats were grouped according to time interval ranging from 0-12 min. In Table 4, it is shown that the group 2, 3 and 6 had zero number of death, this were the groups given antidote at 0 and 4 min and the group not given poison at all. Groups 1 and 5 had the maximum number of deaths, which is the group not given antidote and the group given antidote at 12 minutes after poison administration.

The results obtained at the 4th and 8th min, shows the role of quick intervention in administration of Elaeis guineensis oil as an antidote in cyanide poisoning. Time factor is essential in this form of intervention from the result obtained, the best activity of Elaeis guineensis oil within the earliest possible time is about 4 min as indicated in Table 5. At the 12th min the entire cyanide must have been absorbed into the blood stream, and the application of Elaeis guineensis oil at this stage is unable to revert the injuries caused by this poison on the organs.

CONCLUSION

Elaeis guineensis oil is a rich source of numerous metabolites good for the human health. From the results obtained, Elaeis guineensis can be said to be more effective as an antidote to cyanide poisoning before the poison being ingested is absorbed into the systemic circulation, i.e., into the blood stream. This mechanism can be attributed to the saponification activities, neutralizing action and presence of fatty acids which delays gastric absorption and promotes gastric emptying. According to WHO certain agents used in the treatment of poisoning can be classified under those needed immediately, within 2 hours and within 6 hours.

REFERENCES