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Phytochemical study, biological and homeopathic activity of extract of vomit nut (Strychnos nux-vomica L.)

against toxicity induced by N-acetyl-p-benzoquinone in rats.

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Dédicace

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Abstract

This work aimed to study the effect of aqueous extract of Strychnos *nux-vomica* in the treatment of paracetamol-induced toxicity, in rats. Thirty male white Wistar rats, weighing between 165-247 g, were divided into 5 groups of 6 animals each (n = 6) and treated for 12 days as follows: Group 1: Controls receiving mineral water, Group 2: Animals treated with a toxic dose of Paracetamol 1/4 of the LD_{50} (DL_{50}= 1943 mg/Kg) by gavage (orally). Group 3: Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the aqueous extract of *Strychnos nux-vomica* (0.5 mg / Kg). Group 4: Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the homeopathic trituration (CH 14) of *Strychnos nux-vomica* (0.5 mg / Kg). And Group 5: Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the homeopathic trituration (CH 30) of *Strychnos nux-vomica* (0.5 mg / Kg).Various parameters: hematological, biochemical, and antioxidant markers were estimated. Histopathology of liver and kidney tissues was observed. The results of the qualitative analysis showed the richness of aqueous extract in alkaloids and flavonoids, terpenoids, and reduced compounds. The total phenol and flavonoid content showed the highest concentration in the aqueous extract of *S.nux-vomica* (23.67 mg GA EQ/g , 3.086 mg QEQ/g). The IC50 values for the plant were 6.86 µg/ml. In this study. The change in hematological and some biochemical parameters was observed in poisoned rats and showed significant recovery in aqueous extract and *S. nux-vomica* plant homeopathic trituration groups. The level of MDA was decreased and GSH was increased both with significantly ($P < 0.05$) in rats treated with aqueous extract and trituration plant. Histopathology of liver and kidney tissues confirmed their protection by the aqueous extract and homeopathic trituration of *S.nux-vomica*. The study revealed the presence of antioxidant and anti-hemolytic properties in the aqueous extract of *S.nux-vomica*, with a beneficial effect of the plant homeopathic trituration in reducing oxidative stress, hepatotoxicity and nephrotoxicity occurring in rats.

Key words: Homeopathic trituration, Oxidative stress, *Paracetamol induced toxicit,y* Strychnos *nux-vomica* , wistar rats.

Résumé

Ce travail visait à étudier l'effet de l'extrait aqueux de *Strychnos nux-vomica* dans le traitement de la toxicité induite par le paracétamol, chez le rat. Trente rats blancs Wistar mâles, pesant entre 165 et 247 g, ont été répartis en 5 groupes de 6 animaux chacun (n = 6) et traités pendant 12 jours comme suit : Groupe 1 : Contrôle recevant de l'eau minérale, Groupe 2 : les rats ont été traités avec une dose toxique de Paracétamol 1/4 de la DL50 (DL50= 1943 mg/Kg) par gavage. Groupe 3 : reçu une dose toxique de Paracétamol 1/4 de la DL50 et traités avec l'extrait aqueux de *Strychnos nux-vomica* (0,5 mg/Kg). Groupe 4 : reçu une dose toxique de Paracétamol 1/4 de la DL50 et traités par la trituration homéopathique (CH 14) de *S nux-vomica* (0,5 mg/Kg). Groupe 5 : ont reçu une dose toxique de Paracétamol 1/4 de la DL50 et traités par la trituration homéopathique (CH 30) de *Strychnos nux-vomica* (0,5 mg/Kg). Divers paramètres : marqueurs hématologiques, biochimiques et antioxydants ont été estimés. Une histopathologie des tissus hépatiques et rénaux a été observée. Les résultats de l'analyse qualitative ont montré la richesse de l'extrait aqueux en alcaloïdes et flavonoïdes, terpénoïdes et composés réduits. La teneur totale en phénols et flavonoïdes a montré la concentration la plus élevée dans l'extrait aqueux de S.nux-vomica (23,67 mg GA EQ/g, 3,086 mg QEQ/g). Les valeurs IC50 pour la plante étaient de 6,86 µg/ml. Le changement des paramètres hématologiques et de certains paramètres biochimiques a été observé chez les rats empoisonnés et a montré une récupération significative dans les groupes de trituration homéopathique et de l'extrait aqueux de la plante. Le niveau de MDA a été diminué et le GSH a été augmenté à la fois de manière significative ($P < 0,05$) chez les rats traités avec un extrait aqueux et de la trituration. L'histopathologie des tissus hépatiques et rénaux a confirmé leur protection par l'extrait aqueux et la trituration homéopathique de S.nux-vomica. L'étude a révélé la présence de propriétés antioxydantes et anti-hémolytiques de la plante, avec un effet bénéfique de la trituration homéopathique dans la réduction du stress oxydatif, de l'hépatotoxicité et de la néphrotoxicité survenant chez le rat.

Mots clés : Trituration homéopathique, stress oxydatif, toxicité induite par le paracétamol, *Strychnos nux-vomica* , rats wistar.

الملخص

يهدف هذا العمل إلى دراسة تأثير المستخلص المائي لنبات إسطركن الجوز المقيء (*Strychnos nux-vomica*) في علاج السمية التي يسببها الباراسيتامول في الفئران. تم تقسيم ثلاثين من ذكور جرذ ويستار البيضاء ، التي يتراوح وزنها بين 165 و 247 جرامًا ، إلى 5 مجموعات كل منها 6 حيوانات (عدد = 6) وعولجت لمدة 12 يومًا على النحو التالي: المجموعة 1: عولجت بالمياه المعدنية ، المجموعة 2: الحيوانات المعالجة بجرعة سامة من الباراسيتامول 4/1 من LD50 .المجموعة 3: تلقت الحيوانات جرعة سامة من الباراسيتامول 4/1 من الجرعة المميتة النصفية (LD50) عن طريق الفم وعولجت بالمستخلص المائي من نبات الجوز المقيء 0.5 مجم / كجم. المجموعة 4: تلقت الحيوانات جرعة سامة من الباراسيتامول 4/1 من الجرعة المميتة النصفية (LD50) وعولجت بالطريقة المثلية (CH 14) من نبات الجوز المقيء0.5 مجم / كجم. والمجموعة 5: تلقت الحيوانات جرعة سامة من الباراسيتامول 4/1 من الجرعة المميتة النصفية (LD50) وعولجت بالطريقة المثلية (CH 30 . تم تقدير مؤشرات الدم والكيمياء الحيوية ومضادات الأكسدة و لوحظ التشريح المرضي لأنسجة الكبد والكلى. أظهرت نتائج التحليل النوعي ثراء المستخلص المائي في قلويدات وفلافونيدات وتربينويدات ومركبات مخفضة. أظهر إجمالي محتوى الفينول والفلافونويد أعلى تركيز في المستخلص المائي من23.67 مجم GA EQ / جم ، 3.086 مجم QEQ / جم). كانت قيم IC50 للنبات 6.86 ميكروجرام / مل. في هذه الدراسة. لوحظ التغيير في الدم وبعض المتغيرات البيوكيميائية في الفئران المصابة بالتسمم وأظهرت انتعاشاً معنوياً من المستخلص المائي ومجموعات المعالجة المثلية للنبات. انخفض مستوى MDA وزاد مستوى GSH معنوياً ($P < 0.05$) في الفئران المعالجة بالمستخلص المائي والمعالجة المثلية. أكد التشريح المرضي لأنسجة الكبد والكلى حمايتها من خلال المستخلص المائي والسحن المثلي لـ S.nux-vomica. كشفت الدراسة عن وجود خصائص مضادة للأكسدة ومضادة للانحلال في المستخلص المائي من S.nux-vomica ، مع تأثير مفيد للسحن المثلي للنبات في تقليل الإجهاد التأكسدي والسمية الكبدية والسمية الكلوية التي تحدث في الفئران.

الكلمات المفتاحية: المعالجة المثلية بالسحق ، الإجهاد التأكسدي ، السموم المستحثة بالباراسيتامول ، جرذان ويستار.

Abbreviation list

BHT: Butyl hydroxy toluene

CAT: catalase

CBC : complete blood count

CNS: central nervous system

CYP450: Cytochrome P450

DPPH: 1,1-diphenyl-2-picryhydrazyl

DTNB: Acide 5,5'-dithiobis(2-nitrobenzoique) ou reactif d'Ellman

EDTA: ethylenediaminetetraacetate

EGCG: EGC 3-gallate

GOT: Glutamic Oxaloacetate Transaminase.

GPT: Glutamic Pyruvate Transaminase.

GSH: reduced Glutathione

HCC : hepatocellular carcinoma

HCT: haematocrit

HGB: haemoglobin

HPLC: High-performance liquid chromatography

IC50: Inhibitory Concentration of 50%

IL-10 : interleukins 10

IL-1β : interleukins 1β

IL-6 : interleukins 6

IL-8: interleukins 8

MDA: Determination of malondialdehyde

NAPQI: N-acétyl-p-benzoquinone imin

NF-κB: nuclear factor-kappa B

PGE2: prostaglandine

RBC: red blood cell

ROS : reactive oxygen species

SOD : Superoxyde dismutase

TBA : Thiobarbituric acid: L'acide thiobarbiturique

TCA: Trichloroacétique

PARA: Paracetamol

TCM: teneur corpusculaire moyenne

VEGF: Vascular endothelial growth factor

WBC: white blood cell

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Toxicity of N-acetyl-p-benzoquinone

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Introduction

Poisoning is usually an acute, short-lived event which necessitates immediate care, though complications such as rhabdomyolysis may persist for a few days. Less commonly, symptoms may arise only after prolonged exposure, as occurs with many heavy metals. Rarely, sequelae may not occur until many years after exposure, e.g. with vinyl chloride, acetaminophen, *N*-acetyl-*p*-benzoquinone imine . **(Bagdasarian, 2011).**

Paracetamol (acetaminophen, 4 hydroxyacetanilide, N-acetyl-para-aminophenol, 4 acetamidophenol) is the most popular analgesic in most countries in the world. In normal doses, it is remarkably free from adverse effects and interactions with other drugs, even in patients with established liver disease. Paracetamol poisoning caused by intentional overdose remains a common cause of morbidity. In high doses and / or chronically, paracetamol can cause severe cytolytic hepatitis and, more rarely, organic renal failure. Drug poisoning is one of the main reasons for emergency room visits and intensive care admissions, making this molecule one of the most feared molecules**. (Benson, 1983).**

World Health Organization has estimated that 80% of the population in developing countries rely on traditional medicines for their disease remedy . Traditional medicine in rural India has taken a front seat with the use of the available herbal resources from nature in healing diseases. Even though western allopathic medicine has succeeded in eliminating certain diseases, alleviating pain, and vaccinating people as a preventive measure, it has also contributed to the recent emerging problems like multi-drug resistant bacterial strains, which are a real threat to the population. With many reported side effects of modern drug therapy, there is always a search for disease remedy in alternative medicines like Yoga, Naturopathy, Ayurvedha, and Siddha. **(Mohesh, 2015).**

Strychnos nux-vomica is an evergreen tree native to South East Asia and India belonging to family Loganiaceae. It is a medium size tree found mostly in open habitats. It iscultivated commercially in the different part of world such as United States, European Union, Fujian, Guangdong, Guangxi, Hainan, Taiwan, and throughout tropical Asia. (**Li and Leeuwenberg, 1996).**

Pharmacologically Strychnos nux-vomica showed anticancer, antimicrobial, antiinflamatory, antioxidant, and antifeederent activity. Their specific effects on gastrointestinal problem, nervous system, blood glucose level, bones cells and cardiovascular systems have been also investigated **(Morton, 1934).**

The traditional medicinal component of Strychnos nux-vomica L. is its seed, called Semen Strychni, Nux vomica, or Ma qian zi (Chinese:). Nux vomica has long been used by Ayurvedic physicians for the treatment of different kinds of ailments, such as dyspepsia, nervous system diseases, chronic rheumatism, urinary incontinence, sexual impotence and rejuvenation. **(McIntosh, 1940).**

According to the Pharmacopoeia of the People's Republic of China, Nux vomica is bitter in flavor, warm in nature and attributives to the liver and spleen meridians. It has been shown to remove obstruction in the channels, relieve pain and subdue swelling. However, due to its severe toxicity, Nux vomica should be processed before oral usage, should not be taken over a long time period of time and its dosage should be strictly controlled **(Chinese Pharmacopoeia, 2015)**

Homeopathy is defined as a therapy based on a simple experimental principle: the law of analogy using remedies in infinitesimal doses obtained by a system of specific dilutions.

Homeopathic medicines are prepared by a pharmacological process of potentization /trituration in any of the three scales of potencies: Decimal (X), Centesimal (C) and Millesimal (LM). Each dilution step divides the original quantity by 10, 100 and 50,0000 respectively. Trituration is the method of dilution of insoluble solid substances by grinding them with lactose in a particular ratio for preparation of homeopathic medicines. **(Schmidt, 1842).**

The objective of our work is to study the effect of *Strychnos nux-vomica* extracts on the toxicity induced by Paracetamol and the role of plant trituration in the detoxification of paracetamol.

The present work is structured as follows:

- A bibliographic study summarizes the main knowledge on the plant studied and the principles of homeopathy and trituration.
- An experimental study presenting some phytochemical assay; in order to understand the constituents in secondary metabolites of the plant. As well as biochemical parameters used in the control of toxicity and oxidative stress.

Chapter I

I.1.Introduction

The traditional medicinal component of Strychnos nux-vomica L. is its seed, called Semen Strychni, Nux vomica, or Ma qian zi (Chinese:). Nux vomica has long been used by Ayurvedic physicians for the treatment of different kinds of ailments, such as dyspepsia, nervous system diseases, chronic rheumatism, urinary incontinence, sexual impotence and rejuvenation. In the 17th century, Nux vomica was introduced to America and several European countries, where it was typically sold in its powdered form for the purpose of poisoning rats and other rodents. With the discovery of strychnine and brucine from Nux vomica approximately two centuries ago, knowledge about its physiological effects became more precise, and the drug gradually became an important therapeutic agent in both human and veterinary medicine (McIntosh, 1940). There were a number of official preparations of Nux vomica for medical usage one to two centuries ago, with Nux vomica and its preparations recorded in several Pharmacopoeias, including the United States Pharmacopoeia, the British Pharmacopoeia and the Edinburgh and Dublin Pharmacopoeias. These preparations included the powdered drug, a tincture and a liquid extract. For hypodermic and oral administration, there were three salts of strychnine available: the sulphate, the nitrate and hydrochloride. In addition to official preparations, Nux vomica and strychnine were also used in non-official preparations. Although these preparations are not employed in modern medicine, Nux vomica is still being used in China, India and some Southeast Asian countries today.

I.2. Botanical Description

Strychnosnux-vomica L. **(Fig. 1)** belongs to the genus Strychnos of the family Logania-ceae and grows in Sri Lanka, India and Australia. It is a shrub or small tree that is 5–25min height. Its leaves have an opposite decussate arrangement and are papery, while the leafblade is suborbicular, broadly elliptic or ovate, 5–18 cm in length and 4–13 cm in width.Its 5-merous flower is dull-green and white. Its fruit consists of brownish-yellow berries thesize of small oranges, and contains a gelatinous pulp in which one to five seeds areembedded. The dried ripe seed is very hard, 1.5–3 cm in diameter and 3–6mm in thickness.The seed has a flattened disk shape, is slightly concave and completely covered with hairs radiating from the center to the sides; thus giving the seed a very characteristic sheen. The seed with dark gray horny endosperm, where the small embryo is housed, has no odor buttastes very bitter. These seeds can be kept for long periods.**(Guo et *al*.2018).**

Botanical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Gentianales

Family : Loganiaceae

Genus : Strychnos

Species : *Nux-vomica*

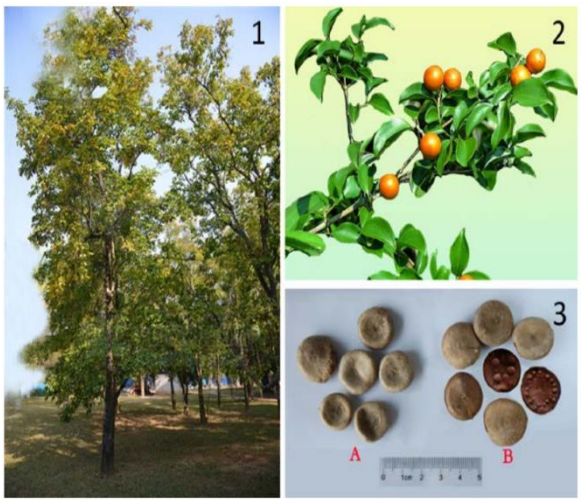

Figure 01 : Strychnosnux-vomica L. (1) whole plant, (2) the fruit and (3) the seeds: (A) crude seeds and (B) processed seeds. The seeds are processed using the hot sand processing method.

I.3.Phenology

The plant is deciduous in nature with leaf fall occurring in the cold season, mostly in December and January. New shiny leaves unfolds at the advent of summer season(1st week of February), followed by flowering initiation. Blooming progress through March and terminates at the end of April with peak of 30 days between mid February to mid March. Fruits take about 10-11 months for maturing and 3-4 fully matured seeds per fruit are common. Fruits attain orange red colour on ripening. Fruit shedding occurs immediately. The seeding was moderate in occurrence and dispersed by vertebrates (mammals and birds). The pulp of the fruit is eaten by monkeys (Bonnet Macaque), bats (fruit bats), rhinoceros hornbills, parakeets and other birds **(Balasubramanian et *al.*2004).**Germination percentage is low, but sprouted seedlings showed very good withstanding capacity in the shaded condition and even though its growth rate is slow, most of them established well**(Joy et *al.*1998).**

I.4. Ecology

S. Nux-vomica is native to tropical and subtropical regions of South East Asia and Australia **(Pawar et *al.*2017)**. In India it is commonly observed in moist deciduousand semi

evergreen forests of West Bengal, Bihar, Maharashtra, Odisha, Central and South India up to altitude of 500m **(Pulliah and Rani.2011).** Also, observed in plains, shifting cultivation areas, degraded hillocks and up lands in 03, 06, 07, 08, 09, 12, 13, 18, 19 Agro-ecological regions of India **(Shiva .1998)** (Fig 02). In its natural habitat Strychnosnux-vomica occurs at the edges of dense forest, on river banks and along the shores. It is an emergent tree in mature and "undisturbed" forests and thought to be the transition stage of the succession between the pioneers and the "climax" forest. Under favourable climatic condition seedlings can get established in a wide variety of soils ranging from loamy or loamy-sandy soil, lateritic and clayey loam soils but growth is very slow **(Schmelzer and Gurib-Fakim.2008).**

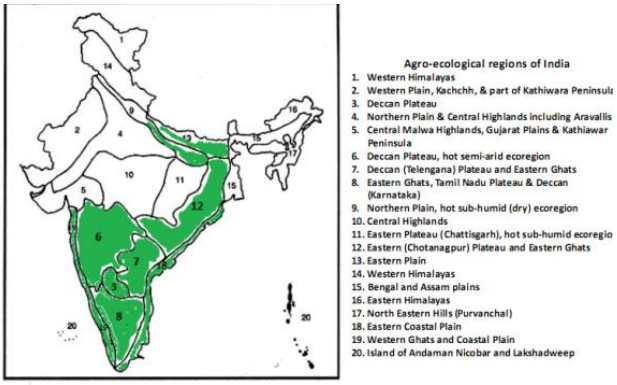

Figure 02 : Distribution of*S. nux vomica* in Agro-ecological Regions of India.

I.5. Phytochemistry

Up to the present day, many chemical compounds, including alkaloids, iridoidglycosides, flavonoid glycosides, triterpenoids, steroids and organic acids, among others, have beenisolated and identified from *S. nux-vomica*. Of these, the alkaloids are the principlechemical component in this plant. Among these alkaloids, strychnine and brucine areconsidered to be the major bioactive and principal toxic compounds. All of the known chemical constituents and isolated components of the plant are listed in **Table 01**.

Table 01 Different chemical components and chemical Formula isolated from *S. nux-vomica*

N°	Chemical Component	Part of Plant	Chemical Formula	Exact Mass	Reference
	Alkaloids				

1	Strychnine	Processed seeds	C21H22N2O2	334.1681	**Zhang et al. (2012)**
2	Strychnine-N-oxide	Seeds	C21H22N2O3	350.1630	**Cai et al. (1994)**
3	Brucine-N-oxide	Seeds seeds	C23H26N2O5	410.1842	**Cai et al. (1994)**
4	Strychnine methiodide	Fruit	C22H24N2O3	364.1787	**Liu (2010)**
5	Isostrychnine N-oxide	Seeds	C21H22N2O3	350.1630	**Cai et al. (1994)**
6	Isobrucine N-oxide	Seeds	C23H26N2O5	410.1842	**Cai et al. (1994)**
	Iridoid glycosides				
7	Loganicacid	Seeds	C16H24O10	376.1369	**Zhang et al. (2003)**
8	Loganin	Fruit	C17H26O10	390.1526	**Liu and Li (1998)**
9	Secoxyloganin	Processed seeds	C17H24O11	404.1319	**Zhang et al. (2012a)**
10	Ketologanin	Fruit	C17H24O10	388.1369	**Bisset and Choudhury (1974)**
	Flavonoid glycosides				
11	Kaempferol-7-O-glucoside	Leaves Leaves	C21H20O11	448.1006	**Eldahshan and Abdel-Daim (2015**
12	Rutin	Leaves	C27H30O16	610.1534	
13	Quercetin-3-rhamnoside	Leaves	C27H30O15	594.1585	
14	Kaempferol 3-rutinoside		C21H20O11	448.1006	

	Triterpenoids and steroids				
15	Ursolic acid	Fruit	C30H48O3	456.3603	**Liu (2010)**
16	Uvaol	Processed seeds	C30H50O2	442.3811	**Zhang et al. (2012)**
17	Stigmasta-7,22,25-triene-3-ol	Processed seeds	C29H46O	410.3549	**Zhang et al. (2012)**
18	β-Sitosterol	Fruit	C29H50O	414.3862	**Liu (2010)**
	Organic acids and phenols				
19	Gallic acid	Fruit	C7H6O5	170.0215	**Liu (2010)**
20	Vanillic acid	Fruit	C8H8O4	168.0423	**Liu (2010)**
21	Cinnamic acid	Fruit	C9H8O2	148.0524	**Liu (2010)**
22	Ferulic acid	Fruit	C10H10O4	194.0579	**Liu (2010)**
23	Salicylic acid	Fruit	C7H6O3	138.0317	**Liu (2010)**

- **Alkaloids**

S. nux-vomica contains many types of alkaloids. Among them, several are bisindole alkaloids isolated from the stem bark or roots, while most are indole alkaloids and mainly exist in the seeds. The content of strychnine must be between 1.20% and 2.20%, and brucine not less than 0.80%, in Nux vomica, according to the Chinese Pharmacopoeia (2015 Version). Most indole alkaloids are a white or yellow amorphous powder or colorless needle-like crystals (Zhang et al., 2012b; Zhao et al., 2012b; Shi et al., 2014). The chemical structures of these indole alkaloids differ mainly in several aspects, such as whether the ring G is formed, if

C-3 is linked with N-4, if C-3 and C-5 are oxidized or if N-4 is connected with other groups (Fig. 2). The chemical structures of alkaloids are shown in Fig. 3.

- **Iridoid Glycosides**

Several iridoid glycosides have been isolated from the fruit of S. nux-vomica. These iridoid glycosides are white amorphous solids, linked with glucose or acetylated glucose at the C-1 position of the iridoid core structure. The chemical structures of the iridoid glycosides are shown in Fig. 4.

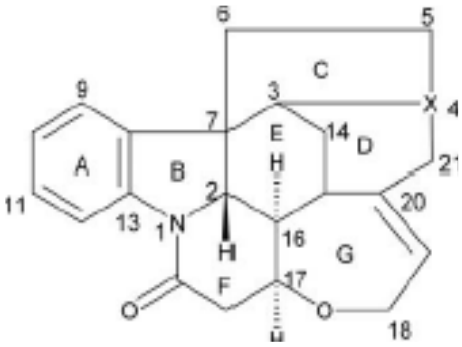

Figure 3. Skeleton structure of a Strychnos alkaloid.

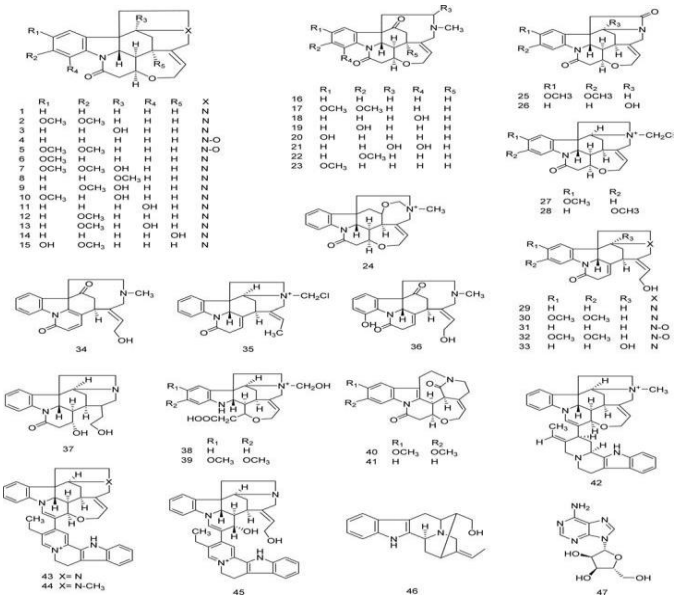

Figure 4. The structures of alkaloids from S. nux-vomica.

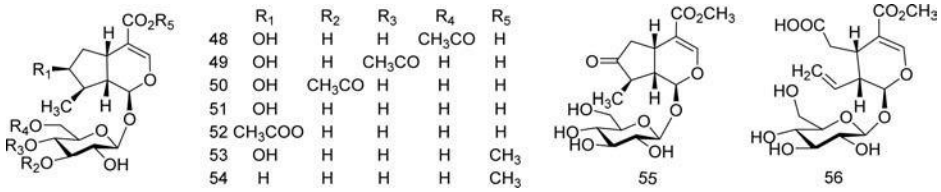

Figure 5. The structures of iridoid glycosides from *S. nux-vomica.*

- **Flavonoid Glycosides**

Some flavonoid glycosides have been isolated from S. nux-vomica leaves, all of them linked to glucose or rhamnose at the C-3 or C-7 position of the flavonoid mother nucleus **(Eldahshan and Abdel-Daim, 2015).**

- **Triterpenoids and Steroids**

Several triterpenoids and steroids have been isolated from the seed or fruit of S. nux-vomica **(Liu, 2010; Zhang et al., 2012a).**

- **Organic Acids and Phenols**

Several types of organic acids and phenols were isolated from the seed or fruit of S. nux-vomica **(Liu, 2010; Zhang et al., 2012a).**

I.6. Pharmacological Activity

I.6.1 Stimulant Effects on the Nervous System

Strychnine and brucine are the most important alkaloids of Nux vomica. Both of them arepotent stimulants of the spinal cord. Thus, the most characteristic feature of Nux vomica isto render the reflex center in the central nervous system (CNS) more sensitive to afferentstimuli (McIntosh, 1940). In addition, strychnine and brucineincrease the secretion ofgastric juices and heighten sensory awareness. There are three important applications ofNux vomica and its alkaloids, which may be related to the nervous system. Firstly, Nuxvomica is used as a stimulant drug in the digestive tract. Secondly, it is used in paralyticconditions arising from dysfunction of the motor nerves in particular. Nux vomica has beenapplied in TCM in this context. Thirdly, it is employed as a respiratory and circulatory stimulant, especiallyin debilitating conditions (McIntosh, 1940).Nuxvomica can stimulate the nervous energy of bowels, has a powerful peristaltic action on the intestines, enables the absorption of nutrients from food by the intestinal lacteals andbrings about the retention of feces by the large intestine, which makes it useful in thetreatment of exhaustive diarrhea. These effects are typically useful in veterinary medicineand are rarely applied to the treatment of humans. In the early literature relating to Nux vomica, it was used in the treatment of constipation, intestinal obstruction, colon and caecumimpactions, chronic digestive ailments and it increased the activity of purgative medicines.Nux vomica was reported to have successfully treated hemiplegia and palsy of the bladder. A tincture of Nux vomica was used to treat cardiac failure, with a possible mechanism of stimulating the motor center and the ganglionic

system to increase activity, thereby rescuing patients from the consequences of obstructed pulmonary circulation and engorgement of the right side of the heart.Nux vomica was used as a nerve tonic to improve a slow pulse. In addition, an alcoholicextract of Nux vomica was used in the treatment of impotence and spermatorrhoea. Powderof Nux vomica combined with ammonium carbonate was used to treat recumbent (unableto rise) cows.

I.6.2 Analgesic and Anti-Inflammatory Actions

The applications of Nux vomica within TCM are closely related to its analgesic and anti-inflammatory effects, and it is especially useful for the treatment of rheumatism bone pain,muscle and joint injury pains. There are many set prescriptions for pain treatment thatinclude Nux vomica, such as Jiu Fen San, Maqian San, Bali San and Ma qianzi San. Modern experimental research has further val-idated its analgesic and anti-inflammatory effects. Nux vomica significantly inhibited pawswelling in adjuvants arthritis (AA) rats (Qin, 2012). Its total alkaloids (6.2525mg/kg)had an antergic effect on AA progress in rats, and it markedly reduced the degree of footswelling, the levels of polyarthritis indicators and relieved arthropathologicalinjury (Zhenget al., 2012). The mechanism of action of Nux vomica could be related to the inhibition ofthe release of inflammatory mediators since levels of interleukin (IL-1), prostaglandin E2(PGE2), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) were reduced in AA rats(Qin, 2012; Zheng et al., 2012). Extracts of S. nuxvomica leaves demonstrated potentanalgesic effects in the writhing test, the hot plate test in mice and the tail immersion test inrats (Eldahshan and Abdel-Daim, 2015). Brucine and brucine N-oxide had significantanalgesic effects on the pain caused by physical and chemical stimulation, such as in thehot plate test, the writhing test and the formalin test (Yin et al., 2003). Both brucine andbrucine N-oxide significantly inhibited the release of PGE2 in inflammatory tissue, and reduced vascular permeability and the content of 5-hydroxytryptamine (5-HT) and 6-keto-PGF1a in the blood plasma of arthritic rats, with a corresponding increase in the content of 5hydroxytryindole-3-acetic acid (5-HIAA) (Yin et al., 2003). In addition, brucine N-oxidehad a stronger inhibitory effects than brucine on carrageenan-induced rat paw oedema,while the associated analgesic actions of the compounds were apparent during differentphases of pain induced by formalin (Yin et al., 2003). This suggests that their analgesic andanti-inflammatory mechanisms are not the same.

I.6.3. Antitumor Effects

Experiments have shown that active components and extracts of Nux vomica have in hibitory effects on many types of tumors, both in vitro and in vivo, including liver cancer, breast cancer, colon cancer and multiple myeloma. The antitumor effects include decreasing vascular endothelial growth factor (VEGF), inhibiting the growth of tumor cells,inducing apoptosis and cytotoxicity, among others. The antitumor mechanisms of Nuxvomica include decrease in VEGF, induction of cell apoptosis, cytotoxic effects and othermechanisms.VEGF induces angiogenesis in vivo, which is a pivotal step in tumor growth, invasiveness and metastasis (Qian et al., 2012; Park et al., 2016; Yang et al., 2016). Studieshave shown that brucine significantly inhibits angiogenesis in the murine sponge implantmodel (25mg/kg/day or 50mg/kg/day for nine days) and in a nude mouse model of bonemetastasis due to breast cancer (1.73, 3.45, or 6.90mg/kg/day for eight consecutive days) through a reduction in VEGF levels, which is associated with a reduction in microvessel density (Agrawal et al., 2011a). Further investigation showed that brucine attenuates VEGF production via the inhibition of the VEGF Receptor 2 (VEGFR2) signaling pathway in vitro and in vivo (Saraswati and Agrawal, 2013a). Strychnine (0.25mg/kg/day or 0.5mg/kg/day for nine days) also brought about a decrease in VEGF, leading to inhibition of inflammatory angiogenesis in the murine sponge implant model (Saraswatiand Agarwal, 2013b)

I.6.4 Effects on Pathogenic Microorganisms

Ethyl acetate extracts of *S.nux-vomica* bark showed antimicrobial activity with respect toboth Gram-positive organisms, such as Staphylococcus aureus, Bacillus subtilis andStreptococcus faecalis, and Gram-negative organisms, such as Escherichia coli, Pseudo-monas aeruginosa and Proteus vulgaris (Thambi and Cherian, 2015). The brucine derivatives, N1-(2,3-dimethoxystrychnidin-10-yliden) ethane-1,2-diamine and 11-[(2-amino-ethylamino)-methyl]-2,3dimethoxystrychnidin-10-one had an inhibitory effect onEscherichia coli and Salmonella typhi growth **(Figueroa-Valverde et al., 2012).**

I.7.Toxicology

I.7.1.Causes and Symptoms of Poisoning

The poisonous properties of Nux vomica have been known for centuries, and in the Pharmacopoeia of the People's Republic of China, it is classified as a highly toxic medicine(Committee for the Pharmacopoeia of China, 2015). Large doses of Nuxvomica cause convulsions and death. There have been many reports of poisoning by Nux vomica and thesevere toxicity caused by strychnine and brucine. Strychnine is highly toxic to humans,

andit can be rapidly absorbed from the gastrointestinal tract to act upon the CNS, causing general CNS excitation (Marques et al., 2000). The absorption half-life of strychnine is approximately 15min (Ellenhorn, 1997). Death by strychnine poisoning is usually caused by spasmodic fixation of the respiratory muscles or exhaustion of the CNS, particularly the respiratory center (McIntosh.1940).Nux vomica and strychnine poisoning is characterized by violent convulsions. Regarding this, Dr. Christison stated that "Larger doses cause violent starting of the muscles, or even a tendency to lockjaw, which are succeeded by stiffness, weariness, pain or rending in the limbs". In their highest degree these amount to violent tetanic spasm, occurring infrequent fits, with brief intervals of repose, acute sensibility and dreadful alarm. Convulsions are usually followed by intervals of complete relaxation of muscles, until the nervous system recovers from exhaustion, whereupon convulsions recur. Consciousness is retained during convulsions, and intense pain is felt from the violent contracting or stretching ofmuscles (McIntosh, 1940). In addition to convulsions, poisoning by Nux vomica andstrychnine causes agitation and ataxia, slightly accelerated breathing, respiratory and cardiac arrest, a hard and fast pulse and strabismus, among other symptoms (Wood et al.2002). Postmortem examination following Nux vomica or strychnine poisoning revealsevidence of severe muscle spasms and cardiac arrest, and the lungs, heart and mucousmembrane of the stomach show congestion (Kasun et al., 2015). Furthermore, the abuseof Nux vomica (low doses over a long time period) can induce delirium tremens andexcitable, cramp-type pains in the abdomen and legs

I.7.2.Detoxification Methods

Considering the high toxicity of Nux vomica, the exploration of detoxification methods andmechanisms is critical. Processing and compatibility are the most commonly used andimportant detoxification methods for Nux vomica. Processing is the most commonly used detoxification method for most poisonous medicines in TCM. There are presently two main types of processing methods for Nux vomica in China: the hot sand and fried processing methods. For the hot sand processing method, sand is firstly put into a hot pot, and heated until it is malleable. Nux vomica is then placed in the heated sand, and turned constantly until the seeds became swollen and the skin turns to brown. Finally, the sand is removed or sieved. For the fried processing method, pure sesame oil is firstly put into a pot and heated to approximately 230C. Nuxvomica is then placed in the pot and fried to a yellow color before immediately removing the oil (Gong, 2013). In ancient Ayurvedic texts, Nux vomica seeds were soaked in cow'smilk for 24 h, and their outer coating was then removed by scraping

with a knife. Theywere cut into small pieces and boiled in cow's milk for three days for approximately fourhours each day. After three days, they were dried in the shade and fried with cow ghee to be used as a therapeutic agent (Kumar et al., 2012). In addition to the processing methods described above, in some parts of China and other countries in Southeast Asia, alternative processing methods are used. These include the vinegar-boiled method (Wu et al., 1994), the aloe or ginger juice application method in India (Katiyar et al., 2010) and the milk impregnated processing method in Uighur medicine **(Tursun et al., 2015).**

Table 02 . The LD of Strychnine in the Rat, Mouse and Dog**(Lewis, 1996)**

LD50 (µg/Kg)			
Animal	**Oral**	**I.P**	**I.V**
Rat	2350	1100	582
Mouse	2000	980	410
Dog	500	/	800

Chapter II

Triturations

II.1 Introduction

All systems of medicine are prepared and administered in doses whose concentration can be quantified to be below Avogadro's limit except for homeopathic medicine which is available both below (hormetic) as well as above (beyond the reciprocal of Avogadro's Number, also known as avogram) Avogadro's limit. Homeopathic medicines are prepared by a pharmacological process of Potentisation/trituration in any of the three scales of potencies: Decimal(X), Centesimal (C) and Millesimal (LM). Each dilution step divides the original quantity by 10, 100 and 50,0000 respectively.

1 mole of a substance contains as many grams of substance as its atomic mass and contains 6.02254 x 1023 molecules. Conventional medicine is generally available at concentrations between micromolar (10-6M, the equivalent in homeopathy is 6X/3C) and nanomolar (10-9M, the equivalent in homeopathy is 9X/4.5C). In the decimal and centesimal scale of dilution, concentration of active medicinal substance in the water-ethanol solution reduces to 1/10 and 1/100 of the original concentration respectively. At 23X, the number of molecules reduces theoretically to 6.022 and 0.6022 at 24X. At 11C, the number of molecules reduces theoretically to 60.22 and 0.6022 at 12C. In terms of moles per litre, 12C contains 10-24 mol/L i.e. 0.6022 molecules/L i.e. 1 molecule/L. Even a single molecule can trigger or regulate a series of chain reactions that leads to activation of specific cell functions [Bellavite, 2014]. The probability of finding 1 molecule of the n molecules of the original substance in 24X/12C potency and above is extremely small, but not zero [Nancy,2015].

The Avogadro's limit (10-23M) for homeopathic solution is 23X/11C/LM4. Potencies up to23X/11C/LM4 contain concentrations (molecules) of the remedy source material and nanoparticles also. Measurable quantities of pharmacologically active compounds have a clear-cut, objective effect on the human body.

II.2 Emergence of Nanomedicine

In a paper [Hahnemann,1801] published in 1801, Hahnemann mentioned the prophylactic properties of Belladonna for the treatment of scarlet fever as well as the method of preparation for potentised Belladonna to1/24,000,000 dilution. He wrote, "we dissolve a grain (1 grain = 0.06479891 grams) of this powder …

in one hundred drops (1 drop=0.05 ml)of common distilled water, by rubbing it up in a small mortar; we pour the thick solution into a one-ounce bottle, and rinse the mortar and the pestle with three hundred drops of diluted alcohol (five parts of water to one of spirit), and we then add this to the solution, and render the union perfect, by diligently shaking the liquid. We label the bottle strong solution of belladonna. One drop of this is intimately mixed with three hundred drops of diluted alcohol by shaking it for a minute, and this is marked medium solution of belladonna. Of this second mixture one drop is mixed with two hundred drops of the diluted alcohol, by shaking for a minute, and marked weak solution of belladonna; and this is our prophylactic remedy for scarlet-fever, each drop of which contains the twenty-four millionth part of a grain of the dry belladonna juice." [Hahnemann,1852]

Hahnemann recommended the administration of one drop which is equivalent to 0.0416 nanograms of Belladonna and to repeat the dose every 72 hours. This is the first recorded nanodose of medicine used in the treatment of any disease in medical history. Samuel Hahnemann is credited with the method of preparation and prescription of nanodoses of medicine.

In 1805, Hahnemann crossed Avogadro's limit (Avogadro's limit was however unknown at that time, it was discovered only in 1860), and the 18c potency appears for the first time. Homeopathic medicines above Avogadro's limit are known as super-Avogadro dilutions [Chikramane,2012] or potentised high dilutions.

II.3 Nanoparticles

Reducing particle size increases surface area. Nanoparticles are nano-size particles (1-100 nanometers diameter) having a large surface area to volume ratio which gives them different properties from those bulk forms of the same material [Roduner,2006]. Nanodoses of medicinal agents have several benefits over crude doses of the same substance, including enhanced bioavailability, chemical and biological reactivity, adsorptive capacity, intracellular accessibility, increased ability to cross cell membranes and even the blood brain barrier, and of course, a substantial better safety profile

(because of reduced toxicity) [Ullman,2014]. Nanoparticles can distinguish between healthy and unhealthy cells while bulk concentrations of medicine cannot.

The body runs on micro and nanodoses. The levels of hormones and cell signaling agents in our body is in the picograms/mililitre range such as 10–900 pg/ml for estradiol,

300–10,000 pg/ml for testosterone, and 8–27 pg/ml for T4. These small amounts are known to have a profound effect on the human body.

II.4 Search for Nanoparticles in Homeopathic Solutions

The presence of molecules of the original substance beyond Avogadro's limit was first mentioned in the French book Recherché expérimentale moderne en homéopathie (Modern Experimental Research in Homeopathy) authored by Maurice Plazy and Robert Mergerin in 1967 .

Research carried out by a team from the Department of Chemical Engineering, Indian Institute of Technology Bombay (IIT-B) and published in Homeopathy (Elsevier) in 2010 indicates the presence of 1-4000 picograms/mililitre of fine nanoparticles (5-15 nanometer size) of the original starting material (one molar concentration) in 200c (dilution of 1 part in 10 raised to 400 parts) potency of metal-derived homeopathic medicines. This study [Chikramane,2010] concluded that by using transmission electron microscopy, electron diffraction and atomic spectroscopy it is possible to measure the quantity of nanoparticles found in homeopathic medicines which retain their potency even when diluted to a nanometre or one-billionth of a metre.

In an article published in The Times of India, the third largest circulating newspaper in the world, dated 16 Dec 2010 titled: "IIT-B Team Shows How Homeopathy Works", the co-author of the paper and Head of Chemical Engineering Department at IIT-B, Dr. Jayesh Bellare, said, "Homeopathy has been a conundrum for modern medicine. Its practitioners maintained that homeopathic pills got more potent on dilution, but they could never explain the mechanism scientifically enough for modern scientists"[IIT-B,2010].

In his lecture, "Homeopathy as Nanomedicine", at the World Homeopathic Congress 2011 , held in New Delhi, India from 1-4 Dec 2011, Dr. Jayesh said, "Even the highest dilutions of remedies have left signatures of respective original molecules in their nanoparticle form, when subjected to electron microscopy studies, validated later by wavelength analysis." He related how the succussion process could 'break the quantum domain' of a medical substance and accelerate-decelerate protein activity, like electro-magnetic waves do.

But what about potencies greater than 200c. Do the potencies above 200c fall under the ambit of nanomedicine? As of now it has not been possible to quantify the amount of

active ingredient in potencies above 200c. Logically potencies above 200c should contain even less amount of active ingredient.

Research carried out jointly by the Indian Institute of Technology Delhi (IIT-D) and the Central Council for Research in Homeopathy (Government of India) and published in the International Journal of High Dilution Research in 2011, indicates the presence of crystalline nanoparticles (100 nanometre) in 15c potencies of plant-derived homeopathic medicines. They used energy dispersive X-ray analysis, selected area nanodiffraction and trace element analysis to determine the presence of nanoparticles [Nayak ,2011].

Research in the Indian Institute of Technology Kharagpur (IIT-Kharagpur), published in Journal of Analytical Methods in Chemistry (2012) found the presence of biologically active digoxin-like substance in Digitalis Purpurea 30c and 200c. They used Fluorescence Spectroscopy and Cyclic-Voltammetry to determine the presence of active ingredients [Anup,2012].

A study [Anup,2012] published in 2012 in Langmuir found that nanoparticles of six original medicinal agents persisted in 6c, 30c and 200c solutions and explained the process by which these nanoparticles are transferred and retained beyond Avogadro's limit which has been documented with high speed videography. They also found 325 nanoclusters of lactose.

Research in the Department of Chemistry, Ramnarian Ruia College, Mumbai in 2013 using dynamic light scattering technique confirms 13.8 nanometer size particle in Terminalia Arjuna Mother Tincture [Brave,2013]. This study shows that the dose of medicine required is reduced by orders of magnitude when it is in nanoparticle size which is in conformity with the principles of homeopathy.

II.5 Nanopharmacology

Homeopathy is, in fact, a form of nanopharmacology [Bellavite,2015], medicine prepared through a specific pharmacological process of potentisation/trituration. Trituration (repeated grinding with lactose powder) is carried out by pulverisation in a grinding machine. This results in breaking down of medicinal substance into nanoparticles. Nanoparticles have biological activity. Nanoparticles in homeopathic medicines exert biological effects on the body. Nanoparticles can cause hormesis [Iavicoli,2010]. Hormesis is an adaptive response that is induced by stimulation at low doses and inhibition at high doses. Hormesis is akin to Arndt-Schultz Law which is about non-linearity between dose and response. Hence, it appears that homeopathically prepared remedies may also stimulate a similar response, causing a

specific hormesis which, in turn, ensures that homeostasis is maintained, and thus, counteracts illness in a curative manner.

II.6.Triturations

Hahnemann knew of the medicinal use of powdered gold from his study of 8th-century medical literature and 12th-century Arabic medicine. Initially he experimented with solu-ble gold compounds. The acid radicals altered the properties of the metal, however, and he therefore tried to find a method of processing the pure metal. In 1818 he triturated gold leaf with lactose and found the le potency to be highly effective in the treatment of suicidal depression.3 He then also triturated Aurum to produce higher potencies and made tritura-tion the general processing method in homreopathic pharmacy.

II.6.1.Importance of the 3c trituration in the manufacture of homreopathic medicines

Based on the original trituration of gold made in 1818, Hahnemann introduced tri-turation to the 3c as a general method for use in homreopathic pharmacy from 1835 onwards, thus distancing himself from the use of solutions and mother tinctures. He found that 3c triturated medicines were more effective, with constituents retained to a much higher degree and improved storage qualities.

II.6.2.Advantages of 3c trituration

Initially, Hahnemann triturated medicines up to the 12c,4 and in 1835 changed to the 3c ('1:1,000,000 dilution in powder form'), producing higher potencies in fluid form. Com-pared to medicines produced from mother tinctures and solutions, this offered the fol-lowing advantages:

--More powerful action

-Constituents retained

-Guaranteed shelf life

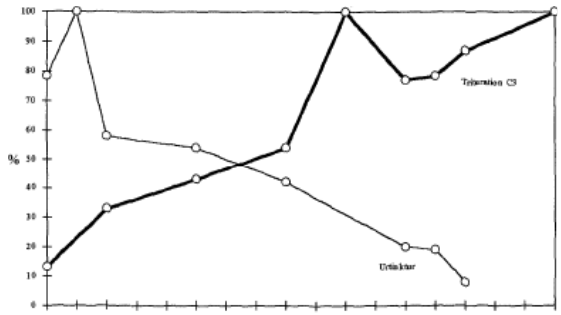

Figure 6. Hahnemann's pharmaceutical methods: Percentage frequency of reference to 3c triturations and mother tinctures in Materia Medica Pura and Chronic Diseases 1825-1842.

II.6.3. More powerful action

In the first edition of his Chronic Diseases, published in 1828, Hahnemann stated that by using the 3c trituration of lodum, evolution of medicinal power was 'much more complete' than with solution-based potencies:

II.7. Paradoxical effect with low dilution

The minimum of T1/T2 observed in the dilution range 3 D-6 D was unexpected. But several authors have also described more or less acute physicochemical transitions to 10-4 - 10-7 M in solutions of electrolytes and non-electrolytes . The phenomenon has been discussed by some authors in terms of the transition between a molecular or ionic regime at high concentration and a regime of large clusters of water beyond a critical dilution, which is in line with my hypotheses. The apparent paradox of my NMR results is that the water appears more unstructured in dilution than in its pure state. In reality, this is explained because water in its pure state is already strongly organized by intermolecular hydrogen bonds into a polyhedral three-dimensional lattice, proof of this is the diffraction of X-rays by liquid water, a phenomenon peculiar to crystalline states. This is therefore a "chaotropic" or "structure-breaking" effect common to many substances, including chlorides ; the mobility of water molecules is higher than in pure water. A completely different interpretation of these physicochemical transitions comes from the QED (Quantum Electrodynamics) theory which predicts that ions or non-electrolyte solutes entlvaated form microdomains of coherence at high concentration that evolve abruptly into macrodomains beyond a critical dilution (of the order of 10-4 M for ions) , which is similar to , coherence in addition, the hypothesis of large clusters of water with high dilution. (Demangeat, 2015).

Curiously, this type of physicochemical transition is also described in pharmacology and toxicology, and generally occurs in the same concentration range 10-3 - 10-6 M. The phenomenon, called "hormesis", consists of an inverted dose effect, inhibitory or toxic at high doses, stimulator or protector at low doses ; its mechanism, probably very complex, is not elucidated (see special issue of Homeopathy . I put forward the idea of bringing the physical transitions of T1 / T2 observed in the low dilutions of chlorides closer to the dose-response curves of chlorides reported in toxicology; the underlying hypothesis is that biological activity could differ, or be inverted, between highly concentrated regimes where the non- or incompletely ly entavated solute, or even in the form of aggregates, acts mainly by its exposed molecular radicals, and highly diluted regimes where it is the totally lylated state, or integrated into aqueous superstructures such as clusters or clathrates, that governs the interaction. It is known that the function of biological macromole cules, enzymes, proteins, DNA, is crucially dependent on their degree of hydration . (Demangeat, 2015).

II.8.Water memory

At present there are only theories, listed above, water polymers [23], clathrates [24,25], fields of consistency [19–21], epitaxy [26,27]. However, admitting the existence of superstructures, even very stereospecific, the mechanisms evoked to explain the transmission of bio-information, empty imprints (polymeric or epitaxial), empty clathrates, or em wave emission, remain specific. My NB theory would provide a solution to this insurmountable difficulty by admitting the persistence, beyond 12 C, of the active ingredient within a nano-metric superstructure. This would be a rational explanation by which the specificity of the remedy would be ensured by the active ingredient

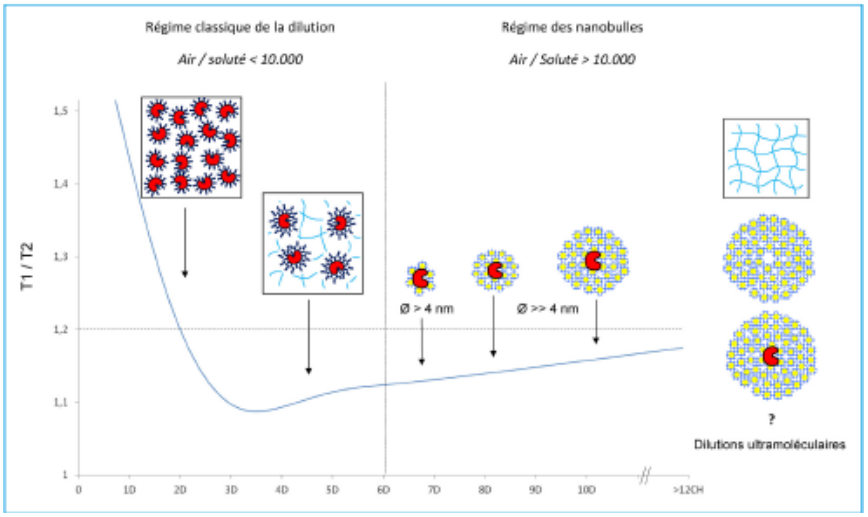

Figure 7. Assumption of interpretation of NMR results. (Demangeat, 2015).

In red, solute molecule surrounded by its solvent water, mono- and bilayer (small blue strokes perpendicular). In yellow, nanobubbles surrounded by their ice-type water clathrates (blue crowns). Blue grid, schematic representation of the organized structure of pure water. At high concentrations, the NMR signal is dominated by highly organized solute solvent water, resulting in a high T1/T2 ratio. When diluting, there is no longer enough solute for the signal of the solvent water to be detectable, but too much solute nevertheless to allow the middle water to recover its initial structuring (represented by the blue grid). T1/T2 goes through a minimum. This is the "classic regime" of dilution (the NB resulting from energization, with no structuring role in relation to the solute at this stage, have not been represented). Beyond that, the environment is characterized by plenty of water; it is the "NB plan." The NBs surrounded by their clathrates come to cash in the solute to form a stereospecific nanostructure of the order of 4 nm. As the dilution continues, it will behave like a new solute and surround itself with more external layers of NB/clathrates, and so on. The increase in superstructures explains the increase in the T1/T2 ratio. Beyond 12 C, while the structure of pure water should theoretically be regained, the superstructures remain present and even increase even more, as my NMR experiment demonstrates. I postulate the possibility (the NMR cannot affirm, being sensitive only to water) of the persistence of residual solute, which would have been transferred from dilution to dilution, within the superstructures, via a vector mechanism (banal) of bubble/pipette interactions. (Demangeat, 2015).

the superstructure may condition its bioavailability or even reactivity. If on the contrary there are no more molecules, my theory is part of the speculative logic of the previous ones by the demonstration of superstructures, but empty, which act via their stereospecificity.

For the first time in an approach to "water memory" were taken into account the physical factors of preparation, energization producing NB and pipetting likely to disturb the environment. These collateral factors may have so far held the key to the mystery of homeopathy. And what about the impurities that contaminate any solution, even under drastic conditions of preparation under laminar flow hood? Hydrophobic for the most part, they behave like NB traps (and probably also NB-rich superstructures) and are subject to the hydrophobic pull of the sampling pipette. Such a parasitic factor cannot be overlooked in the transfer process. (Demangeat, 2015).

II.9.Sinusoidal activity with the height of dilution

In homeopathy, successive dilutions exert non-linear or even oscillating sinusoidal effects (reviewed in . Challenged by the remarkable "too" regularity of oscillations in some studies .I am interested in a possible physical origin of such a response. Without wishing to deny the possibility of complex biological processes such as cybernetics or feedback (of which hormesis could be the first manifestation), I would be inclined to first seek a systematic phenomenon related to the preparation of dilutions. Barnard and Stephenson had already hypothesized that water polymer chains grew with dilution to a break point related to shear forces, and that during suc-cessive dilutions the average molecular weight of these chains oscillated between a minimum and maximum value. This hypothesis is transposable to the superstructures of my theory, consisting of NB iterative appositions, collapsing after magnifying critically (while retaining the ultimate structure of the heart), then reforming, and so on, with near-perfect mechanistic regularity. However, I have no experiential arguments to support it other than the experiment on arsenic anhydride where the suggested oscillation at 12 C cannot be confirmed given the absence of intermediate dilutions between 15C and 30C. (Demangeat, 2015).

Chapter III

Toxicity of N-acetyl-p-benzoquinone

III.1.Pathophysiology

The principal toxic metabolite of acetaminophen, *N*-acetyl-*p*-benzoquinone imine (NAPQI), is produced by the hepatic cytochrome P-450 enzyme system; glutathione stores in the liver detoxify this metabolite. An acute overdose depletes glutathione stores in the liver. As a result, NAPQI accumulates, causing hepatocellular necrosis and possibly damage to other organs (eg, kidneys, pancreas). Theoretically, alcoholic liver disease or undernutrition could increase risk of toxicity because hepatic enzyme preconditioning may increase formation of NAPQI and because undernutrition (also common among alcoholics) reduces hepatic glutathione stores. However, therapeutic doses of acetaminophen in alcoholic patients are not associated with hepatic injury.**(Hinson, 2010)**

III.2. Acute Acetaminophen Poisoning

To cause toxicity, an acute oral overdose must total ≥ 150 mg/kg (about 7.5 g in adults) within 24 hours.

III.2.1. IV acetaminophen

An IV formulation of acetaminophen that is designed for use in hospitals and in patients > 2 years of age has been associated with several hundred reports of overdoses, including several dozen fatalities, several in children. Most of these adverse events were the result of dosing errors because the drug is dosed in milligrams but dispensed in milliliters. Because these overdoses are iatrogenic, reliable information regarding time and total dose is available. The Rumack-Matthew nomogram has thus been used with success to predict toxicity. Overdoses < 150 mg/kg are unlikely to result in toxicity. However, definitive treatment of IV acetaminophen overdose has not been determined, and consultation with a toxicologist or a poison control center is recommended.**(Bailey, 2017).**

III.2.2. Symptoms and Signs

Mild poisoning may not cause symptoms, and when present, symptoms of acute acetaminophen poisoning are usually minor until ≥ 48 hours after ingestion. Symptoms, which occur in 4 stages, include anorexia, nausea, vomiting, and right upper quadrant abdominal pain. Renal failure and pancreatitis may occur, occasionally without liver failure. After > 5 days, hepatotoxicity resolves or progresses to multiple organ failure, which can be fatal.**(Saljoughian, 2016).**

III.2.3. Diagnosis

- Serum acetaminophen levels
- Rumack-Matthew nomogram

Acetaminophen overdose should be considered in all patients with nonaccidental ingestions that may be suicide attempts and in children with ingestions because formulations containing acetaminophen are frequently ingested in such overdoses and are not reported. Also, because acetaminophen often causes minimal symptoms during the early stages and is potentially lethal but treatable, ingestion should be considered in all patients with accidental ingestions as well.

Likelihood and severity of hepatotoxicity caused by an acute ingestion can be predicted by the amount ingested or, more accurately, by the serum acetaminophen level. If the time of acute ingestion is known, the Rumack-Matthew nomogram is used to estimate likelihood of hepatotoxicity; if the time of acute ingestion is unknown, the nomogram cannot be used. For a single acute overdose of traditional acetaminophen or rapid-relief acetaminophen (which is absorbed 7 to 8 minutes faster), levels are measured ≥ 4 hours after ingestion and plotted on the nomogram. A level ≤ 150 mcg/mL (≤ 990 micromol/L) and absence of toxic symptoms indicate that hepatotoxicity is very unlikely. Higher levels indicate possible hepatotoxicity. For a single acute overdose with extended-relief acetaminophen (which has 2 peak serum levels about 4 hours apart), acetaminophen levels are measured ≥ 4 hours after ingestion and 4 hours later; if either level is above the Rumack-Matthew line of toxicity, treatment is required.

If poisoning is confirmed or strongly suspected or if the time of ingestion is unclear or unknown, additional testing is indicated. Liver tests are done and, in suspected severe poisoning, prothrombin time is measured. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) results correlate with the stage of poisoning AST levels > 1000 IU/L are more likely to result from acetaminophen poisoning than from chronic hepatitis or alcoholic liver disease. If poisoning is severe, bilirubin and international normalized ratio may be elevated.

Low-level transaminase elevations (eg, up to 2 or 3 times the upper limit of normal) may occur in adults taking therapeutic doses of acetaminophen for days or weeks. These elevations appear to be transient, usually resolve or decrease (even with continued acetaminophen use), are usually clinically asymptomatic, and are probably insignificant.

Acetaminophen/cysteine protein adducts are new biomarkers developed and marketed as indicators of acetaminophen-induced hepatotoxicity. Although the biomarkers may indicate exposure to acetaminophen, they do not conclusively indicate acetaminophen-induced hepatotoxicity. Other biomarkers such as microRNA are under investigation but are not standard diagnostic tools.**(Agrawal, 2020)**

III.3. Prognosis

With appropriate treatment, mortality is uncommon.

Poor prognostic indicators at 24 to 48 hours postingestion include all of the following:

- pH < 7.3 after adequate resuscitation
- International normalized ratio (INR) > 3
- Serum creatinine > 2.6
- Hepatic encephalopathy grade III (confusion and somnolence) or grade IV (stupor and coma)
- Hypoglycemia
- Thrombocytopenia

Acute acetaminophen toxicity does not predispose patients to cirrhosis.**(Yoon, 2016).**

III.3.1. Treatment

- Oral or IV *N*-acetylcysteine
- Possibly activated charcoal

Activated charcoal may be given if acetaminophen is likely to still remain in the gastrointestinal (GI) tract.

N-Acetylcysteine is an antidote for acetaminophen poisoning. This drug is a glutathione precursor that decreases acetaminophen toxicity by increasing hepatic glutathione stores and possibly via other mechanisms. It helps prevent hepatic toxicity by inactivating the toxic acetaminophen metabolite NAPQI (*N*-acetyl-*p*-benzoquinone imine) before it can injure liver cells. However, it does not reverse damage to liver cells that has already occurred.

For acute poisoning, *N*-acetylcysteine is given if hepatotoxicity is likely based on acetaminophen dose or serum level. The drug is most effective if given within 8 hours

of acetaminophen ingestion. After 24 hours, the benefit of the antidote is questionable, but it should still be given. If degree of toxicity is uncertain, *N*-acetylcysteine should be given until toxicity is ruled out.

N-Acetylcysteine is equally effective given IV or orally. IV therapy is given as a continuous infusion. A loading dose of 150 mg/kg in 200 mL of 5% D/W given over 15 minutes is followed by maintenance doses of 50 mg/kg in 500 mL of 5% D/W given over 4 hours, then 100 mg/kg in 1000 mL of 5% D/W given over 16 hours. For children, dosing may need to be adjusted to decrease the total volume of fluid delivered; consultation with a poison control center is recommended.

The oral loading dose of *N*-acetylcysteine is 140 mg/kg. This dose is followed by 17 additional doses of 70 mg/kg every 4 hours. Oral acetylcysteine is unpalatable; it is given diluted 1:4 in a carbonated beverage or fruit juice and may still cause vomiting. If vomiting occurs, an antiemetic can be used; if vomiting occurs within 1 hour of a dose, the dose is repeated. However, vomiting may be protracted and may limit oral use. Allergic reactions are unusual but have occurred with oral and IV use.

Liver failure is treated supportively. Patients with fulminant liver failure may require liver transplantation.**(Heard, 2018).**

Key Points

- Because acetaminophen is ubiquitous and initially asymptomatic and treatable in overdose, consider toxicity in all possibly poisoned patients.
- Use the Rumack-Matthew nomogram when time of ingestion is known to predict risk of hepatotoxicity based on serum acetaminophen levels.
- If hepatotoxicity is likely, give oral or IV *N*-acetylcysteine.
- If acetaminophen is still probably in the GI tract, give activated charcoal.
- If degree of toxicity is uncertain, begin IV or oral *N*-acetylcysteine until more conclusive definitive information is available.**(Gerald ,2020)**

III.4. Chronic Acetaminophen Poisoning

Chronic excessive use or repeated overdoses cause hepatotoxicity in a few patients. Usually, chronic overdose is not an attempt at self-injury but instead results from taking

inappropriately high doses to treat pain. Symptoms may be absent or may include any of those symptoms that occur with acute overdose.**(Gerald ,2020)**

III.4.1 Diagnosis

- Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum acetaminophen levels

The Rumack-Matthew nomogram cannot be used, but likelihood of clinically significant hepatotoxicity can be estimated based on AST, ALT, and serum acetaminophen levels.

- If AST and ALT levels are normal (< 50 IU/L [0.83 microkat/L]), and the acetaminophen level is < 10 mcg/mL (< 66 micromol/L), significant hepatotoxicity is very unlikely.
- If AST and ALT levels are normal but the acetaminophen level is ≥ 10 mcg/mL (> 66 micromol/L), significant hepatotoxicity is possible; AST and ALT levels are remeasured after 24 hours. If repeat AST and ALT levels are normal, significant hepatotoxicity is unlikely; if the levels are high, significant hepatotoxicity is assumed.
- If initial AST and ALT levels are high, regardless of the acetaminophen level, significant hepatotoxicity is assumed.**(Gerald ,2020)**

III.4.2 Treatment

- Sometimes *N*-acetylcysteine

The role of *N*-acetylcysteine in treatment of chronic acetaminophen toxicity or in the presence of established acute hepatotoxicity is unclear. Theoretically, the antidote may have some benefit if given > 24 hours after an ingestion if residual (unmetabolized) acetaminophen is present. The following approach has not been proved effective but may be used:

- If hepatotoxicity is possible (if aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are normal and acetaminophen level is initially elevated), *N*-acetylcysteine is given 140 mg/kg orally loading dose and 70 mg/kg orally every 4 hours for the first 24 hours. If repeat AST and ALT levels (after 24 hours) are normal, *N*-acetylcysteine is stopped; if repeat levels are high, they are remeasured daily, and *N*-acetylcysteine is continued until levels are normal.

- If hepatotoxicity is likely (especially if initial AST and ALT levels are high), a full course of *N*-acetylcysteine is given.

Prognostic factors are similar to those in acute acetaminophen poisoning.**(Gerald ,2020)**

Second Part

Experimental Part

Chapter I

Materials and Methods

I. Material

I.1. In vitro study

I.1.1 Plant material

The plant used *Strychnos nux-vomica* (the fruit) was obtained by an herbalist in the wilaya of El oued, then it was authenticated by a specialist in taxonomy. The part used was washed and air-dried. Then it was crushed and stored in glass vials until use.

I.1.2 Aqueous extract preparation

The aqueous extracts were prepared by adding 150 ml of distilled water to (10 g) of *Strychnosnux-vomica* dans powder in an Erlenmeyer flask. The extraction was carried out on a magnetic heating stirrer at 80 ° C. for 2 h, and the maceration at room temperature for 12 h. after filtration, the filtrate was evaporated to dryness at 65 ° C using a rotary evaporator (Majhenic et al., 2007).

I.3 Preparation of Homeopathic Treatments

The extraction makes it possible to obtain a mother tincture which is the first preparation making it possible to carry out the Hahnemannian dilutions. The soluble substances macerate in a water / alcohol mixture at 80% V / V for three weeks (21 days). After filtration, the insoluble substances, used in the pure state, are triturated, that is to say crushed with lactose. The strains are then deconcentrated by adding lactose until the solubility threshold is obtained (generally 3 CH or 4 CH). **(Bitencourt and Bonato 2008).**

- **Vehicles**

Lactose for which the obligatory prior checking is carried out by the supplier.

- **Souche** hydro alcoholic extract of nut of *Strychnos nux-vomica*

➢ **Operating mode.**

- **Preparations for delutions Hahnemannian process**

Take 1 drop of mother tincture and dilute it with 99 drops of 70% V / V alcohol. By a dynamization action, the mixture is homogenized to obtain the substance "X" 1 CH. To repeat this operation, a drop of this preparation is placed in a flask and 99 drops of 70% V / V alcohol are again added, the mixture is stirred to obtain the substance "X" 2 CH. This process is repeated each time in a new vial to arrive at the desired dilution.

For insoluble products, preparation by maceration is impossible, so you have to work in a dry environment. The 1 CH dilution is obtained by gradually adding 99 parts of lactose to the parent substance while finely grinding the mixture. This operation is called "trituration". From 3-4 CH, the substances are considered soluble, therefore prepared according to the conventional Hahnemannian method described above. **(Bitencourt and Bonato 2008).**

Fig. 8 : process of potentisation (dilution and succussion) homeopathic preparations **(Bitencourt and Bonato 2008).**

I.4. Animal materials

I.4.1 Animal and husbandry condition

In this study, 30 male Wister rats weighting between **165-247g**, were obtained at the Animal Service of the Pasteur Institute, Algeria. The animals were carried under the same conditions, photoperiod (12h of light/12h of black) with a relative humidity of 65.3%

and an ambient temperature of (25 ± 2) C° for four weeks. Animals have free access to water and food by a standard diet (Southon et al, 1984) .

I.4.2 Treatment animals

After a period of adaptation, the animals were divided into five experimental groups of 6 animals each as follows:

- **Group 1:** Controls receiving distilled water for 12 days
- **Group 2:** Animals treated with a toxic dose of Paracetamol 1/4 of the LD_{50} (DL_{50}= 1943 mg/Kg) by gavage (orally) for 12 days.
- **Group 3:** Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the aqueous extract of *Strychnos nux-vomica* (0.5 mg / Kg) for 12 days.
- **Group 4:** Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the homeopathic trituration (CH 14) of *Strychnos nux-vomica* (0.5 mg / Kg) for 12 days.
- **Group 5:** Animals received a toxic dose of Paracetamol 1/4 of the LD_{50} by gavage (oral route) and treated with the homeopathic trituration (CH 30) of *Strychnos nux-vomica* (0.5 mg / Kg) for 12 days.

I.4.3 Sacrifice, blood and tissues collection

The rats are fasted for 12 hours, then anesthetized by an intraperitonial injection of 10% hydrated chloral (0.3 ml / 100 g of the body weight of the rat) using a 2 ml syringe.

Blood samples are taken from the abdominal artery to obtain all of the blood after an abdominal incision. An aliquot is collected on heparinized tubes to perform the biochemical study.

After the dissection the liver, the heart, and the kidneys are carefully removed, rinsed with physiological water, weighed. the liver and kidneys were used for the histopathological study. then the homogenates of the organs were used for the determination of the parameters of oxidative stress (glutathione and MDA)

II. Methods

II.1 Phytochemical analysis

The phytochemical analysis were carried out on the aqueous extracts prepared from the plant by qualitative characterization method according to (Evans., 2009; Harborne., 1998; Wadood et al, 2013 &Harborne, 1973)).

II.1.1 The yield of the extract

The yield of the plant in extract is the ratio between the weight of the extract and the weight of the plant to be treated (Harborne, 1998), The yield of the isolated macerated aqueous crude extract was quantified according to the formula:

$$R\% = PEB / PMV \times 100$$

A: Yield.

PEB: Weight of the Raw Extract (g).

PMV: Weight of Plant Material (g).

II.1.2 Phenols

1 ml of plant extract, two drops of 2% alcoholic ferric chloride solution are added. The appearance of a blackish blue or more or less dark green color indicates the presence of polyphenolic compounds (Bidie et al., 2011).

II.1.3 Flavonoids

Flavonoids are sought by the reaction to cyanidin (Bruneton, 2009). 5 ml of 5% infused test tube are placed in a test tube and mixed with 5 ml of hydrochloric alcohol (4 ml of ethanol and 1 ml of concentrated HCl) and 1 ml of isoamyl alcohol and then 2 or 3 magnesium shavings. A precipitation reaction occurs for a few minutes. The appearance in the supernatant layer of isoamyl alcohol of a color:

- Orange pink indicates the presence of flavones.
- Purplish pink characterizes flavanones.
- Red indicates the presence of flavonols and flavanonols.

Alkaloids

1 ml of aqueous and hydro-ethanolic plant extract are added with a drop of concentrated HCl, the solution obtained is added 2 drops of Drajendorf's reagent. The appearance of a precipitate or a reddish-brown color indicates the presence of alkaloids (Bagre et al.,2007)

Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride (FeCl3). The presence of tannins was indicated by a greenish or bluish-blackish coloration. (Karumi et *al.*, 2004; Rizk, 1982).

Terpenoids

The formation of a reddish brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract. (Chebrouk ,2009).

Reducing compound

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, the appearance of a brick-red precipitate indicates the presence of reducing sugars. Harborne (1998)

Saponins

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins. (Makhloufi, 2013)

Steroids

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H2SO4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H2SO4 was added. The presence of the red color indicates the presence of steroid derivatives. (Bagré *et al*., 2007).

Determination of total polyphenols

The reagent used is Folin-Ciocalteu, which is a mixture of complexes of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40) of yellow

color. The principle of the method is based on the oxidation of phenolic compounds by this reagent. It causes the formation of a new blue molybdenum-tungsten complex which absorbs at 765 nm..

The determination of the total polyphenols was carried out according to a colorimetric method described by Nickvar et al. (2008): 50 µl of the sample or one of the concentrations of the standard range (0 to 400 µg / ml of gallic acid) are mixed with 1 ml of the Folin-Ciocalteu reagent (0.2 N) and incubated at room temperature. After 10 min, 0.8 ml of sodium carbonate solution (Na2CO3) (75%) are added. The final solution is mixed well and kept in the dark for 30 min, the absorbance is read at 765 nm. The content of phenolic compounds is determined by referring to the calibration curve obtained with gallic acid.

The results are expressed in micrograms gallic acid equivalent per milligram of dry matter of the extract obtained (mg EAG / g DW)

- **Determination of flavonoids**

The reagents used are colorless solutions of sodium nitrite (NaNO2) and aluminum chloride (AlCl3). The principle of the method is based on the oxidation of flavonoids by these reagents. It causes the formation of a pink complex which absorbs at 510 nm.

The determination of the flavonoids was carried out according to a olorimetricmethod described by Zhishen et al., (1999): 250 µl of the sample or one of the concentrations of the standard range (20 to 260 µg / ml of quercetin) are mixed with 1 ml of distilled water, then 75 µl of an NaNO2 solution (15%) are added. After 6 min of incubation at room temperature, 75 µl of aluminum chloride AlCl3 (10%) are added. After standing for 6 min at room temperature 1 ml of sodium hydroxide NaOH (4%) is brought to the mixture. The total volume is made up to 2.5 ml of distilled water.

The final solution is mixed well and kept in the dark for 30 min, the absorbance is read at 510 nm. The flavonoid content is determined by referring to the calibration curve obtained with quercetin. The results obtained are expressed in micrograms of quercetin equivalent per milligram of the dry matter of the extract obtained (µg EQ / g DW)

II.2 DPPH radical-scavenging assay

Radical-scavenging activity of plant extracts against stable DPPH (1,1diphenyl-2 picryl hydrazyl radical) was determined spectrophotometrically. The DPPH assay was carried out as described by **(Samarth et *al*.,2008).** Stock solutions of crude extracts were prepared as

1 mg/ml in methanol. Fifty microlitres of different concentration samples were added to 5 ml of 0.004% methanol solution of DPPH After 30 min of incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate and percentage of inhibition was calculated using the following formula

DPPH scavenging-radical (%) = [(**A0 - As**) / **A0**] x 100

A0: is the absorbance of control reaction

As: is the absorbance of sample solution containing the test compound.

The ascorbic acid and BHT were used as a positive control. The IC50 of extract was calculated from the graph of inhibition percentage plotted against extract concentrations

II.3 Hemolysis assay

Hemolysis assay was done as described by **(Henkelman.S. et *al*.,2009)** 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4oC and used within 6 h for the hemolysis assay. 50 uL of 10 dilutions (100 uL Erythrocytes suspension: 900 uL1XPBS) of erythrocytes suspension was mixed with 100 uL of test samples (*Strychnosnux-vomica*) (20-80ng/mL), 100 uL of 1XPBS was used as negative control and 100 uL of 1% SDS as positive controls.

Reaction mixture was incubated at 37ºC water bath for 60 min. Volume of reaction mixture was made up to 1 mL by adding 850 uL of 1XPB. Finally, it was centrifuged at 300rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. Percentage haemolysis was calculated as follows

$$\textbf{Hemolysis inhibition (\%)} = 100 - [\textbf{Sample} \div \textbf{Control}] \times 100$$

II.4 Method for assaying serum biochemical parameters

Blood glucose test method

blood glucose measurement was performed by the enzymatic glucose oxidase method by a type autoanalyzer (BIOLIS24j) using the Blood Glucose Reagent Kit (Trinder, 1996). Glucose is converted by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide (H2O2). The latter, in the presence of peroxidase (POD), oxidizes the colorless chromogen (4-aminophenazone) to a compound colored in red-violet (quinoneimine). The absorbances of the samples, the standard and the blank are read at 505nm. The final color is stable for at least 30 minutes.

Urea assay method

Serum urea was determined by a colorimetric method by a type Autoanalyzer (BIOLIS24j) using the Serum Urea Reagent Kit **(KAPLAN, 1984).**

Creatinine dosage

Creatinine was determined according to a colorimetric method by a type autoanalyzer (BIOLIS24j) using the JAFFE Reagent Kit (Murray, 1984). The test is based on the reaction of creatinine with sodium picrate: this is the reaction of JAFFE. Creatinine reacts with the alkaline picrate to give a color complex, measured over a defined time interval and proportional to the creatinine concentration in the sample. The reading is done by spectrophotometry at a wavelength $\lambda = 492$ nm.

Method for assaying alanine aminotransferase (ALAT) activity

In our study, Alanine aminotransferase (ALAT) was determined following a colorimetric method by a type autoanalyzer (BIOLIS24j) using the Kit of Alanine Aminotransferase Reagent (MURRAY., 1984).

Aspartate aminotransferase (ASAT) activity assay method

In our study, Aspartate aminotransferase (ASAT) was determined colorimetrically by a type autoanalyzer (BIOLIS24j) using the Aspartate aminotransferase Reagent Kit (MURRAY., 1984b).

II.5 Method for determining oxidative stress parameters

Preparation of organ homogenates

One gram of tissue (liver, heart and kidneys) from each rat of the different groups studies, has been used. After grinding and homogenization of the tissues in TBS (50 mM Tris, 150m M NaCl, pH 7.4), the cell suspension was centrifuged (3000 holes / min, 15 min), then the supernatant obtained is stored at -20 ° C while waiting to be carried out the determinations of the parameters of oxidative stress.

Tissue Malondialdehyde (MDA) assay method

Carbonyl compounds like malondialdehyde react with thiobarbituric acid (TBA) to give pink chromophores absorbing at 532 nm (YAGI., 1976).

Pipette into glass and screw test tubes, 100ul sample, 400ul TBA reagent and close tightly. Heat the mixture in a water bath at 100 ° C for 15 minutes. Then cool in a cold water bath for 30 minutes leaving the tubes open to allow the evacuation of the gases formed during the reaction. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using aspectrophotometer.

Tissue Reduced Glutathione (GSH) Assay Method

glutathione was determined according to a colorimetric method of (WECKBERCKER and CORY., 1988) by a spectrophotometer of the SHIMATZU type, the measurement of the optical density results from the formation of 2-nitro-5 mercocapturic acid from the reduction dithio-bis2-nitrobenzoic acid the so-called Ellman's reagent with SH groups exist in GSH.

II.6 Statistical analyzes

The results are presented as the mean ± standard deviations (SE). The comparison of the means is carried out by the Student's t test. This analysis is carried out using MINITAB and EXCEL software. The differences are considered significant at $P < 0.05$, highly significant at $P < 0.01$, and very highly significant at $P < 0.001$

Chapter II

Results & Discussion

III.1. Phytochemical study for aqueous extract of *S.nux-vomica*

III.1.1 Qualitative phytochemical analysis

Results of phytochemical essays shows that aqueous extract of *S.vomica* rich on different important chemical compounds such as flavonoïds, phenols, carbohydrates, , alkaloïds, tannins and terpenoïds but our extract plant is poured from saponins.

Table 03: Phytochemical assays for aqueous extract of *S.vomica*

Compounds	Alkaloids	Flavonoïds	Terpenoids	Phenols	Tannins	Reducing compound	Saponins
Aqueous *S.vomica* extract	+	+	+	+	+	+	-

(+): Present , (-): Absent

III.1.2 Qualitative phytochemical analysis

- **Dosage of polyphenols**

The quantitative study of the extract carried out by spectrophotometric assay using the method of Folin Ciocalteu aimed to determine the total content of polyphenols present in aqueous extract of *S.vomica*. The content of these compounds was calculated from of the calibration curve for Gallic acid, expressed in mg of Gallic acid / g of dry extract.

- **Determination of flavonoids**

From the Quercitin calibration curve, the flavonoid content was determined in the extract, expressed as mg of Quercetin / g of dry extract. The results of this assays is shown in table 04**.**

Table 04: Total Phenols and Flavonoïds concentration in aqueous extract of *S.vomica*

Compounds	**Polyphenols (mg of GAEq/g of extract)**	**Flavonoïds (mg QEq/g of extract)**
Aqueous Extract of *S.aromaticum*	**23,67 ± 5,33**	**3,086 ± 0,199**

III.2. Antioxidant activity

DPPH radicals scavenging activity and IC50 value

The scavenging activity of aqueous extract of *S.nux-vomica* and BHT on DPPH radicals at various concentrations showed in figure 9. The greatest inhibitory activity observed was in the case of queous extract of *S.nux-vomica*, reaching as high as 97.35% at 0.5 mg/mL, while for BHT a concentration at 0.5 mg/mL the inhibition of DPPH radicals 77.63%.The concentration of aqueous extract of *S.nux-vomica* resulting in a 50% inhibition of the free radical, IC50, was 6.86µg/mL. The standard BHT had IC50 values of 257 µg/mL .

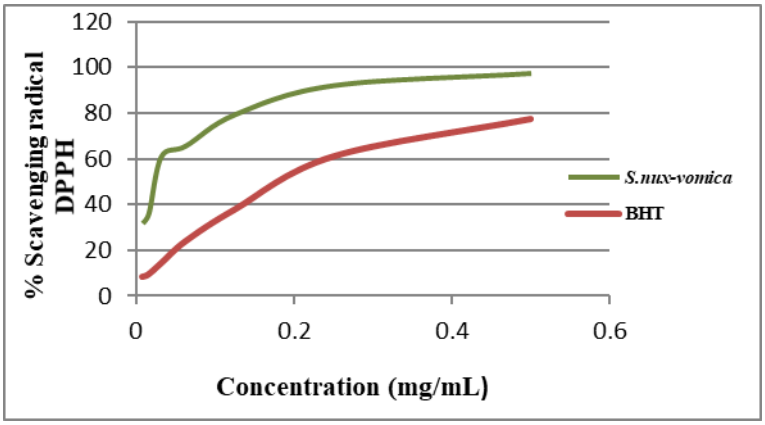

Fig 9 : Scavenging radical DPPH of aqueous extract of *S.nux-vomica* and standart BHT

III.3 Hemolysis assay

The antihemolytic activity of the various Concentration (20-80 µg/mL) of aqueous extract of *S.nux-vomica* on human blood erythrocytes are presented in Fig 10.

At the concentration 80µg/mL of aqueous extract of *S.vomica* showed maximal antihemolysis activity (95.80 %) . We notice the exact at various concentrations of aqueous extract of *S.nux-vomica*, the higher the concentration, the higher of antihemolysis activity. antihemolysis activity with regression coefficient (R^2 =0.895 aqueous extract of *S.nux-vomica*).

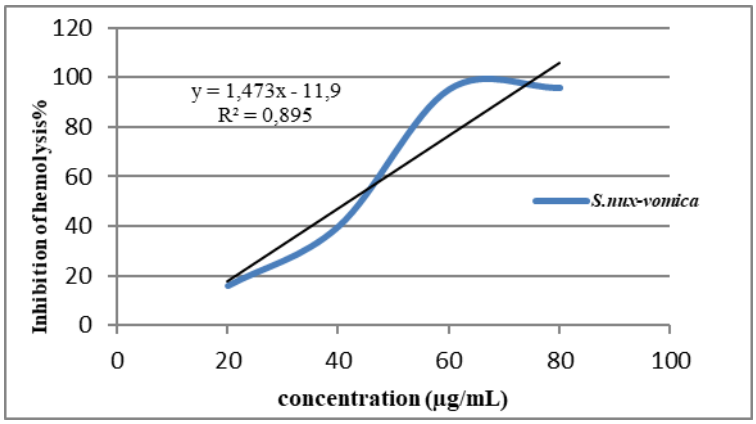

Fig 10 : HPLC of aqueous extract results *S.vomica*

HPLC chromatogram results of *S.vomica* barks showing in fig. 11 appeared that *S.vomica* had a deferent number of molecules detected, but we identified just one molecule which is Quercitin (12.69 µg/mL).

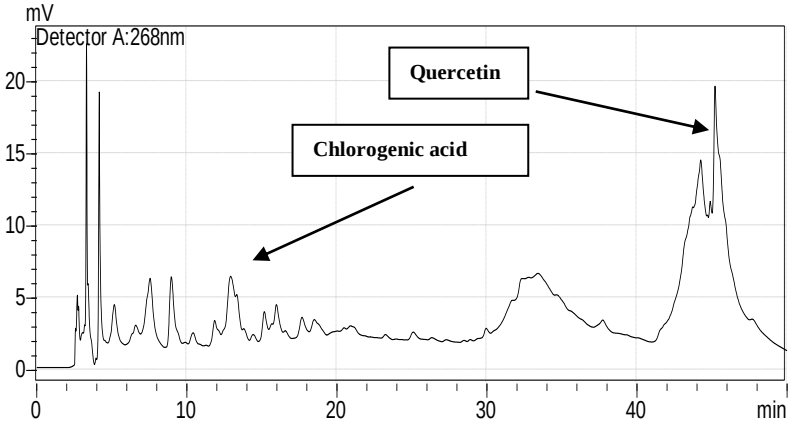

Figure 11: HPLC chromatogram of the aqueous extract of *S.vomica*

- **Hematological parameters**

Hematological parameters are illustrated in (Figure 12). The results show that, there is no effect of Paracetamol or different treatment except the aqueous extract group was significantly increased with (P<0.001) on RBC levels. In hole the experimental rats but WBC, number are increase with very high signification (p<0.001) in PARA group and the rest groups (EA, CH ^{14} and CH ^{30}) compared to the control. Also treatment in EA, CH ^{14}, CH ^{30} and PARA groups shows a signification increase in LYMPHO level with very high signification (p<0.001) compared to control group.

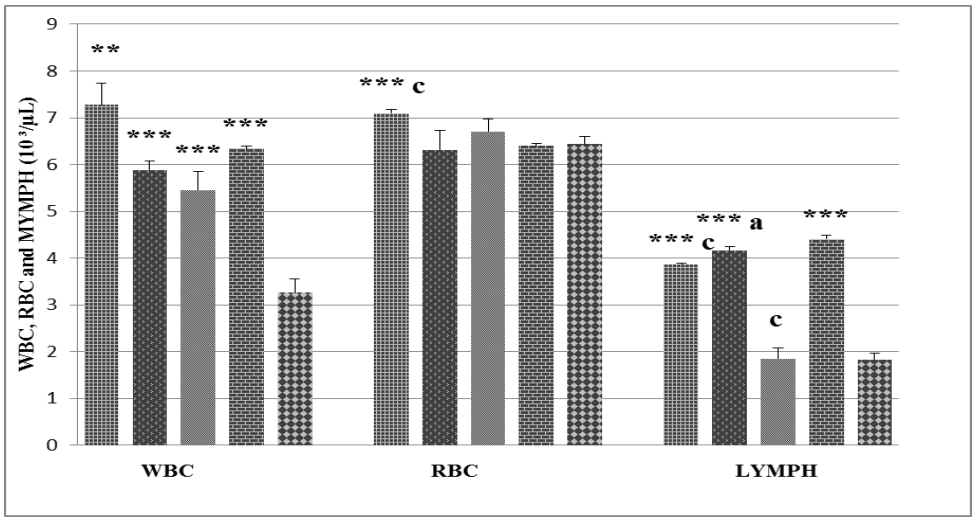

Figure 12: WBC, RBC and LYMPHO levels of control and experimental groups

Results show also a signification increase in HGB, HCT and PLT level with very high signification ($p<0.001$) compared to control group(Fig .13)

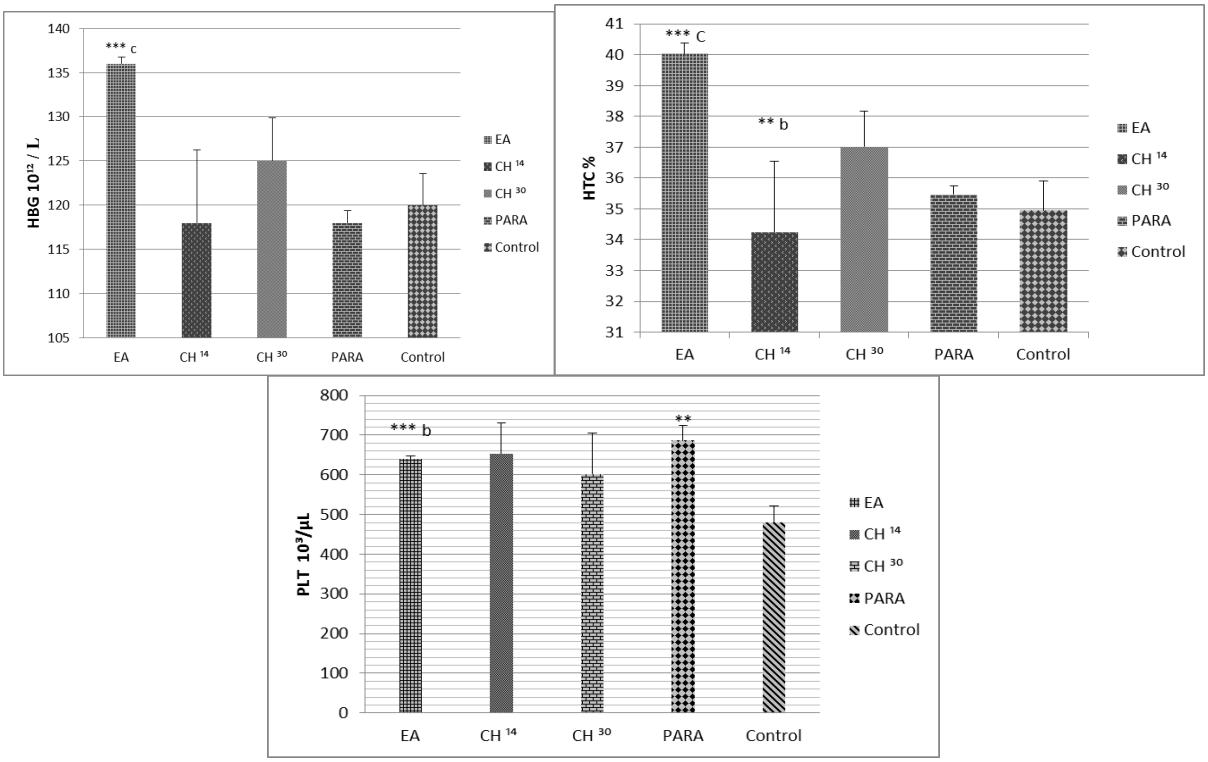

Figure 13: HGB, HTC and PLT levels of control and experimental groups

As shown in (Figure 14). The results of renal enzymes (Urea and creatinie) appeared increase with very high signification ($p<0.001$) in PARA group compared to control.

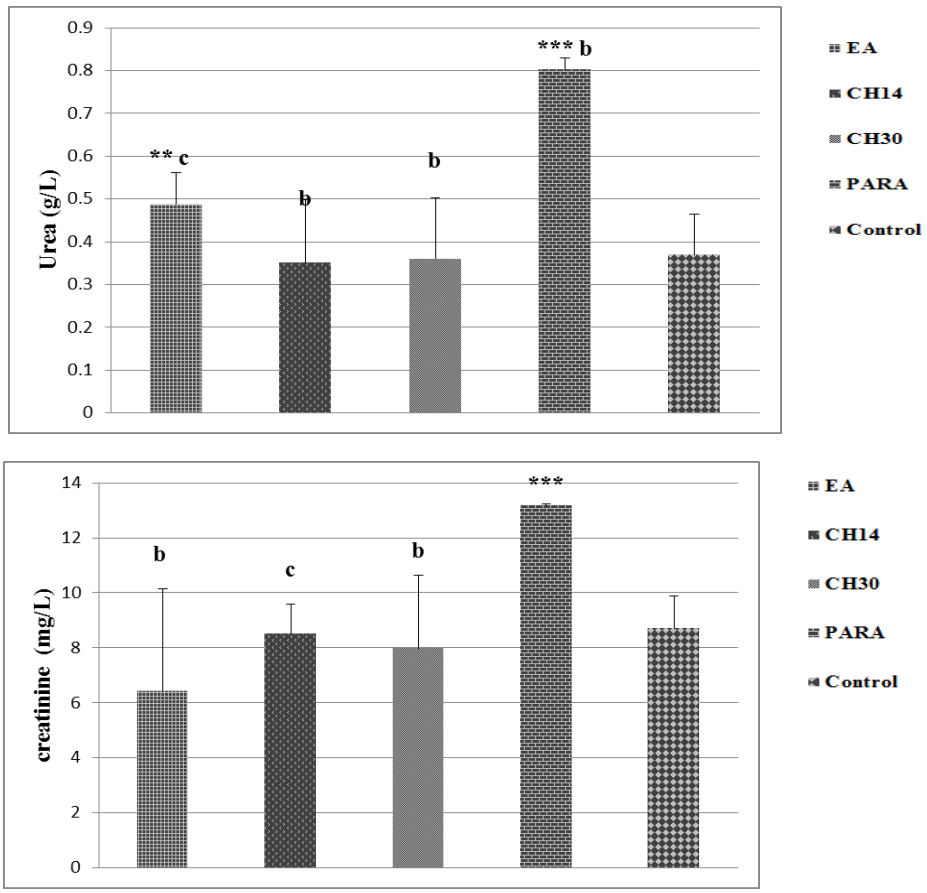

Figure 14 Urea and creatinie levels of control and experimental groups

Also in (Figure 15).showing results of transaminases enzymes activities appeared very high significant increase of GPT and GOT with ($p<0.001$) in PARA group compared to the control. Treatment with (EA, CH ^{14} and CH ^{30}) groups showed a decrease in the transaminases enzymes activities compared to PARA group with ($p<0.001$)

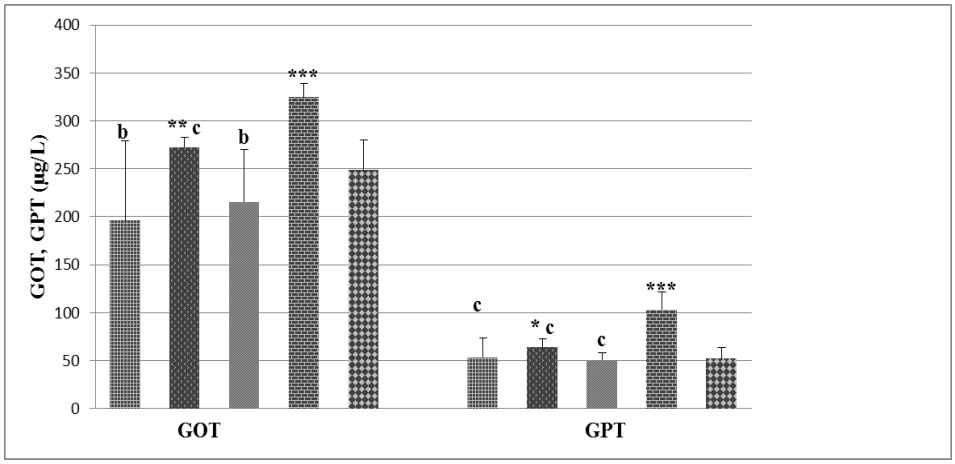

Figure 15 GPT, GOT activities in the control and experimental groups.

III.4 Oxidative stress parameters

- **Malondialdehyde (MDA) levels**

The results obtained are presented in Figure 16. Liver and Kidney MDA levels have a significant decrease in experimental (EA, CH ^{14} and CH ^{30}) groups and control group; compared to the PARA ,while in Brain GSH level show that not significant deference in experimental groups.

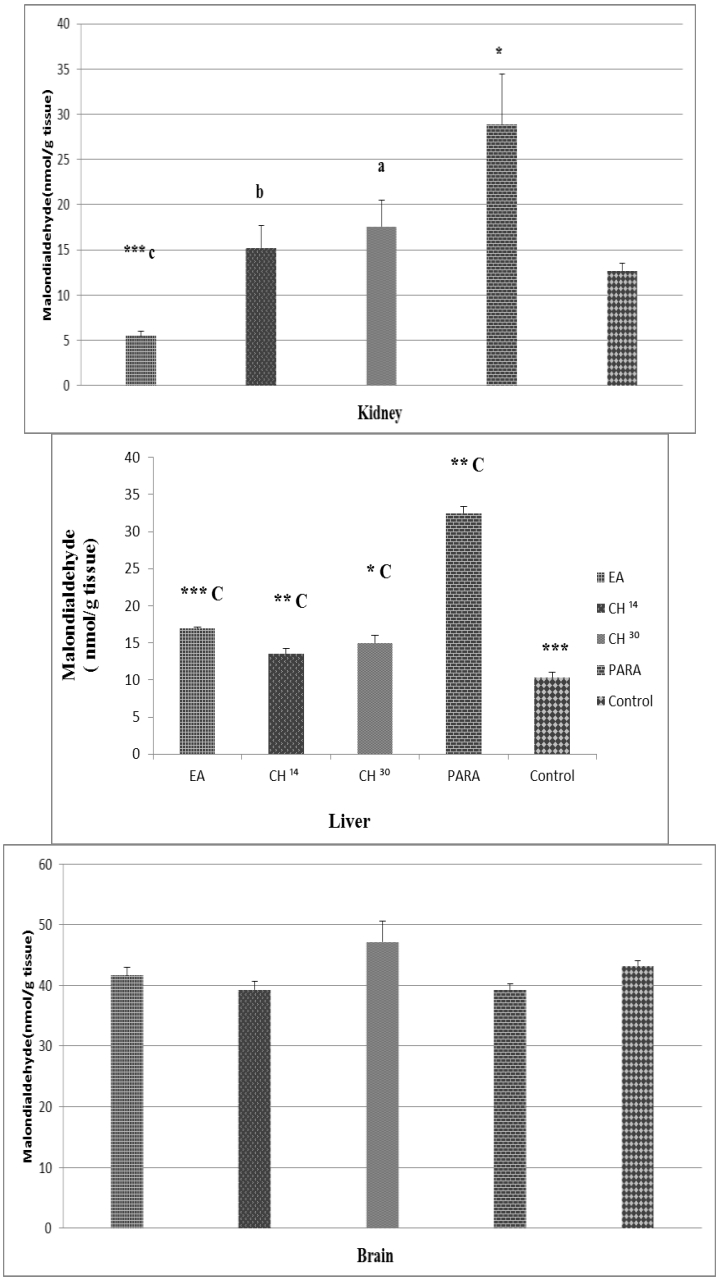

Figure 16 MDA levels in Liver, Kidney and brain in control and experimental groups.

- **Reduced glutathione (GSH) level**

GSH levels are shown in Figure 17. Liver, Kidney and Brain GSH levels have a significant increase in experimental (EA, CH ^{14} and CH ^{30}) groups compared to the PARA and control group

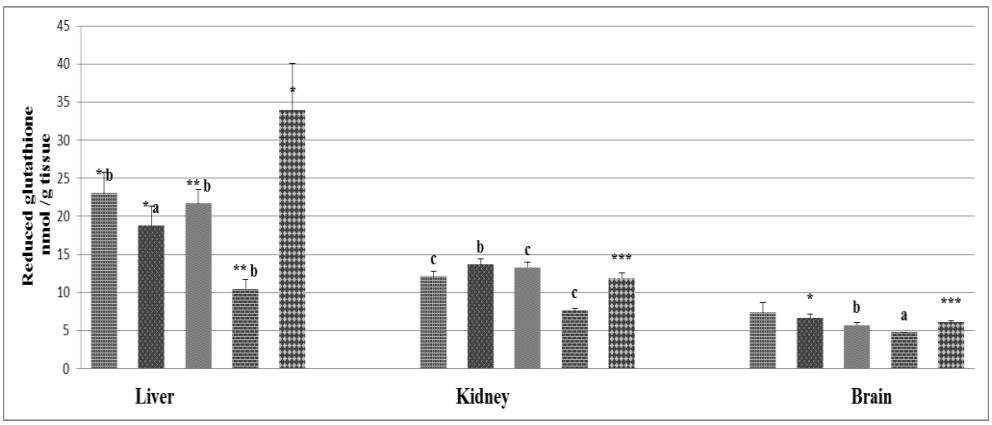

Figure 17 GSH levels of control and experimental groups

III.5 Histological results

Histological results of experimental rats for liver and kidney are shown in figure 17 and 18 with magnification (×40).

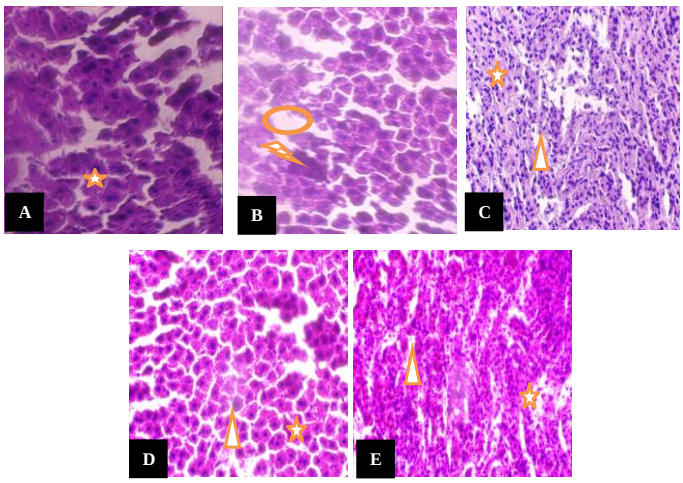

Fig. 18: Histopathological changes in liver of wistar rat. (A) control group showing normal hepatocyte (★), (B) PARAcontrol group showing severe centrilobular necrosis surrounded by moderate vacuolar degeneration (⚡),hyperplasia of bile duct and necrotic area (○), (C , D, and E rats groups EA, CH14 and CH30 respetivtly showing normal central vein(▲), normal hepatocyte (★).

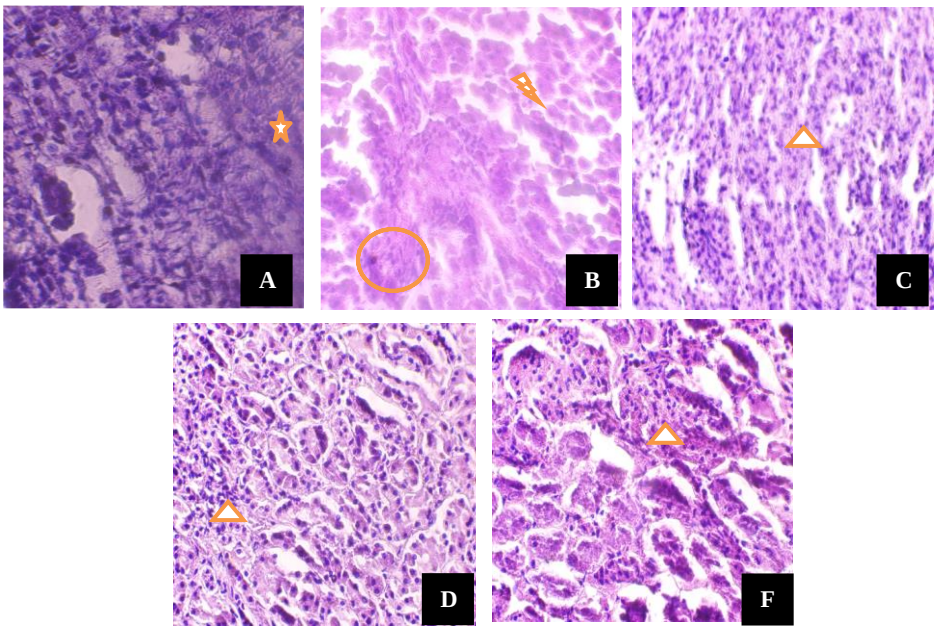

Fig. 19: Histopathological changes in Kidney of Wistar rat. (A) control group showing normal glomerul and normal renal tubules (★), (B) PARA control group showingreducedglomerular size(⚡), vacuolated of epithelial cells and hemorrhagic area(○), (C , D, and E rats groups EA, CH14 and CH30 respetivtly showing normal RTs

with normal glomerulus (▲)

IV.Discussion

The objective of our study is to determinate the characteristics compound of *S.vomica* and evaluated their protection property associated with biological elimination effect of trituration using *S.vomic* (CH14, CH30) against hepatic/renal deficit induced by Paracetamol drugs in Wistar *albino* rats.

IV.1. In vitro *S.vomica* characterization

The results of phytochemical essays (Table 3) chows that aqueous extract of *S.vomica*rich with different important second metabolic (alkaloïds, phenols,flavonoïds, carbohydrates, saponoside, tannins and terpenoïds). secondary plant metabolites have biological properties such as analgesic and anti-inflammatory properties, anticancer and anti-tumor activity, antioxidant, anticonvulsant, antiamnesic, antiallergic, antialcoholic,antimicrobial activity

detoxification and immunomodulatory property increases the importance of this plant in modern medicines.**(Tripathi et *al.*1996 ; Yin et *al.*2003 ; Behpour et *al.*2011).** Among its negatives is the richness of this plant in alkaloids, as it contains a high percentage of the compound strychnine 1.5–2.5% **(Mitra and Acharya.2012).**According to **(Kumar et *al.*2012)** detoxification of *Strychnos nux-vomica* seeds are very essential before its therapeutic use. Use of different factors like cow's urine, milk, ghee (purified butter), Kanji (sour gruel), castor oil, ginger juice, sand, vinegar etc. Additionally **(Mitra et *al.*2011),** every purification process directs towards reducing the levels of alkaloids and has some therapeutic rationale. On heat treatment the content of major alkaloids strychnine and brucine declines with the increase in the proportion of isostrychine, isobrucine, strychnine N-oxide and brucine N-oxide. This change in alkaloid content renders *Strychnos nux-vomica*to be used as a drug **(Choi et *al.*2004).**

The anti-oxidant activity results obtained from *Strychnosnux-vomica*, its seems to be from presence of our plant from polyphenols and flavonoids, and the presence of Alkaloïds in their compounds plant extracts, such as flavonoids and phenolics, have raised public interest in their potential to act as antioxidants. Natural antioxidants can strengthen the endogenous antioxidant defence from ROS ravage and restore the optimal balance by neutralizing the reactive species **(Jiao-K et *al.* 2015).** The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. Tannins are a widely distributed phenolic antioxidant, present in various barks, leaves, fruits and vegetables, phenolic compounds are known to exhibit antioxidant activity by virtue of their redox property because of which they can scavenge oxygen radicals and donate hydrogen to neutralise reactive oxygen species **(Debosree .2015).** Condensed tannins, which result from the condensation of monomers of flavan-3-ol units. it has been reported that to be used in treating detoxification of hepatic and renal, inflammations, anti-diabetic , antidiarrheal potential **(Patel et *al.*2012 ; Bhati et *al.*2012 ; Shoba et *al.*2001).**Flavan-3-ols have been shown to behave as antioxidants via several mechanisms including the scavengingof free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes. These properties are due to the presence of the phenolic hydroxyl groups on the B ring in un galloylatedcatechins (EC and EGC) and on the B and D rings of the galloylatedcatechins (ECG and EGCG) **(Luís et *al* 2016).** Antioxidant activity of phenolic compounds was correlated to their chemical structures. Structure-activity relationship of many phenolic compounds (e.g. flavonoids, phenolic acids, quercetin, tannins, etc.) has been studied. In general, free radical scavenging

and antioxidant activity of these classes of compounds mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules **(I. Kostova et *al.*2011)**. DPPH radical scavenging assay is the most popular and simple in vitro radical scavenging assay method. DPPH is a stable, nitrogen centered, commercially available, organic free radical and has an absorption maxima at 515-517nm in methanol. On accepting hydrogen from donor (antioxidant), the solution of DPPH loses the characteristic deep purple colourand becomes yellow coloureddiphenylpicryl hydrazine**(Dhatri et *al.*2015)**. Our results on antioxidant property is similar to that established with high antioxidant property of the seeds of this plant **(Chitra et *al.*2010).** The methanolic extract of the flowers exhibited 78.49%, while us found potent inhibition percentage 97.35% of *S.vomica*extract for scavenging activity. This confirms the presence of antioxidant property in aqueous *S.vomica*extract.

IV.2. Oxidative Stress

In this study revealed a significant increase in MDA and decrease GSH level in Liver and kidney of rats treated with Paracetamol group compared to control and (EA, CH_{14}, and CH_{30}) groups. Our results are according to **(yousef et *al.* 2010),** show treatment with Paracetamol caused a significant elevation in MDA levels with simultaneous inhibition in the activities of antioxidant enzymes; GST inrathomogenate, liver and kidney. Furthermore it decreased GSH content significantly in rat liver, kidney in rats treated with paracetamol only. These features might be attributed to the metabolic activation of paracetamol, which is considered a major mechanism of its toxicity. It was found to trigger a rapid loss of GSH and lipid peroxidation in both liver and kidney **(Jaeschke et *al.*, 2003, Newton et *al.*,1986)**. The basic mechanism of paracetamol toxicity in the liver is the covalent binding of N-acetyl p-benzoquinone imine (NAPQI), the reactive metabolite of paracetamol, to sulfhydryl groups ofGSH and various proteins and their subsequent oxidation **(Leeet al., 2003).** On the other hand, several mechanisms were suggested as probable pathways for paracetamol-mediated nephrotoxicity. These included the oxidative metabolism to NAPQI similar to that in the liver, deacetylationto p-aminophenol and further oxidation to an aminophenoxy radical and benzoquinoneimine **(Mugford and Tarloff, 1997; Harmon et *al.*, 2005).** Also involved are the hepatically-derived metabolites from paracetamol-GSH conjugates **(Trumper et *al.*, 1998)**.The elevation in MDA level is an indicator of lipid peroxidation, which has been suggested to be closely related to paracetamol-induced tissue damage **(Sener et *al.*, 2003).** It has been proven that hydrogen peroxide and superoxide anion are produced during metabolic activation of

paracetamol in the CYP450 system **(Dai and Cederbaum, 1995)** and from mitochondria during paracetamol intoxication **(Knight et *al*., 2001).** It has been further suggested that the generation of ROS appears as an early eventwhich precedes intracellular GSH depletion and cell damage in paracetamol hepatotoxicity **(Manov et *al*., 2002).** The superoxide formation may promote peroxynitrite generation and protein nitration that may further result into oxidative damage to proteins, DNA and lipids (**Abdel-Zaher et *al*., 2008).** In addition, both paracetamol and NAPQI can interact with mitochondria, thereby inducing depletion of mitochondrial GSH content, decline in ATP content, and uncoupling of the mitochondrial respiratory chain combined with electron leakage **(Donnellyet *al*., 1994).**Glutathione is a ubiquitous tripeptide present in all cell types in millimolar concentrations. The major roles of GSH are to maintain the intracellular redox balance and to eliminate xenobiotics and reactive oxygen species (ROS) **(Myhrstad et *al*., 2002).**

In the present work, we had chosen the liver and kidney to estimate thechanges in GSH because they possess a high content of this protein. According to the decline in hepatic and renal GSH content, it was evident that paracetamolinduced toxicity involved a changein cellular redox status toward a state of oxidative stress. A widevariety of oxidizing molecules such as ROS and/or depleting agentscan alter glutathione redox state, which is normally maintained by the activity of GSH-depleting (GPx, GST) and GSH-replenishing (GR) enzymes **(Halliwell, 1996).**Therefore, it can be assumed thatthe decrease in GSH concentration might cause the effectiveness of GST and GPx activity to be restricted, as evident by the intensification of lipid peroxidation **(Czeczot et *al*., 2006).**

IV.3. Hematological parameters

This study aime to investigate S-nux-vomica as protection against paracetamol-induced toxicity. Oral administration of paracetamol-induced significant lower ($p \leq 0.05$) in B.W compared with negative control group may be due to the loss of appetite observed in the course of study. Furthermore, our results also observed that administration of the aqueous extract of S-nux-vomica led to improved B.W gaining in rats, through its contents which were flavonoids, proteins, and carbohydrates, which are necessary for growth, body repair, and maintenance. This study demonstrated significant decline ($p \leq 0.05$) in RBC count, Hb, PCV, MCV, MCH, and MCHC levels in positive control group which treated with paracetamol when compared with negative control group, that is, paracetamol has potential to prevent erythropoietin release from the kidneys. The low in erythrocyte value may be due to rise free radicals, reactive oxygen species, and peroxide radicals after paracetamol administration

which lead to hemolysis anemia **(Gonzales et *al.*2009).** As well paracetamol lead to hepatotoxicity and impairs protein synthesis and reduction in the serum TP, albumin, and globulin concentration, consequently, insufficiency of protein synthesis that specifically induces decline of essential amino acids and shortage of energy. Source of protein synthesis incorporated in Hb production and anemia **(Gonzales et *al.*2009).** This result in agrees with previous research that paracetamol caused destruction RBC and cause thrombocytopenia and hemolytic anemia **(Kornberg et *al.*1978).** These results show that these plants product may have therapeutic effect agonist hematotoxicity induce by paracetamol in the group which treated with the ethanolic extract of S.nux-vomica and paracetamol when compared with positive control group which treated with paracetamol may be due S.nux-vomica contain phytochemical compound include alkaloids, saponins, Alkaloids, terpenes, polyphenol, flavonoids, sterols, tannins, and glycosides. These compounds are well known homeopathic factors that have a direct influence on the production of blood and antioxidant substance serve on inhibition free radical inhibite hemolytic anemia and ameliorate blood components **(Ma et *al.*2000 ; Pandey et *al.*2012).** On other hand, **(Kadhem.2019)** study show demonstrated significant decline ($p \leq 0.05$) in WBC of rats received paracetamol in positive control group comparison with negative control group indicated a suppressing of the immune system. The low in WBCs count may be due to the inability of the hematopoietic tissues to production new WBCs **(Tan et *al.*1992).** From the result of the differential white cells count that carried out in our study, the rise in lymphocytes count in paracetamol treated group might be due to the interaction between paracetamol and gastrointestinal macrophages, which act as a toxic material. The macrophages caused the activation of the helper T cells and the B lymphocytes, through serving as antigen presenting cell and the antigenic products **(Sembulingam.2012).** They as well excrete materials called interleukin-1/-cytokines that stimulate the activation of lymphocytes and rise their count **(Sembulingam.2012).**

The major role of lymphocyte is the response to antigen (foreign bodies) through the expansion of cellular immunity and forming antibodies circulating in the blood **(Frandson et *al.*2003).** Paracetamol might be a toxic effect on the neutrophils in the blood, or it has a serious impact on the bone marrow, causing the lowering of these blood cells production. Neutrophils considered as the first-line defense versus toxic materials, foreign substances, and microorganisms **(Hall et *al.*2011).** This might be an indicator of the disruption of immune status in the treated animals responding to the toxic effect of paracetamol. Coadministration of S. lappa and paracetamol showed increase WBC as a compared with control positive

group and restore differential count near to control group may be due to active materials known as dehydrocostuslactone and costunolide in *S.nux-vomica* **(Alnahdi.2017 ; Choih.2009)** that refers to *S.nux-vomica* enhanced the immunity of rabbits treated with Paracetamol **(Kang et *al.* 2004).**

IV.4. Biochimical and blood suger parameters

The larger dose of paracetamol causes hepatotoxicity. The obtain result indicated chronic paracetamol consumption induces severe liver injury and liver necrosis as observed by the rising liver enzyme GOT and GPT in PARA group which administered paracetamol, may overdose of this caused forming reactive oxygen species and induce oxidative stress. which lead to the hepatotoxicity, well rise may be attributed to the libration of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular injury of hepatocytes and necrosis. According to **Valentine et *al.* (1990)** Serum GOT and GPT are biomarkers in the diagnosis of hepatic injury because they are liberated into the blood circulation after cellular damage. Moreover, hepatotoxin impaired the ability of the liver to synthesize albumin **(Dubey et *al.*1994).** In our study, decline total serum protein level in paracetamol treated rats may be attributed to impaired protein synthesis by damaged liver tissue **(Kanchana et *al.*2011).** While administration of the aqueous extract of *Strychnosnux-vomica* ameliorated effect agonist hepatotoxicity which induces by paracetamol. In our present study shown lowering liver enzymes GOT and GPT in Groups (EA, CH14 and CH30) when compared with PARA group. Which exposure to paracetamol may be due to phytochemical compounds such as flavonoids and chlorogenic acid which acts as antioxidant substance serve a suppression free radicals induced lipid peroxidation and prevents paracetamol toxicity **(Ravindran et *al.* 2019 ; Mayne et *al.*1994)**

In this study, paracetamol caused nephrotoxicity was characterized by apparent elevations in serum creatinine and urea in PARA group. We can explain this as follow, in nephrotoxicity and kidney diseases, the serum urea and creatinine accumulate due to the rate of production exceeds, the rate of clearance due to the deficiency in renal function **(Mayne et *al.*1994).** Additionally Paracetamol nephrotoxicity occurs because its highly reactive metabolite NAPQI- which arylates proteins in the proximal tubule, at beginning cell death of renal tubular cells **(Mugford and Tarloff.1997)**. The kidneys include the excretion of various xenobiotic, pollutants, and toxins and hence they are prone to liberate rise quantities of free radicals which participate in high oxidative stress. This is included in the pathogenesis of kidney damage (**Trumperet *al.*1996)**. The present results in Groups (EA, CH14 and CH30)

toxicated with paracetamol and treated with the extract of *S-nux-vomica*until. The results observed nearly values of creatinine and urea compared to control group that means nephroprotective properties of *S-nux-vomica*on toxic effect of paracetamol, due to the high concentration of flavonoids and alkaloids they contain as antioxidant and/or free-radical scavenging activities **(Giri et *al.*2019 ; Adeneye and Benebo.2008)**.

IV.5. Histological results

histological studies on liver tissues following administration acetaminophen overdoses can cause liver damage and even failure cell death may occur as a result of apoptosis and necrosis, our results similar of **(Gujral et *al.*2002)** study. Microscopic examination of histological preparations of the kidney observed decrease in glomerular size, severe tubular vacuolar, necrosis with degeneration, and glomerular bleeding. According to **Fouad et *al.* (2009)** Also the histological studies on liver and kidney tissues following administration of *S.nux-vomica*did not present any visible lesions on the tissues (**Khorsandi et *al.*2008).**

Conclusion

Poisoning by excessive consumption of Paracetamol is among the dangerous factors that cause total or partial damage to the functions of the liver and kidneys. Any defect in the organs leads to the failure to perform its vital functions.

One of these main problems is toxicity to the cells of the liver and kidneys. Therefore, the aim of this study is to evaluate the biological protection effect of *S.nux-vomica* aqueous extract with the effect of trituration technique against paracetamol-induced poisoning.

Phytochemical analysis showed the richness of the aqueous extract of *S.nux-vomica* from the second metabolic compounds such as: alkaloids, polyphenols, flavonoids, tannins, saponins, carbohydrates…etc, . In vitro study of *S.nux-vomica* aqueous extract showed high activity against DPPH free radicals.

Blood analysis concluded that rats treated with *S.nux-vomica* extracts may have a positive effect in the hematopoietic system, and interestingly, there is a very clear effect that was treated by the plant trituration technique. As for the biochemical parameters, the aqueous extract and the two groups of plants showed protection in reducing the percentage of hepatic and renal enzymes such as: (GOT, GPT, Creatinin, Urea).

The ability of the plant is not limited to its antioxidant properties in the laboratory, but extends to the microscopic and in vivo levels, which indicates its high treatment to filter paracetamol residues from liver and kidney cells that cause oxidative stress, and return them to normal cells. In the groups of rats treated with plant extract and the plant trituration groups, there was a significant decrease in MDA and an increase in the activity of GSH level.

The present study is a preliminary work, which must be continued, by other work in order to test the homeopathic treatments against the various diseases, as well as to find new molecules of medical interest in the objective to use in trituration homeopathic to eliminate the secondary effect of drug and valorized the naturel product.

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- Strychnine is one of the oldest poisons from the tree Strychnos nux vomica which contains the alkaloids brucine and strychnine in greater quantity[2].Any part of the tree if consumed will bring effects that can be fatal.
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Annex

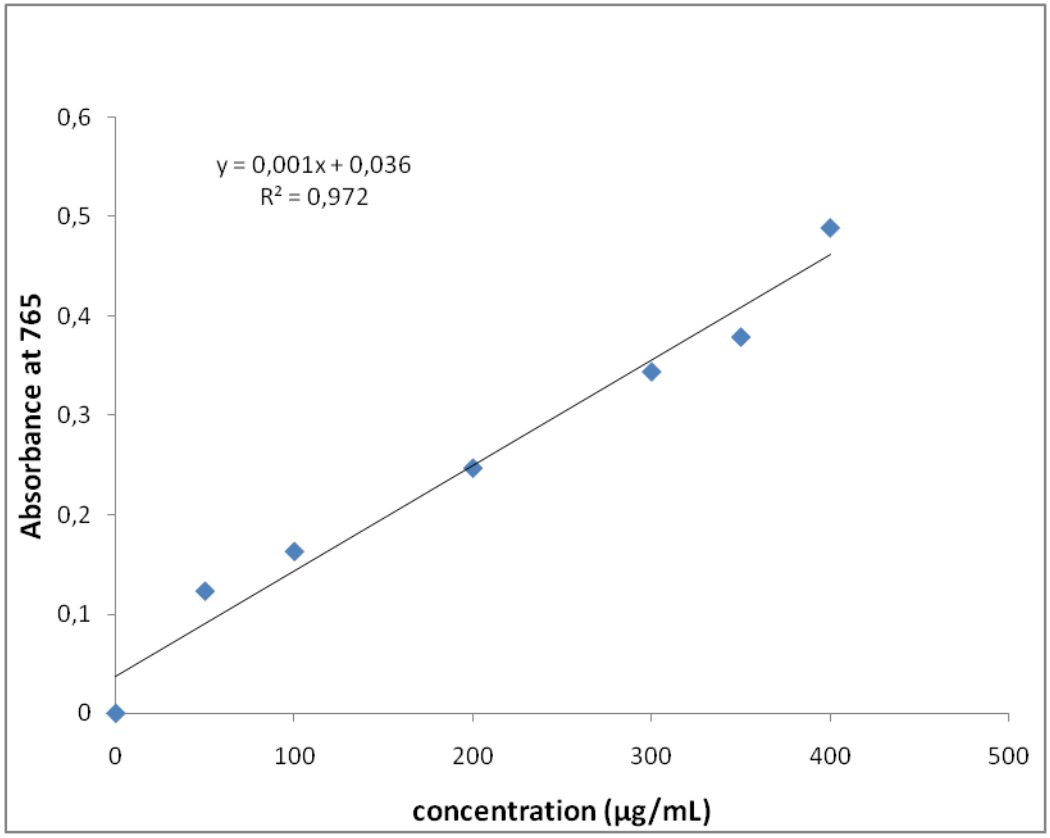

Figure: Calibration curve for Gallic acid determination of polyphenols

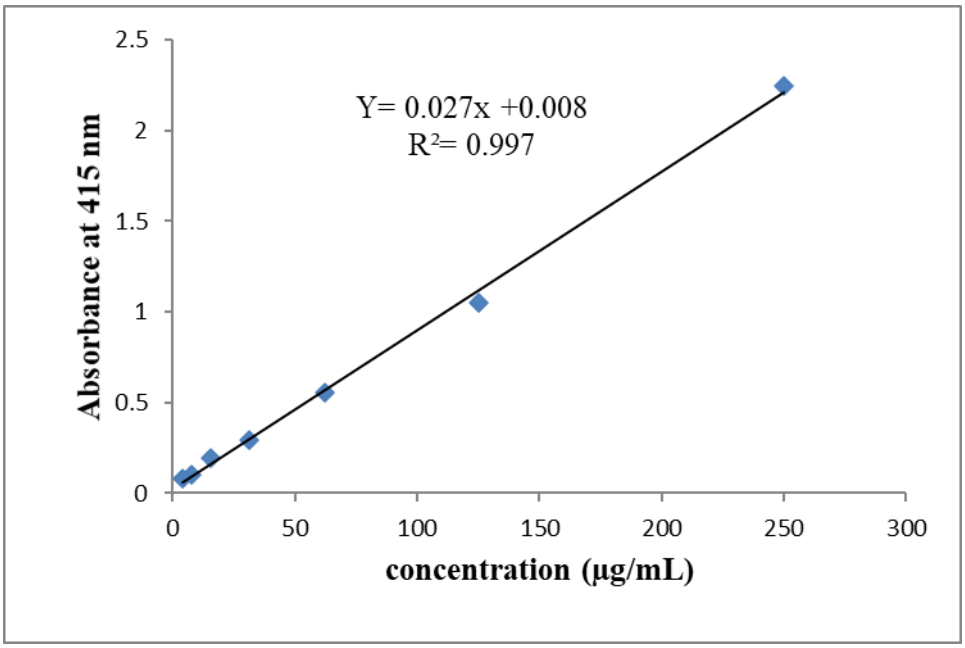

Figure: Calibration curve for Quercetin for determination of total flavonoids

المركب	Tr	المعادلة	R^2
Gallic Acid	5,29	$y = 54681x$	$R^2 = 0.9956$
Chlorogenic Acid	13,392	$y = 21665x$	R^2 =0.9853
Vanilic Acid	15,531	$y = 65077x$	R^2 =0.9921
Caffiec Acid	16,277	$y = 84066x$	R^2 =0.9974
Vanilin	21,46	$y = 58930x$	R^2 =0.9966
p-Coumaric Acid	23,817	$y = 49495x$	R^2 =0.9961
Rutin	28,37	$y = 28144x$	R^2 =0.9869
Naringin	34,788	$y = 19379x$	R^2 =0.9968
Quercetin	45,047	$y = 45378x$	R^2 =0.9962

Lamda= 68 nm

Figure : HPLC chromatogram of flavonoids standard.