

Studies on Franch Oil - NH*

A Multispectrum Oil

CONTENTS

CHAPTERS	Page No.
1. GENERAL INTRODUCTION	1
2. ANTI-MICROBIAL AND ANTI-FUNGAL	20
3. WOUND HEALING AND STRETCH MARK	28
4. ANTI-INFLAMMATORY AND ANALGESIC	43
5. TOXICOLOGICAL STUDY	50
6. GENERAL SUMMARY	76

CHAPTER 1

GENERAL INTRODUCTION

The use of medicinal plants in the world contributes significantly to primary health care. The indigenous people for centuries have relied on herbal medicine for all aspects of their primary health care. It is estimated in South Africa that between 12 and 15 million people still use traditional remedies from as many as 700 indigenous plant species.

Although many rural communities now have access to mobile clinics and hospitals, there is still to a large extent, the belief in herbal medicine, possibly due to an inherent distrust in any thing western. Although free health care has become entrenched in India, many rural people still rely on the cheaper traditional healing methods rather than the expensive treatments by western practitioners.

Franch oil is an herbal medicine, which is a combination of cold pressed extraction of Ricinus Cummunis Linn seed and root with Ocimum Sanctum oil.

Ricinus Cummins Linn (Euphorbiaceae)

A monotypic genus comprising R.communis, widely cultivated in me tropics and warm regions for its seeds, which yeld the well known Ricinus oil. The Ricinus oil is one of the major oil seed crops of India and infact; India is second largest producer of Ricinus seed in the world.

Habitat:

Some scholars agree that the birth place of the Ricinus bean is Tropical Africa, others Abyssinia or Egypt, whilst others assert that the origin must be sought in the tropics, Southern Asia or India What appears certain is that the ancient Egyptians knew this plant: witness its presence in the sarcophagus, around the mummies of famous personages, in particular priests, that are 4000 years old. In this region the Ricinus bean was worshipped. Herodotus mentions that it was very well known in Egypt from where, most probably, it was first introduced into Greece and further among the Latin peoples who, as Pliny reports, appreciated it for its therapeutic qualities. According to Roi, from Egypt the Ricinus bean would have reached India and China, where we can find the first reference to the plant dated from Tang in a mention to Hou . Also, Strabone and. Dioscoride relate the Ricinus bean as having Egyptian origins and being used there as oil for Lamoade and unguent, from 500 b.c, Ricinus oil was also known as an aperient among the Greeks and the Romans

Infectious diseases

The pygmies of equatorial Africa use the seeds internally against small pox. The vitamins and the enzymes present in large quantities in the seeds of the Ricinus bean and the ricine, which causes production of antibodies endowed with a specific immunological action, could account for their utilization in infectious diseases

Oncology

In Africa they suggest drinking a liquid prepared from the minced rind of the Ricinus bean for mammary tumors. It may be that the mammary tumors are not real tumors, but blocked mammary ducts. Dermatology Alzate states, referring to Columbia, that the oil removes spots from the face and hands of old persons, though it is necessary, morning and night, to massage over and over again in order that the oil can penetrate well. The oil is also suggested for large spots that appear on the body.

In Brazil, the oil put on scalds relieves pain and promotes healing. In Russia they use the oil externally for bums, eczema and to soften the skin. In the Transvaal they use the powder from roasted seeds to put on sores and furuncles of children

In the field of cosmetology Ricinus oil is used in alcohol solution to grease the body with soap and oil. The substances contained in the castor oil can have a biodynamic activity in the field of dermatology. This oil contains riboflavin, vitamins, lipids, protein and the glyceride of oleic acid and vitamin E. Nicotinic acid which is present in the oil, due to its irritant action, could act as a hemostat and cicatrizing agent, especially for bums, wounds, sores, etc.

Otorlunolaryngology

The Ricinus bean, according to Chinese medicine, cures troubles of speech and hearing. Recently it has been demonstrated that in rats, a diet poor in tryptophan causes a marked reduction in the synthesis of cerebral serotonin, which in turn controls a greater sensitivity of the senses in general, and acoustics in particular. The presence, in ricin of this amino acid could explain the above mentioned uses.

Ophthalmology

In Columbia one drop of oil in the eye reduces reddening and inflammation; one drop put on the eyelids treats sties

Obstetrics and gynecology

In India and Pakistan the oil is greased on the abdomen to promote menstrual flux (19). In the Dominican Republic the oil is used as an ingredient of an infusion that is given after childbirth (24) and against inflammation and metritis, in Brazil

Nervous system

In Algeria, against paralysis of the limbs they rub in the oil locally (43). During the tenth century, the oil was used in Abu Mansur to treat facial paralysis.

Ocimum Linn

A genus of aromatic herb undershrubs or shrubs distributed in the tropical and warm temperature regions of the world. Nine species are found in India of which three are exotic. Several Ocimum species yield essential oils, which are valued in medicine and perfumery; a few are rich sources of camphor.

pose them and not greater than can be readily borne by the hand. The object of this step is to render the oil sufficiently liquid for easy expression. The seeds are then introduced into a powerful hydraulic press. whitish oily liquid thus obtained, is transferred to clean iron bolers, supplied with a considerable quantity of water. The mixture is boiled for some time and the impurities being skimmed off as they rise to the surface. A clear oil is at length left at the top of the water, the mucilage and starch having been dissolved by this liquid and albumin coagulated by the heat. The latter ingredient forms a whitish layer between the oil and water. The clear oil is now carefully removed and the process is completed by boiling with a minute preparation of water and continuing the application of heat till aqueous vapour ceases to rise and till small portion of the liquid taken out in a vial continues to be perfectly transparent when it cools. The effect of their last operation is to clarify the oil and to render it less irritating by driving off the acrid volatile matter. But care is requisite not to push the heat too far as the oil then acquires a brown hue and an acrid peppery taste. After completion of the process, the oil is just put into ban-els and sent into the market.

Extraction with alcohol

The process for obtaining Ricinus oil by means of alcohol has been practiced in France, but the product is said to become rancid more speedily than that procured in the ordinary mode. Such a preparation has been employed in Italy and is asserted to be less disagreeable to the taste, and more effective than the common oil obtained by expression.

Refining

Crude Ricinus oil is yellow to brown in colour and contains mucilaginous matter, which is removed by treatment with hot water, followed by filtration through activated earth or bone black. The residual ricin and lipase get inactivated and the refined oil obtained is nontoxic and has little tendency to become rancid. Expelled Ricinus oil is generally of low free acidity (acid val. <3) and is suitable for a majority of its uses while solvent extracted oils have acid values higher than the required standards.

Characteristics

Ricinus oil is nearly colourless or very pale greenish yellow viscous liquid, having a mild taste and odour which soon become unpleasant. The oil is distinguished from most other oils by its high viscosity, specific gravity and acetyl value and by its solubility in absolute alcohol and glacial acetic acid and poor solubility in petroleum ether, gasoline, kerosene etc. Ricinus oil is dextro rotatory ($[\alpha]_D$, +7.6 to 9.7) due to the asymmetric carbon atom of ricinolic acid present in it. On cooling, it deposits 3-4 percent of tristearin and triricinolein.

The characteristics of Ricinus oil usually vary within the following ranges: sp.gr, 0.958, 0.968; n_D²⁰, 1.4790-1.4813; n_D²⁵, 1.4771, n₄₀, 1.4659- 1.473; iod.val, 0.2-0.3, viscosity (red wood seconds at 38), 1,160-90; and unsapon matter, 0.3-0.7%. The acid value of the oil ranges up to c 10 depending on the quantity. One exposure to air, the specific gravity of Ricinus oil increases while the iodine and acid values are unaffected. When heated and blown with air, the specific gravity increases and the iodine

values decreases. Viscosity of several sample of Ricinus oil varied between 935 and 1,033 cp. A sample with a viscosity of 1,000 cp at 20°C had the following value at other temperatures: 30°C - 453, 40°C - 232 and 50°C - 128cp.

Composition

Ricinus oil consists principally of ricinoleic acid (12 hydroxy oleic acid) which occurs to the extent of c90 percent and is responsible for the high viscosity and other peculiar characteristics of the oil. Stearic, oleic, linoleic and dihydroxystearic acids are also present in small amounts. A study of oils from seeds at different stages of maturity showed that ricinoleic acid content gradually increases with the ripening of seeds. The unsaponifiable matter contains B-sitosterol, Squalene (38mg/100g) and tocopherols. Ricinus oil is not normally stored. It keeps well only for 3 months, the lipase present in it remaining mostly inactive. Refined Ricinus oil can be stored for 6-12 months at ordinary temperatures and for 3 months at high atmospheric temperatures under similar conditions of storage, the increase in free acidity of refined oil is less than that occurring in the crude oil. Ricinus oil is very stable to oxidative rancidity and even after one or two years, there is no significant change in peroxide.

Grades and specifications

Grade specifications based on clarity and colour of the product as well as other physico-chemical characteristics have been laid down for Ricinus oil. Four grades viz, medicinal, first special, firsts and commercial have been recognized. The medicinal grade is intended for medicinal purposes and for making hydraulic brake fluids, first special for use in cosmetics and lubricants. First for use in lubricants and commercial for other industrial uses such as paints, sulphated oil, soaps and printing inks. All the four grades shall be free from sediment and suspended matter and also from admixture with other oils or substances. In U.S.A, two grades are recognized viz nol. The medicinal cold drawn oil and no. 3, the technical grades oil obtained from the preserved cake and used for industrial purpose.

Utilization

In the following sections 'are listed the therapeutic applications of the Ricinus bean and its different parts as observed in different parts of the world, and grouped under the pharmacological action of interest to the various medical specialties.

Respiratory apparatus

In China to recover from rhinitis they instill Ricinus bean latex in the ear of the patient. In Haiti they use Ricinus oil with an infusion of orange leaves against bronchitis. Pierre-Noel states that in Haiti asthma is treated by a spoonful of Ricinus oil with parsley, the effect being immediate. In India the root is used against pleurodynia

The above mentioned uses can be partially justified by observations made in the laboratory that extracts of the leaves possess specific activity against Mycobacterium tuberculosis and Aspergillus niger. The action of terpenic esters present in different amounts in plants of the Euphorbiaceae should be noted.

Cardiovascular apparatus

In the 10th century in Iran, the oil was used for apoplexy, and in Haiti, together with the juice of the pais Congo (cajanus indica); the oil is suggested for cerebral

congestion. In Ceylon congestion are treated by rubbing with castor oil (instantly warming the blood), whilst in Haiti lymphangitis is treated by lubricating the inflamed part with Ricinus oil (18). From the pharmacological point of view, the presence of ricinine, an alkaloid derived from pyridine, and the depressant effect on blood pressure (in the dog) caused by extracts of the trunk should be considered.

Digestive apparatus

The different parts of the Ricinus bean have many applications for infections of the digestive apparatus, whilst in SouthEast Africa the Zulu utilize the root in cases of dentalgia. In China, according to Wallnofer, the Ricinus bean is used against swelling of the tongue. Among the Zulu they use the Ricinus bean to relieve gastralgia; for the same syndrome in Columbia they use an infusion of white bark and in Mexico they masticate the seeds. In Iran the oil has been used for intestinal colic. In Brazil the oil is used to stop vomiting.

The Ricinus bean has been widely used as an anthelmintic. Littré and Gilbert mention in their treatise its use for the above property as well as a purgative. In Brazil and in Italy the oil is used with other anthelmintics. Wallnofer refers to the Chinese use for the vermifugal properties of the seeds.

In East Africa the oil is used as a taeniafuge. In the Transvaal Ricinus oil is still used against diarrhea, as in Somaliland. In Mexico, they have the custom of giving the root, which is naturally, jelly-like and refreshing, to stop diarrhea and dysentery-hence its popular name Apitzapatii de la Tehoitztea. Bally states that in Tanganyika they masticate the root against bellyache and diarrhea.

In Italy, Negri asserts that the Ricinus bean overcomes coprostasis due to inflammation of the abdominal organs in general and of the intestinal canal. Arietti, Palma and Viola extol the properties of the oil. Pomini asserts that 2-10g of the oil act like a laxative, 20-40g with lemon, coffee or tea, like a purgative for adults.

In Ceylon they rub the oil on the abdomen, whilst in China they prefer to use mashed seeds. In Haiti they use an enema with 30-40g of oil in a decoction of senna seeds or distill the oil for internal use. In Indonesia and the Philippines Ricinus oil is the purgative par excellence. It is very widespread also in Latin America, and in Columbia, Argentina, Brazil and Mexico where it is indicated as a purgative.

Articulations, bones and muscles

Rubbing with oil is practiced in Algeria for cases of bone deformities, whilst in the Transvaal, acute osteomyelitis is treated with the oil

Urogenital apparatus

According to Pomini, in Italy the roots of the Ricinus bean are known also for their diuretic action. In India and Pakistan the Ricinus bean is used against inflammation of the genital organs and in particular the purified seed is used against vaginal and uterine disease. In India the oil is considered to have a spermatopoietic action. In Brazil the root is used against pains due to renal calculus. In Ceylon, in the case of hydrocele, the oil is massaged on the afflicted part. The anti-inflammatory action could be due to the toxic action of ricine, which causes vasodilatation followed by an increase of the platelets.

Result

Table 1 shows the anti-microbial and anti-fungal activity (mm inhibition zone) of Franch oil - NH on *Staphylococcus aureus* (Plate 1), *Escherichia coli*, *Pseudomonas*, *Candida albicans* and yeast. The Franch oil -

TABLE -1
Anti - microbial activity of Franch oil NH

<i>Microbes</i>	<i>Anti-microbial activities Diameter of Inhibition (mm)</i>
Staphylococcus aureus	21.0 ±4.5
Escherichia coli	19.6 ±3.5
Pseudomonas	13.7 ±2.0
Yeast	6.4 ±0.8

Diameter of the inhibitory zone ± S.D. (mm) Means of 30 measurements

TABLE - 2
Minimal Inhibitory Concentration for Franch oil NH on
bacterial and fungal strains

<i>Tested Micro organisms</i>	<i>Minimal Inhibitory Concentration (mg/ml)</i>
Staphylococcus aureus	10.4
Escherichia coli	15.7
Pseudomonas	12.1
Candia albicans	13.0

TABLE 3
Minimal inhibitory concentration of Franch Oil NH antibiotic
standards on bacterial and fungal strains

<i>Tested Microorganism</i>	<i>Antibacterial activity staphylococcus aureus (n=3)</i>	<i>Anti fungal activity candia albicans (n-3)</i>
Franch oil NH	10.4	13.0
Tested antibiotic tetracycline	2.07 x 10 ⁻⁴ mg/ml	-
Tested antibiotic miconazole	-	6.5 x 10 ⁻⁵ mg/ml

NH showed a good (> 13mm) pattern of inhibition against *staphylococcus aureus*, *E. coli* and *Pseudomonas* spp; a moderated inhibition (> 8mm) on *Candida albicans* and mild inhibition was observed in yeast. Plate 1 shows staphylococcus aureus and pseudomonas showing sensitive to Franch oil NH. Minimal inhibition concentration of Franch oil - NH required to find out the bactericidal fungicidal activity were de-

The nomenclature of ocimum spp and varieties is complicated and confused and it is difficult to classify the oils reported in literature according to the bacterial nomenclature of plants from which they are derived. In several instances, oil derived from morphologically identical plant show different physico-chemical properties; such plants may be typical cases of physiological forms. There are also cases of hybrids, which yield oils similar to those of the parent plants. It has been suggested that ocimum oils should be classified on the basis of their chemical composition rather than by their botanical origin.

O. Sanctum Linn.

D.E.P., V, 443:Fl.Br. Ind., IV, 609: Mukerjies, Rec.bot.surv. India, 1940,14(1), 19. SAS-Ajaka brinda, manjari, pamasa, patra pushpha, suvasa tulasi; HINDI- tulsi, barananda, Kala tulsi; BENG-Tulsi; MAR- Tulasa, tulasi chajadha; Guj-Tursi; Tel-Tulasi, brynda, gaggera, Krishna tulasi, nalla tulasi, Tam- Thulasi; Kan.-vishnu tulasi, Kari tulasi, Sri tulasi; Mal.Trittavu Mundari- tunrusi.

An erect, herbaceous, much-branched, softly hairy annual, 30-75cm high, found throughout India ascending up to 1,800m in the Himalayas, and in Andaman and Nicobar Islands. Leaves elliptic oblong, acute or obtuse, entire or serrate, pubescent on both sides, minutely gland-dotted; flowers purplish or crimson, in raceme, close whorled; nutlets sub-globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish, with small black markings.

Habitat

O. Sanctum is commonly cultivated in gardens; it is frequently found as an escape. The plant is held sacred by Hindus all over India and frequently grown in courtyards and temples. At least two types of O.Sanctum are met with in cultivation, the green type (Sri tulsi) is the most common, and the second type (Krishna tulsi) bears purple leaves. The plant is propagated by seed.

The leaves on steam distillation yield a bright yellow volatile oil possessing a pleasant odor characteristic of the plant with an appreciable note of cloves. The yield of oil varies with type, season and the place of origin. The yields and characteristics of the oils distilled from leaves and flowering tops of plants grown in Ghazipur (2 types) and Jammu were as follows; Ghazipur- type Krishna tulsi (yield of oil, 0.23%): sp.gr.0.9421-1.0280; acid val., 1.1- 1.6; phenols, 45-76%; and aldehydes, 15-25%, type sri tulsi (yield of oil, 0.20- 0.33%) sp.gr.,0.9255-1.1242, acid val., 1.0-2.4; phenols, 50-76% and aldehydes, 10-15% , Jammu (yield of oil, 0.9%); sp gr 151, 0.967; n20 ; 1.5197; sap.val., 86; 501 in all proportions of 90% alcohol. A sample of oil from Alahabad gave on analysis; eugenol, 71%, ergenol methyl ether, 20% and carvacrol 3%. The oil distilled from plants growing in Philippines is reported to possess a sweet anise like odor ; it contains methyl chavicol, cineole and linalool.

The seed of plant give a greenish yellow fixed oil (17.8%) with good drying properties. It has the following characteristics: sp.gr.300, 0.9063; nd30, 1.4789; acid val., 2.0; sap. Val., 181.6; iod val., 173.0; thiocyanogen val., 104.6; acet.val., 12.1; R.M. val., 1.2; polenske val., 0.2; Hehner val., 93.6 and unsapon. Matter (contains sisto sterol), 2.3%. The fatty acid composition of the oil is as follows. Palmitic, 6.9;

stearic,2.1; oleic ,9.0; linoleic, 66.1; and linolenic, 15.7%. the seeds contain a mucilage (hexouronic acid ,27.2; pentoses, 38.9; and ash ,0.2) which on hydrolysis yields xylose and glucuronic acid in 2:1 molar ratio.

Uses

The plant is used as a pot herb, leaves are used as condiment in salads and other foods . It is also reputed to have medicinal properties. Besides the volatile oil, the plant is reported to contain alkaloids, glycosides, saponins and tannins. The leaves contain ascorbic acid (83mg/100g) and carotene (2.5mg/100g)

The juice of leaves possesses diaphoretic, antiperiodic, stimulating and expectorant properties; it is used in catarrh and bronchitis, applied to the skin in ring worm and other cutaneous diseases and dropped into the ear to relieve earache. The infusion of the leaves is used as a stomachache in gastric disorders of children. A decoction of the root is given as a diaphoretic in malarial fever . The seeds are mucilaginous and demulcent and are given in disorders of genito-urinary system. They contain antistaphylocoagulase which can be extracted with water and alcohol.

The oil is reported to possess antibacterial and insecticidal properties. It inhibits the in vitro growth of mycobacterium tuberculosis and micrococcus pyogenes van aureus, in antitubercular activity, it has one tenth the potency of streptomycin and one fourth of that of isoniazid. It has marked insecticidal activity against mosquitoes, though it is not comparable to that of pyrethrin; the mosquito repellent action lasts for 2 hr. The oil from the green type is active against salmonella typhosa; it has a rideal walker(R.W) coefficient of 6, while the R.W coefficient of the oil from red type is 3. Ether and alcohol extracts of leaves are active against Escherichia coli

Scope of the present study

This study is conducted to evaluate the medicinal potential of the Franch oil NH under the following headings.

1. Anti-Microbial and Anti-Fungal studies; To find out the supportive efficacy of Franch oil NH on athlete's foot, bums, cracked heels, external ulcer, skin disorders, wounds and itches.
2. Wound healing and Stretch Mark: To find the wound healing property of Franch oil NH by inducing the wound and treating with Franch oil NH and to find out the efficacy of Franch oil-NH on stretch marks.
3. Anti-Inflammatory and Analgesic activity of Franch oil NH: To find the effect on menstrual pain, joint pain, muscular and body pain
4. Toxicity study :To find out any toxic effect of Franch oil NH in heart, liver, intestine, kidney and testis by doing the basic biochemical analysis like sugar, urea,uric acid, creatinine, SGOT, SGPT, y-GT, total protein, albumin, LDH, CPK, cholesterol, triglyceride, HDL.alkaline phosphatase, acid phosphatase and histopathological studies.

CHAPTER 2

ANTI MICROBIAL AND ANTI FUNGAL ACTIVITY OF FRANCH OIL - NH* On Skin Microbes

Introduction

More and more people in developing countries utilize traditional medicine for their major primary health care needs. This has been the case of Franch oil - NH whose major composition of it are the extracts of *Ricinus Cummins Linn* seeds, root and root extract of *Ocimum Sanctum*. Franch oil - NH is used for wounds, bums ulcers, cracked heels, athletic foot, itches, pimples and other skin disorders. The *Ricinus Cummins* oil is commonly used on various disorders wounds, bums, ulcers. Oil of *Ocimum Sanctum* possesses marked antibacterial activity against *Micobacterium tuberculosis*, *Pyogenes*, *Escherichia coli* *Micrococuss pyogenes*, *Streptococcus* and *Salmonella typhosa*. This study is to find the effect of anti microbial and anti fungal activity of Franch oil - NH, on the commonly occurring microorganisms in the skin disorders.

Materials and Methods

Franch oil - NH was obtained from Mother Land Laboratories Limited, Chennai for evaluation. Microbial cultures and growth conditions of the common skin microbes of *Staphylococcus aureus*, *Escherichia coli*, *Pseuchmonas*, *Candida albicans* and yeast isolates from infected wounds, cracks and burns were used as a test microorganisms. Culture of the bacteria were grown for 10 hour in 50 ml of nutrient both at 37°C and were maintained on nutrient agar slants at 4°C. Cultures of filamentous fungi and yeast were grown in malt broth at 28°C and were maintained at 4°C in Potato dextrose agar plates. Anti microbial activity assay of Franch oil - NH was tested using the Agar diffusion method, sterile 5mm diameter filter paper discs were impregnated with 1000u g of the test material and placed in duplicates onto nutrient agar plates, surface spread with 0.2 ml bacteria or yeast cultures (Ca. 108 cells / ml). The plates were then incubated for 24 hours at 37°C for bacteria and for 48 hours at 28°C for fungi. The experiments were carried out in duplicate three times. The results (mean values, n=3) were recorded by measuring the zones of growth inhibition surrounding the discs. Inhibition zone values were corrected by subtracting the disk diameter from the value of the inhibition zone;

Minimal bactericidal concentration assay: Cylindric pieces (of 4 mm in diameter) were extracted, forms the inhibition - zones of *Staphylococcus aureus* produced by the higher concentrations of the Franch oil - NH. The pieces were transferred to sterile tubes containing triptose phosphate broth. The tubes were incubated at 36°C for 24hres. An aliquot of its broth was spread over petri plates containing sterile nutrient agar. This was incubated at 36°C for 24 hr and the development of microorganisms was checked. For comparative purposed, the standard tetracycline and the antifungal miconazole nitrate were included in the assay. As the diameter of the disc was 5mm, inhibition zones less than 6mm were not evaluated.

methyl cellosolve and 50 ml buffer were added. The solution was kept in a glass stoppered flask.

2. Buffer; 50 gm of citric acid monohydrate, 12 ml of glacial acetic acid, 120 gm sodium acetate trihydrate and 34 gm , NaOH were made to a final volume of 1 liter in distilled water. The pH was carefully adjusted to 6.0 and buffer, stored in fridge.
3. Methyl cellosolve(Ethylene Glycol Monomethyl Ether) Preparation free of interfering substances was obtained.
4. Perchloric acid-.A3.15 M solution was obtained by diluting 27 ml of 70% perchloric acid to 100 ml with water.
5. P- dimethylamino benzaldehyde,(PDAB):A 20% solution was prepared shortly before use by adding methyl cellosolve to 20 gm of p-dimethyl amine benzaldehyde to give a final volume of 100 ml. This was warmed to 60c to facilitate solubilizations. Hydroxy proline standard: A standard solution was prepared by dissolving 10 mg L-Hydroxy proline in 100 ml of 0.001N'HCL standard was prepared by diluting the stock with water to obtain a concentration of 1-5 ug/2.0 ml.

Sample preparation

100 mg of tissue was homogenized with 100 ml of 5% TCA and kept at 90c for 30 minutes to extract protein, DNA and collagen. The solution was filtered and the filtrate was used for estimation. Aliquots of the 5% TCA extract were hydrolyzed by adding HCL to a final concentration of 6N in sealed tubes for 3 hrs at 130c After hydrolysis the sample was evaporated to dryness. The residue was dissolved in water and made upto a known volume.

Procedure

2.0 ml portions containing 1-5 ug hydroxy proline were placed in test tubes. A series of standards were prepared containing 0.5 ug hydroxy proline in a total volume of 2.0 ml. Hydroxy proline oxidation was initiated by adding 1.0 ml chloramine-T to each tube. The tube contents were mixed by shaking a few times and allowed to stand for 20 minutes at room temperature. The chloramine-T was then destroyed by adding 1.0 ml perchloric acid to each tube. The contents were mixed and allowed to stand for 5 minutes. Finally 1.0 ml PDAB solution was added and the mixture was shaken . The tubes were placed in a 60c water bath for 20 minutes then cooled in tap water for 5 minutes. The colour developed was read in a Shimadzu UV Spectrophotometer at 557 nm

The collagen content was expressed as ug/g tissue.

Estimation of Collagen fractions

Collagen fractions were estimated by the method ofPiez et al

Neutral salt soluble collagen

The tissue were cut into small fragments, minced, homogenized in a warring blender using 1.0 M Nad in 0.05 M Tris-HCL buffer, pH 7.2 for 10 minutes at 4c by stirring gently using a magnetic stirrer. The supematants were collected and the remaining residue was further extracted twice with the same buffer containing 1.0 M

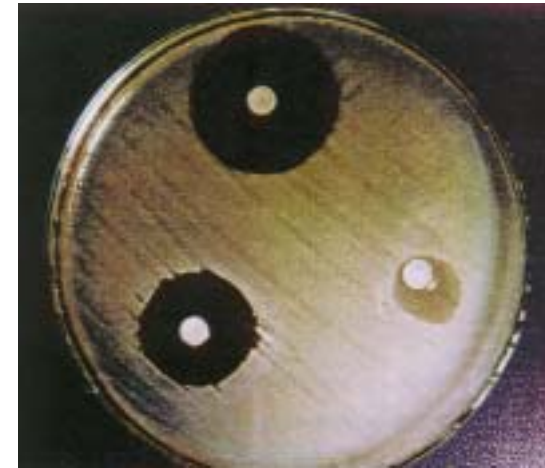


PLATE 1 :

A. Antimicrobial activity of Recinus oil and Franch oil NH on Staphylococcus aureus

(a) Control (b) Cold pressed Recinus oil (c) Franch Oil NH



B. Antimicrobial activity of Recinus oil and Franch oil NH on Pseudomonas

(a) Control (b) Cold pressed Recinus oil (c) Franch Oil NH

picts in table 2. Table 3 shows the comparative minimal inhibitory concentration of Franch oil - NH with antibiotic standards on bacterial and fungal strains.

Discussion

The present study demonstrated the anti-microbial and anti-fungal activity of Franch oil - NH against the common microorganisms present in the wounds, cracks and bums. In Malaya and China *Ricinus oil* were used to treat Scrofulous ulcers and on chronic wounds of the legs. In Columbia the *Ricinus oil* is the ideal applicant against ulcerated feet.

The *Ocimum Sanctum* oil is reported to posses anti-bacterial activity against *Mycobaacterium tuberculosis*, *Micrococcus pyogens*, *var.aureus*, and *Escherchia coli*. *Ricinnus oil* acts as fungicide in the treatment of skin diseases like athletic foot. Siddique et al has reported the anti-microbial effect of Ricinus oil based antiseptic having more bactericidal effect when compared to other base.

In the field of cosmetology in Tanganyika the *Ricinus oil* is used as grease to the body. Some of the substances contained in the *Ricinus oil* have a biodynamic activity in the field of dermatology. The *Ricinus oil* contains riboflavin, vitamin E, lipids, protein and the glyceride of oleic acid. It also contain nicotinic acid which, due to its irritant action, could act as a hemostat and cicatrizing agent, especially for bums wounds, sores etc.

The mechanism of anti-bacterial action of Franch oil - NH may be due to smoothening and disruption of the bacterial cell wall or the presence of bactericidal property of *Ocimum sanctum*. Thus this study concludes that Franch oil NH is an effective bactericidal and fungicidal agent.

CHAPTER 3

WOUND HEALING AND STRETCH MARK

Introduction

Cutaneous injury is characterized by fibroplasia, angiogenesis and re-epithelisation and involves the migration and proliferation of cells such as fibroblasts, endothelial cells and epithelial cells, deposition of connective tissue and contraction of the wound.

These steps are orchestrated in a controlled manner by a variety of bioactive molecules like growth factors, cytokines, their receptors and matrix • molecules. Such a controlled phenomenon can be disrupted in diseases like diabetes, immunocompromised persons, ischaemia etc. thus leading to the development of a chronic wound prolonged or incomplete wound healing is then a troublesome complication

Healing is a physiological process and does not normally require much help but still wounds cause discomfort and are prone to infection and other complications. Therefore, use of agents expediting healing is indicated. Further, some diseases like diabetes, immunocomprised conditions, ischaemia and conditions like malnourishment, aging local infection, and local bum or gunshot wounds leads to delay in healing(3-6). Such conditions specially require the use of agents, which can facilitate healing.

Auhough a very large number antibiotics and antibacterial chemotherapeutics exist today, their usage is becoming restricted not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria.

Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and saw the patient from amputation or other severe complications. In primary screening Franch oil-NH has showed a significant wound healing activity. Detailed evaluation of the wound healing activity of Franch oil-NH has been carried out.

Materials and methods

Male albino rats weighing 150-200g were divided into 4 groups. A 1- cm² standard full thickness wound was created on the back of the rat under light ether anesthesia. The first group, which served as the control, was left untreated. To the second group, 0.5ml of Franch oil NH was applied on the wounds. The control and treated rats were housed individually and were given feed and water ad libitum. The rats were sacrificed on 8 and 16 days after wound creations and the granulation tissue formed was removed and used for further studies.The wound tissues were used to analyze the total collagen and their fractions. To the third group Betadiene was applied.

Estimation of Collagen

Collagen was estimated by the method of Woessner

Reagents

1. Chloramine-T (sodium p-toluene sulfochloramide): A 0.05 M solution was prepared freshly by dissolving 1.41 gm choramine-T in 20 ml water. 30 ml of

Nad for a total period of 48 hrs. The combined extract was centrifuged for 1 hour at 20,000 x g at 4c in a refrigerated centrifuge. An aliquot of the supernatant was hydrolyzed with an equal volume of 12N HCL and the collagen content was determined by the method of woessner

Acid soluble collagen

The residue left after NaCl extraction was again extracted with 0.5 M acetic acid. It was left in a magnetic stirrer overnight and the solution was centrifuged at 20,000x g for 30 minutes. An aliquot of the supernatant was subjected to the determination of collagen content by the method of Woessner.

Insoluble collagen

Neutral salt soluble collagen and acid soluble collagen contents were expressed as percentage of total collagen . Insoluble collagen content was calculated by subtracting the sums of soluble collagen contents from 100.

The collagen fractions were expressed as percentage.

Histopathology of wound samples

The rats were sacrificed and skin samples with the incisions were removed periodically, cleaned well in cold physiological saline to remove blood and the adhering tissues. The samples were then fixed in 10% Formalin- saline and embedded in paraffin. Serial sections were cut at 5 mem and stained with haemotoxylin and eosin and also with Van Gieson's stain. The sections were examined under light microscope and photomicrographs were taken.

A 6 cm linear incisional wound was created on either side of the mid line of the male rats weighing 150-200 g. After mopping the wound dry, intermittent sutures were placed 1 cm apart with cotton threads. The animals were separated into 2 groups and were treated with Franch oil NH as previously given on the 7th day sutures were removed and on the 10th day. The tensile strength of the wounded skin was measured.

In many of the reports the effectiveness of Franch oil NH as a dressing on infected wounds is attributed in part to its antibacterial properties. The antibacterial properties of it have been well established; but there are also many clinical observations and animals experiments that make it clear that Franch oil NH has additional properties that make it an ideal wound dressing material.

It provides a moist healing environment

It has an anti-inflammatory action

It stimulates granulation and epithelialisation.

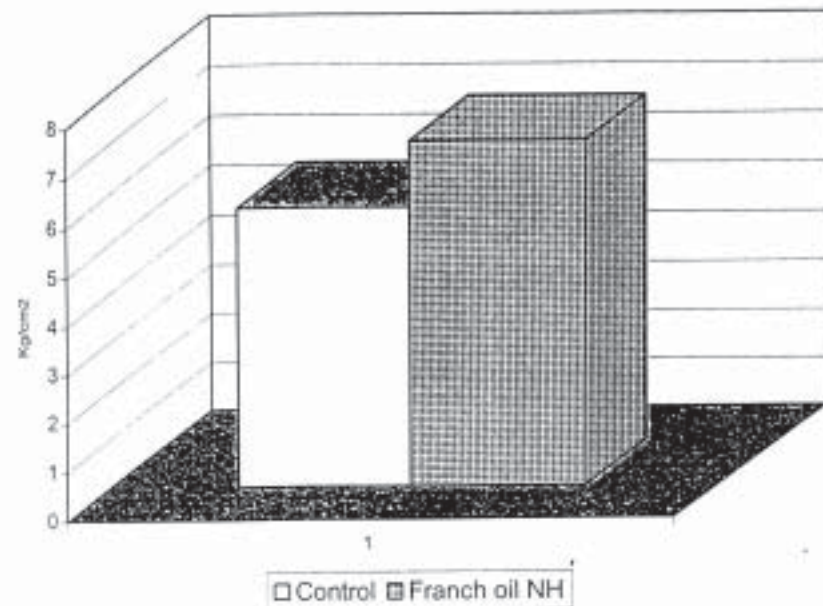
Results and discussion

Franch oil-NH gives good results on a very wide range of wounds of varied etiology, including: surgical wounds, traumatic wounds, abrasion wounds (especially those containing particles of food surface, which it lifts out from the wound), bum, and leg ulcers.

Surgical wounds

Franch oil NH is used to good effect in the treatment of infected surgical wounds. The stimulation of the healing process in addition to the antibacterial action helps to give very good results. (Plate 1 and 2).

Fig. 1 Streach testing using tissues of Normal and Experimental rats



The viscosity of Franch oil NH acts as a barrier, and along with the antibacterial properties of Franch oil NH prevents wounds from becoming infected, and thus Franch oil NH dressings protect patients from cross- infection.

Anti bacterial action

In vitro studies established that Franch oil NH has significant antibacterial activity.

Anti-inflammatory action

The reduction in inflammation. It also reduces edema and exudation, and the soothing effect when Franch oil NH is applied to wounds is a direct anti-inflammatory effect, not secondary to the antibacterial action removing inflammation causing bacteria. The anti-inflammatory effects of Franch oil NH have been demonstrated in hispathological studies of experimental wounds in animals where there was no infection involved.

Stimulation of tissue growth

Franch oil NH as a wound dressing gives rapid healing of wounds. It promotes the formation of clean healthy granulation tissue and epithelialisation of the wound. Thus it helps skin regenerates, making plastic reconstruction unnecessary.

This growth-stimulating property of Franch oil NH has been demonstrated histologically in many animal studies, as has a stimulation of the synthesis of collagen and other connective tissue components, improvement of the strength of collagen, and stimulation of angiogenesis.

Table 2 depicts the changes in the content of total collagen in the granulation tissue of Franch oil NH treated rats and the controls. The total collagen content is found to increase in the granulation tissue of experimental rats after Franch oil NH treatment.

The time kinetics of the total collagen is as follows. Collagen content increases sharply on the fourth day and reaches maximum level on the 8th day. A rapid fall in the collagen content is observed till day twelve and thereafter the fall is slow. This confirms to the earlier findings of Grille et al

To evaluate the healing of the wound, histopathological studies are most important. Keeping this in view the histological sections taken from the biopsies have been studied. For the healing pattern of the wound, both in control and in experimental animals.

Treatment of rats with Franch oil NH is found to produce significant increase in total collagen content of the granulation inhibition of its synthesis. Determination of the solubility of collagen after the infection of labeled proline in the individual fraction of skin collagen has yielded valuable information about the metabolism of collagen. 0.5 M acetic acid is known to extract almost all the soluble collagen including the newly formed collagen. The increase in total collagen content of wounds treated with Franch oil NH, with a corresponding increase in percentage extractability in acid soluble solution clearly indicates that Franch oil NH administration enhances collagen synthesis.

It has been reported that Franch oil NH contains vitamin D. These vitamins present in the Franch oil NH can also help the wound healing process. Yabuki has shown the influence of various vitamins on the healing of wound

Table 3 shows the solubility pattern of collagen into neutral salt soluble collagen (NS), acid soluble (AS) and insoluble collagen. In controls, the amount of AS fraction is 1.5 times higher than that of the NSS fraction, whereas in the IP treated animals; the acid soluble collagen is about 3 times higher than that of tile controls. It appears that there is more and earlier maturation of collagen fiber in Franch oil NH treated animals. The increase in tensile strength Table 2 of the incised wounds in Franch oil NH Treated rats confirms these results.

Fig 1 shows the stress strain behavior of both control and Franch oil NH treated rat-wounded tissues. The increase in the tensile strength (Tab 4) of Fmach oil NH treated animals may be due to the increase in the collagen concentration per unit area and /or stabilization of the fibrils. The increase in tensile strength is directly proportional to the increase in the hydroxy proline content. The increase in the aldehyde content of the acid soluble collagen shows that collagen in the treated animals is more cross- linked than that of the control. Maturation of collagen fibrils results in stable cross- links between several chains and these cross-links are responsible for gain in strength. Tensile strength of rat skin is found to increase with age due to the increase in the cross- linking and also due to the increase in the total collagen content

More clearly defined. Dehydration of unwanted tissue fluid around the wound might also decrease tissue turgor and improve tissue oxygenation and hence wound healing

Kautman et al applied buffered solutions to experimental second degree burns in guinea pigs and noted significantly increased re- epithelization in wounds treated with pH 3.5 solution compared with wounds treated with neutral and alkaline solutions. Leveen et al also demonstrated increase rate of healing in acid pH.

Table 1: Rate of control contraction as percent of original wound size of normal and experimental rats.

Values Are expressed mean + S.D. from rats in each individual experiments

<i>Groups</i>	<i>Experimental days</i>			
	4	8	12	16
Control	35 ±3.6	54 ±3.9	72 ±6.8	80 ± 9.9
Topical	50 ±5.2***	65 ±7.3***	79 ±8.1**	92 ± 7.9

Results and discussion

Table 1 shows Franch oil NH to possess highly significant analgesic property. Absolute nil writhing on Franch oil NH administration at per Kg body weight with respect to 32 number of writhing produced by the untreated rats, leaves no doubt as to the analgesic potential possessed by Franch oil NH

Tab 6 summarize the anti-inflammatory property possessed by Franch oil NH 100ul 200ul /kg showed an inhibition of 29.17% and 56.37% respectively. It is observed that the vehicle PBS used, as seen in the control rats increased the inflammation doubly compared to the untreated carrageenin induced rats. Franch oil NH administration suspended in PBS effectively reduced the inflammation to a significant extent.

The present study demonstrated that Franch oil NH was effective in animal model for acute inflammation. Franch oil NH administration significantly inhibited the paw edema formation induced by carrageenan.

The time course of edema development in carrageenan- induced paw edema model in rats. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also to the serotonin component 13. Prostaglandins play a major role in the development of the second phase of reaction, which is measured around 3-h time. The presence of PGF in the inflammatory exudes from the in-thereafter. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibited the cyclooxygenase involved in prostaglandin synthesis. Based on these reports it can be inferred that the inhibitory effect of oil on Carrageenan, induced inflammation in ratio could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin's synthesis.

TABLE 1: ANALGESIC EFFECT OF FRANCH OIL NH.

<i>Analgesic Treatment Mg/Kg</i>	<i>Averageno Writhing</i>
Normal	32
Control	32
Franch 100ul	1
Franch 200ul	0

TABLE: 2 ANTI INFLAMMATORY EFFECT OF FRANCH OIL NH

<i>Treatment inhibition Mg/Kg</i>	<i>Mean paw weight (g) right hind paw</i>	<i>Left hind paw</i>	<i>Difference</i>	<i>%</i>
Normal	0.705	0.621	0.084	—
Control	0.812	0.625	0.187	—
Franch oil NH 100	0.773	0.616	0.157	29.13
Franch oil NH 200	0.759	0.630	0.123	56.31

Table 2: Solubility pattern of granulation tissue collagen of normal and experimental rats.

Values are expressed as mcg/g wet weight, Mean +- S.D from six rats rats in each individual experiments.

<i>Groups</i>	<i>Neutral salt soluble Collagen(NSS)</i>	<i>Acid soluble Collagen(AS)</i>	<i>Insoluble Collagen</i>
Control	375 ± 23	690 ± 52	3172±215
Topical	429 ±31***	826 ± 79 «	3328 ±290

The presence of heat-labile, light-sensitive antibacterial agent, inhibit (hydrogen peroxide) also helps wound healing by killing bacteria and in clearing the wound healing by its energy producing properties, its hygroscopic effect on the wound and its bactericidal properties.

Franch oil NH which is a nontoxic, non-allergic natural product and which hasten the healing process when applied topically. It is observed that Franch oil NH helps in the entire phase and wound healing like inflammation, granulation tissue formation, collagen synthesis and maturation of wounds when given both systematically and topically.

Conclusions

Franch oil NH as a wound dressing material provides a moist healing environment in which microbial growth cannot occur. The antimicrobial properties of Franch oil NH give rapid clearance of infection with no adverse effects on wound tissues. Inflammation, swelling and pain are quickly reduced, chemical or enzymatic debridement is induced, granulation and epithelialisation are hastened, and healing occurs rapidly with minimal scarring. The film of Franch oil NH in contact with the wound bed prevents adhesion to the tissues of any covering dressing materials, allowing painless dressing changes with no damage to granulation tissue. The anti-inflammatory effect reduces edema and exudation. There is also a growth-promoting effect on granulation and epithelialisation. There is good clinical evidence that Franch oil NH gives better results than commonly used conventional dressings for a wide variety of wounds.



Plate (a)
Immediately after wound creation



Plate (b)
Untreated wound after 16 days



Plate 2 (a)
Betadiene treated wound
after 16 days



Plate 2 (b)
Franch oil NH treated rats
after 16 days

CHAPTER 4

ANTI INFLAMMATORY AND ANALGESIC ACTIVITY

Anti inflammatory activity

Inflammation and skin disorder have been held closely comparable. Inflammation can be described in general terms as a local tissue response to injury by chemical or physical agents. In the acute inflammatory change, there is dilation of the capillaries and of the smaller arterioles and vessels at the site of injury, infection or irritation due to foreign substances. The predominant features of a chronic inflammatory process are proliferation of fibrous tissue.

Analgesic activity

Analgesia is generally defined as a state of reduced awareness to pain and analgesic decrease pain sensation by increasing the threshold of the brain to painful stimuli. Although there are different definitions for pain the most widely accepted one is that of the International Association for the study of pain (IASO) which states pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Since the control of pain with the use of systemic cytotoxic chemotherapy is usually a consequence of objective response with shrinkage of the tumor mass, the method which is often slow and unpredictable) a drug possessing multifunctional activity provides effective treatment.

Natural products of origin are still a major part of traditional medicinal systems in developing countries. There is also a resurgence of interest in herbal medicines in western countries as an alternative source of drugs often for intractable diseases such as rheumatoid arthritis.

The need for safer and effective analgesic and anti-inflammatory drug and the lack of enough scientific data to support the claims made in ancient literature prompted the present study. The Franch oil NH was used for the pharmacological investigations.

Materials and Methods

Acute anti-inflammatory activity was investigated by employing carrageenin induced hind-paw edema in rat's. The animals were divided into four groups, the normal rats, which received no treatment, the control rats receiving only the saline, and the Franch oil -NH treated rats given 100ul and 200ul per rat.

Sample preparation

Franch oil, NH (100ul and 200ul) were dissolved in chloroform (10ml) in a round bottom flask. The solvent was removed by evaporation to produce a thin film on the interior of the flask. Phosphate buffered saline (PBS) 10ml was then added and the film was dispersed by. All the procedures were carried out under sterile conditions. Male albino rats were injected with 0.1 ml of a 1% Carrageenin solution in saline was injected into the sub-plantar region of the right hind paw.

The analgesic activity was investigated in rats employing acetic acid induced writhing.

cooled and diluted to one litter with water. The reagent was then cooled and diluted to one litter with water. The reagent was diluted 1:2 with distilled water just before use.

- Standard bovine serum albumin: 10 mg of crystalline BSA was dissolved in 100 ml of distilled water.

Procedure

An aliquot of the suitably diluted serum (0.1 ml to 10 ml by two serial dilution) was made upto 1.0 ml with water. 4.5 ml of alkaline copper reagent was added to all the tubes including blank. Blank containing 1.0 ml of water and standard containing aliquots of BSA were also treated similarly. The contents were left to stand for 10 minutes at room temperature. Then 0.5 ml of diluted Folin's phenol reagent was added. The blue color developed was read at 640 nm after 20 minutes in a Shimadzu UV spectrophotometer.

The values were expressed as g/dl serum.

Assay of Serum Transaminases

Aspartate amino transferase (Glutamate oxaloacetate transaminase The method of King(9) was adopted for the assay of serum aspartate transaminase.

Reagents

- Phosphate buffer: 0.1 M, pH 7.4
- Substrate : 2.66 g of DL-aspartate and 38 mg of α -oxaloglutarate were dissolved in 20.5 ml of 1.0 N sodium hydroxide with gentle heating. This was made upto 100 ml with water.
- 2,4- dinitro phenylhydrazine reagent (DNPH): 1.0 mM dinitrophenyl hydrazine in 2.0 N hydrochloric acid.
- Sodium hydroxide : 0.4 N solution.
- Standard pyruvate: 10 mg of sodium pyruvate was dissolved in 100ml of phosphate buffer 0.1 M, pH 7.4

Procedure

In different tubes, 1.0ml of the buffered substrate was added to 0.1 ml of serum and incubated at 37°C for 1 hr. Then 1.0 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of serum was added only after the addition of DNPH reagent. The tubes were kept aside for 15 minutes, then 10 ml of 0.4 N sodium hydroxide was added and read at 520 nm in a Shimadzu UV Spectrophotometer. The enzyme activity was expressed as IU/litter.

Alanine amino transferase (glutamate pyruvate transaminase, B.C.2.6.1.2). The reagents and method used were the same as those used for the assay of aspartate transaminase expect for fine substrate solution and the incubation time was reduced to 30 min.

Substrate

1.78 g of DL-alanine and 38 mg of α -keto-oxaloglutarate were dissolved in buffer. 0.5 ml of sodium hydroxide was added and the volume was made upto 100ml with buffer.

The analgesic activity of Franch oil NH on the writhing response is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. It was found that Franch oil NH significantly inhibited the acetic acid induced writhing response in a dose dependent manner. It is therefore possible that Franch oil NH has an analgesic effect probably by inhibiting synthesis or action of Prostaglandin's.

Based on the results of this study we came to the conclusion that Franch oil NH has the potential anti-inflammatory activity against the inflammation and thus support the claimed use of this oil in the ayurvedic medicine. The Franch oil NH has analgesic activity

TOXICOLOGICAL STUDIES ON FRANCH OIL-NH

Introduction

Faced with the mounting adverse drug reactions to synthesized chemical medications efforts are currently being made to look for the products of natural origin. For most among these are medicinal plants and their essence. Ayurvedic formulations are known to play a pivotal role in the management of various disorders and presently considerable attention is being directed towards Franch oil-NH, to establish its mechanism of action. It has got many medicinal properties like Antimicrobial, Antifungal, Anti-inflammatory, Analgesic and wound healing properties. An in-depth study was conducted to find out, the adverse effect of Franch oil-NH, if any.

Experimental set-up

Adult male wistar rats weighing 100-120g were used for this study. They were divided into two groups.

Group 1.-Normal

Group 2. - 100 ul of Franch oil NH administered orally for 30 days.

The animals were fed with commercially available pelleted rat feed which was obtained from Lipton India Ltd., Madras. Food and water were given ad libitum. At the end of the experimental period the rats were sacrificed by cervical decapitation. Blood was collected in plain and heparinized tube immediately after the sacrifice. Liver, kidney, heart, intestine and testis were removed and washed in saline. A part of the tissue was fixed in 10% Formaline saline and used for the histopathological examination. The other part of the tissues was used for biochemical analysis.

Histological Study

For histological examination, a small portion of liver, kidney, heart, intestine and testis were fixed in 10% formal-saline. The tissues were processed for paraffin embedding and sections were stained with hematoxylin and eosin and viewed under a high power microscope.

The following parameters with Blood Glucose, Urea, Creatinine, Uric acid, Total Protein, albumin, Globulin, Cholesterol, Triglycerides, Bilirubin, HDL, SGOT, SGPT, LDH, ALP, γ -GT and CK were estimated. Protein content in the homogenate was estimated by the method of Lowry et al.

MATERIALS AND METHODS

Estimation of Blood Glucose

Blood glucose was estimated by the method of Sasaki et al.

Reagents

1. TCA:10%
2. O-Toluidine reagent: 12.5 g of thiourea and 12.0 g of boric acid were dissolved in 50ml of distilled water by heating 75 ml of O-Toluidine (redistilled) and 375ml of acetic acid (AR) were mixed separately. These two solutions were mixed and the total volume was made upto 500ml with distilled water. The reagent was left overnight in the refrigerator and filtered.
3. Glucose standard: 100 mg of pure glucose was dissolved in 100 ml of distilled water containing 0.01% benzoic acid.

Procedure

0.2 ml of blood was deproteinized with 2.8 ml of 10% TCA. To 2.0ml of water and standards containing 20 to 40ug of glucose were also treated similarly.

The values were expressed as mg/dl blood.

Estimation of Urea

Blood urea was determined by the method of Geyer and Dabich.

Reagents

1. Diacetyl monoxime: Thiosemicarbazide reagent(DAM-TSC) 36mM diacetyl monoxime and 61.7mM thiosemicarbazide in 2% acetic acid.
2. Acid ferric reagent: 3.6 ml sulfuric acid, 0.12 mg ferric chloride and 38.6 ml 0-phosphoric acid.
3. Standard Urea: 10 mg of urea was dissolved in 100ml of distilled water.

Procedure

0.2 ml of blood was deproteinized with 2.8ml of 10% TCA. To 2.0ml of the supernatant obtained by centrifugation, 1.0ml of DAM-TSC reagent and 1.5 of acid ferric reagent were added and the solution was heated in a boiling water bath for 15 minutes.

Aliquots of the standard urea and blank containing 2.0ml water were also treated in a similar manner. After cooling, the color developed was read at 520 nm in Shimadzu UV spectrophotometer.

Determination of serum Uric acid

Serum uric acid was estimated according to the method of Caraway.

Reagents

1. Colouring reagent : 50g of molybdate free sodium tungstate was dissolved in 400ml of distilled water. Added 40 ml of phosphoric acid was added and refluxed for 2 hours. A drop of bromine was added cooled and diluted to 500ml with water.
2. Sodium carbonate reagent: 20 percent aqueous solution.
3. Uric acid standard: 100mg of uric acid was dissolved in 150ml of water containing 60 mg of lithium carbonate by heating at 60 c , the solution was cooled at room temperature and added 2ml of formaldehyde diluted to about 500ml.

4. Working standard: 1.0 ml of the stock standard and 2.0 ml of 30mg/litre BSA were diluted to 10ml with water. The working standard was prepared fresh. Albumin was added to account for the positive error induced by a co-precipitation of uric acid and proteins.

Procedure

5.4 ml of diluted tungstic acid was added to 0.6 ml of serum. The contents were mixed and centrifuged. Into three test tubes 3ml each of supernatant, standard and water(as blank) were taken in these tubes. 0.6 ml of sodium carbonate and 0.6ml of phosphotungstic acid reagent were added, mixed and placed in a 25 c water bath for 10 minutes. The blue color developed was read at 700 nm.

The values were expressed as mg/dl serum. The values were expressed as mg/dl blood.

Estimation of serum Creatinine

Serum creatinine was estimated by the method of slot.

Reagents

1. Picric acid: 1.2 g of picric acid was dissolved in one liter of distilled water.
2. Sodium hydroxide: 30.0 g/litter
3. Alkaline picrate reagent: Equal volumes of solutions and were mixed just before use.
4. Sodium tungstate solution: 50.0 g/litter of water.
5. Sulphuric acid: 0.33 M
6. Creatinine standard: 100 mg of creatinine was dissolved in 100 ml of 0.1 ml HCL. Before use, this stock standard was diluted to 10 fold with water.
7. Glacial acetic acid.

Procedure

To 3.0 ml of deproteinized supernatant (0.1 ml blood + 3.9 ml 10% TCA) added 2.0 ml of alkaline picrate solution. Blank containing 3.0 ml of water and aliquots of standard in 3.0 ml of water were also treated in a similar manner. After 30 minutes the color was measured at 520 nm against the reagent blank.

Determination of Serum Protein

Scrum protein content was estimated by the method of Lowry et al.

Reagents

1. Alkaline copper reagent
Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide
Solution B : 0.5% copper sulphate in water.
Solution C: 1% sodium potassium tartarate in water.
50 ml of solution A was mixed with 0.5 ml of solution B and 1.0 ml of solution C just before use
2. Folin's phenol reagent: Into a 1500 ml round bottomed flask, 100 g of sodium molybdate, 700 ml of water, 50 ml of 85% 0- phosphoric acid and 100 ml of concentrated hydrochloric acid were added and refluxed for 10 hours. Then 150 g of lithium sulphate, 50 ml of distilled water and a few drops of bromine were added. The mixture was boiled to remove excess bromine. It was then

tated cholesteryl digitonide was removed by centrifuging at 1000xg for 15 minutes and the upper solvent phase was carefully decanted and discarded. The precipitate was washed twice with 3.0 ml of acetone- ether mixture (1:2v/v) and finally with 3.0 ml of pure dry ether. The precipitate was then dissolved in 1.0 ml of glacial acetic acid by heating over a water bath. From this solution aliquots were taken for the estimation of cholesterol. The level was expressed as mg/dl.

Determination of Triglycerides

Plasma triglycerides were estimated by the method of Rice.

Reagents

1. Chloroform-Methanol mixture: 2:1(v/v)
2. Saturated sodium chloride solution
3. Activated silicic acid
4. Alcoholic potassium hydroxide: Dissolved 0.5 g of reagent grade potassium hydroxide in 95% ethanol and made up to a final volume of 25ml. The working solution was prepared shortly before use by diluting one volume of this stock solution to 5 volumes with 95% ethanol.
5. Sulfuric acid : 0.2N.
6. Sodium metaperiodate: 5.0%
7. Sodium bisulphite: 5.0%
8. Chromotropic acid reagent: Dissolved 1.0 mg of the disodium salt of chromotropic acid in 60 ml of water. Separately added slowly 300ml of concentrated sulfuric acid to 150 ml water. After cooling to room temperature, the solution was added slowly to chromotropic acid solution and stored in a brown bottle.
9. Thiourea solution: 70%

Standard tripalmitin: 100 mg of tripalmitin was dissolved in 100ml of chloroform. 10 ml of this stock solution was diluted to 100 ml and used as working standard.

Procedure

12 or 15 ml glass stoppered centrifuge tube containing 9.8 ml of chloroform methanol mixture was mixed with 0.2 ml of plasma. The tubes were stoppered, shaken vigorously and left to stand for 30 minutes with intermittent shaking. It was then centrifuged at high speed for several minutes to sediment the proteins. 4.0 ml of the supernatant was transferred to a 15 ml glass stoppered centrifuge tube containing 8.0 ml of saturated saline solution. The tube was stoppered, shaken vigorously and left to stand for 1 hour. After centrifugation the upper layer was carefully removed leaving the washed chloroform layer containing the lipids which was filtered.

0.2 g of activated silicic acid was added to the filtered lipid extract. The mixture was shaken gently and left to stand for 30 minutes. With occasional shaking 0.5 ml of the supernatant was evaporated to dryness in a water bath at 70°C. Standard solutions of tripalmitin (0.50g) were taken and similarly evaporated to dryness together with a blank containing only the solvent.

0.5 ml of alcoholic potassium hydroxide was added to all tubes and the mixtures were saponified in a water bath at 60-70°C for 20 minutes. The tubes were stoppered with glass marbles to minimize evaporation. 0.5 ml of 0.2 N sulfuric acid

The enzyme activity was expressed as IU/litre.

Serum lactate dehydrogenase (L-lactate: NAD oxido-reductase B.C.

1.1.1.27)

The enzyme activity was assayed according to the method of King

Reagents

1. Glycine buffer(0.1M)- 7.5 g of glycine and 5.85 g of sodium chloride were dissolved in 1 litre of distilled water.
2. Buffered substrate: 2.78g of lithium lactate was dissolved in 124ml of glycine buffer containing 7.5 ml of 0.1N sodium hydroxide solution. This was prepared just before use.
3. 2,4- dinitro phenyl hydrazine reagent(DNPH): 200 mg of DNPH was dissolved in one litre of 1.0N hydrochloric acid.
4. Standard pyruvate solution: 11.0 mg of sodium pyruvate was dissolved in 100ml of buffer.

Procedure

To a set of tubes, 1.0 ml of the buffered substrate and 0.1ml serum were added and the tubes were incubated at 37°C for 15 minutes. After adding 0.2 ml of NAD+ solution, the incubation was continued for another 15 minutes.

The reaction was then arrested by adding 1.0 ml of DNPH reagent and the tubes were incubated for a further period of 15 minutes at 37°C. 0.1 ml of serum was added to blank tubes after arresting the reaction with DNPH. 7.0 ml of 0.4 N sodium hydroxide solution was added the colour developed was measured at 420 nm in a Shimadzu UV Spectrophotometer. Suitable aliquots of the standard were also analyzed by the same procedure.

The enzyme activity was expressed as IU/litter

Assay of serum Creatine Kinase (ATP- creatine phosphotransferase E.C.2.7.3.2)

Serum creatine kinase activity was determined by the method of Okinaka et al(11).

Reagents

1. Tris-HCL buffer: 0.1 mM, pH 9.0
2. ATP: 0.0185 M in Tris-HCL buffer(0.1M pH9.0)
3. Magnesium-cysteine reagent : 24.65 mg of magnesium sulphate and 15.76 mg of cysteine-HCL were dissolved in 10 ml of distilled water.
4. Creatine-240nM
5. Ammonium molybdate : 2.5 g of ammonium molybdate was dissolved in 100 ml of 3N sulfuric acid.
6. Amino-naphthol sulfonic acid (ANSA) reagent: 0.5 g of ANSA was dissolved in 195 ml of 15% sodium meta bisulfite was added for complete solubilization. This solution was filtered and stored in a brown bottle,
7. Standard phosphorus: 35.1 mg of potassium dihydrogen phosphate was accurately weighed, dissolved in 100ml double distilled water. 1.0 ml of this solution contained 80 microgram of phosphorus.

Procedure

The incubation mixture containing 0.75 ml of double distilled water, 0.05 ml of serum, 0.1 ml of ATP solution, 0.1 ml of magnesium-cysteine reagent and 0.1 ml of creatine was incubated at 37°C for 20 minutes. The tubes were centrifuged and the supernatant was used for the estimation of phosphorous as described earlier in this chapter.

The enzyme activity was expressed as ILJ/litter.

Assay of acid phosphatase (ortho- phosphoric monoester hydrolase, E.C.3.1.3.1)

Acid phosphatase was assayed by the method of Moog(12) as modified by King (13) using disodium phenyl phosphate as substrate.

Reagents

1. Acetate- acetic acid buffer: pH 4.9, 0.1M
2. Disodium nhenyl phosphate solution: 0.01 M
3. Folin's phenol reagent: This was prepared as described earlier in this chapter
4. Sodium carbonate: 15%

Standard phenol: 100 mg of recrystallized phenol in 100 ml of water. 100 ug of phenol per ml was prepared and used as working standard.

Procedure

The incubation mixture of 3.0 ml contains 1.5 ml of buffer, 1.0 ml of substrate and requisite amount of the enzyme source. The tubes were incubated at 37c for 15 minutes. The reaction was arrested by the addition of 1.0 ml of Folin's phenol reagent. The control tubes received the enzyme after arresting the reaction. The contents were centrifuged and to the supernatant, 1.0 ml of 15% sodium carbonate was added and the mixture incubated for 10 minutes at 37°C The colour was read at 640 nm in a Shimadzu UV Spectro photometer.

The enzyme activity was expressed as micromoles of phenol liberated per minute per mg protein.

Assay of alkaline phosphatase (Ortho-phosphoric monoester phosphohydrolase, B.C.. 1.1)

Alkaline phasphatase was assayed by the method of Moog as modified by King using disodium phenyl phosphate as substrate.

Reagents

1. Carbonate-bicarbonate buffer: 0.1 M, pH 10.0
2. Disodium phenyl phosphate solution: 0.01 M
3. Magnesium chloride solution: 0.1 M
4. Folin's phenol reagent: This was prepared as described earlier in this chapter.
5. Sodium carbonate solution: 15%

Standard phenol: 100 mg of recrystallized phenol in 100 ml of water was prepared 100ug of phenol per ml was then prepared by proper dilution and used as the working standard.

Procedure

The procedure was same as that used for acid phosphatase assay except the addition of 0.1 ml magnesium chloride.

The enzyme activity was expressed as micromoles of phenol liberated per minute per mg protein.

Assay of γ -Glutamyl Transferase

γ -glutamyl transferase in serum was estimated according to the method of Jacob

Reagents

1. Tris buffer: 0.2 M , pH 8.5
2. Glycyl-glycine: 640 nM
3. γ - glutamyl p-mtroanylide 0.04 M in Tris buffer

Procedure

To 1.4 ml of buffered substrate added 0.5 ml of glycyl glycine. The enzyme was initiated by adding 0.5 ml of the sample. Blank contained 1.4 ml of buffered substrate and 0.6 ml of water. The change in absorbance was monitored at 410 nm in a Shimadzu UV spectrophotometer.

The enzyme activity is expressed as IU/L.

Determination of total Cholesterol

Cholesterol was estimated by the method of Parekh and Jung

Reagents

1. Ferric-acetate- Uranyl acetate reagent: 10 ml of water and 3.0 ml of concentrated ammonia were added to 500 mg of crystalline ferric chloride. The precipitate was washed several times with distilled water and was dissolved in glacial acetic acid and made up to 1 litre with acetic acid. 100 mg of uranyl acetate was added, shaken well and kept overnight. The reagent was stored in an amber colored bottle. This reagent was stable for 6 months.
2. Sulfuric acid Ferrous sulphate reagents: To 100 ml of glacial acetic acid, 1 g of anhydrous ferrous sulphate was added and shaken well. 100 ml of concentrated sulphuric acid was added and after cooling, the volume was made up to 1 litre with concentrated sulfuric acid. The reagent was stable for 6 months.

Cholesterol Standard:

The stock standard was prepared by dissolving 200 mg of cholesterol in 100 ml of chloroform. A standard curve was obtained using aliquot containing 0 to 20 ug of cholesterol.

The reagents 6 and 7 were prepared as described previously.

Procedure

To 0.2 ml of plasma , 3.0 ml of acetone - ethanol mixture was added and kept on a boiling water bath to raise its temperature just to boiling point. This was stirred well for 1.5 minutes on a vortex mixture and the precipitated protein was separated by centrifugation. The protein precipitate was again washed with 3.0 ml of acetone- ethanol mixture and the supernatants were combined. 1.0 ml of digitonin solution was added followed by a drop of 10% glacial acetic acid and the contents were mixed well. Then the tubes were securely closed and kept in a dark chamber for 16 hours. The precipi-

Table 5 : Levels of lipid profile in serum of normal and experimental rats.

Values are expressed as mean \pm S.D. for six animals in each groups.

<i>Lipids Profile</i>	<i>Normal</i>	<i>Franch Oil NH</i>
Cholesterol	79 \pm 6	88 \pm 7
Triglyceride	103 \pm 10	121 \pm 9
HDL	32 \pm 3	35 \pm 4
Cholesterol/HDL ratio	2.4 \pm 0.3	2.5 \pm 0.3

significant change in Fr.-inch oil Nil treated rats when compared to normal. This indicates (hat the Franch oi) treated does not affect the *kidney*.

The level of total protein, albumin and bilirubin were depicted in Table 2 and the enzymes SGPT, Alkaline phosphatase and γ -GT in Table-3. The result confirms that the Franch oil NH is not affecting the liver.

The activity of SGOT, CPK and LDH and lipid profile is depicted in table 4 and table 5. The results shows that the Franch oil NH treatment does not affect the heart.

The results of the histopathological variations studied in various tissues of rats treated with Franch oil NH is given in plate 1 to 4.

At the end of the experimental period no significant group's pathological changes were noticed in control rat (plate 1-1f). The liver, kidney, intestine and testis showed normal architecture. Histopathological studies of liver, kidney and heart showed as normal architecture.

Intestine and testis showed significant morphological changes in franch oil NH administered when compared to normal. Hence Franch oil NH is advised to use only externally.

was added to the tubes and they were placed in a boiling water bath for about 10 min. The tubes were cooled and added 1.0 ml of sodium metaperiodate solution, mixed well and allowed to stand for 10 min. 0.1 ml of sodium bisulphite solution was added and allowed to stand for 10 minutes.

0.5 ml of chromotropic acid reagent was added to each tube, mixed thoroughly, stoppered and placed in a boiling water bath for 30 minutes and away from strong direct light. The tubes were cooled and 0.5 ml of thiourea solution was added to all tubes, mixed thoroughly and the absorbance was measured in Shimadzu UV Spectro photometer at 540 nm The values were expressed as mg triglyceride/dl plasma.

Lipoprotein fractionation

Lipoproteins were fractionated by dual precipitation techniques.

HDL Fractionation

Total HDL was separated by the method of Bumstein et al.

Reagents

1. Heparin manganese chloride reagent: 3.167 gm of manganese chloride was added to 1.0 ml of heparin containing 20,000 units/ml. This was made upto 8.0 ml with water.

Procedure

1.0 ml of plasma was added to 0.09 ml of heparin-manganese chloride reagent and mixed well. The solution was allowed to stand at 4°C for 30 minutes and then centrifuged at 2,500 rpm for 30 minutes. The supernatant represented HDL fraction. Aliquot were taken from HDL fraction for the estimations of the cholesterol.

Results and Discussion

The skin is not only a protective covering for the body. It is also a major site of interaction with other body systems and with the environment.

The mass of this complex organ exceeds that of all other organs, and the skin in most places on the body is no more than, 2 mm in thickness. Several questions should be considered before discussing safety testing of skin care products, the most elementary of which is why test for safety at all? If safety testing is done, why should skin care products be considered separately- Are they significantly different from other cosmetic agents in their safety aspects? And why differentiate the skin from other parts of the outer integument, such as nails, hair, and mucosal surfaces?

Figure 1 shows the weight of the experimental rats treated with Franch oil-NH for 30 days . At 30 days there is a non-significant weight change.

The levels of various blood constituents analyzed are given in table-1 to table-5. Table-1 shows the levels of blood glucose, urea, creatinine and uric acid in normal and Franch oil NH treated rats. The results showed, there is no

Table .1 Levels of blood constituents of normal and Franch oil NH administered rats.

The Values are expressed as mean ± S.D. for six animals in each group.

<i>Parameters</i>	<i>Normal</i>	<i>Franch Oil NH</i>
Blood glucose	76.5 ± 5.8	71.0 ± 6.3
Serum Urea	20.1 ± 1.5	26.8 ± 2.1
Serum Creatinine	0.8 ± 0.1	0.9 ± 0.1
Serum. Unc acid	2.7 ± 0.2	3.1 ± 0.2

Table 2: Levels of Bilirubin & Proteins in serum of normal and experimental rats.

Values are expressed as mean ± S.D. for six animals in each group.

<i>Parameters</i>	<i>Normal</i>	<i>Franch Oil NH</i>
Bilirubin (Total)	0.9 ± 0.06	1.0 ± 0.08
Bilirubin (Direct)	0.4 ± 0.02	0.5 ± 0.03
Bilirubin (Indirect)	0.5 ± 0.04	0.5 ± 0.05
Total protein	6.7 ± 0.5	6.4 ± 0.6
Albumin	4.0 ± 0.3	3.8 ± 0.3
Globulin	2.7 ± 0.1	2.6 ± 0.2

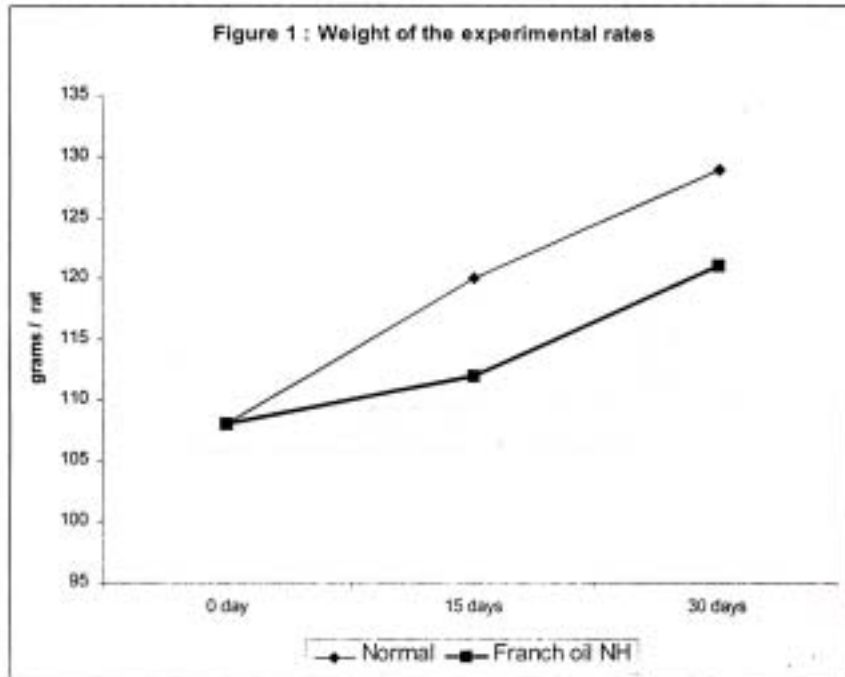
Table 3: Activities of Liver enzymes in serum of normal and experimental rats
values are expressed as mean j: S.D. for six animals in each group.

<i>Enzymes (IU / L)</i>	<i>Normal</i>	<i>Franch Oil NH</i>
SGPT	21 ± 4	25 ± 3
ALP	155 ± 10	152 ± 12
tpT	3.7 ± 0.3	4.0 ± 0.3

Table 4: Activities of Cardiac en/yms in serum of normal and experimental rats.

Values are expressed as mean j: S.D. for six animals in each group.

<i>Enzymes (IU/L)</i>	<i>Normal</i>	<i>Franch Oil NH</i>
SGOT	28 ± 3	29 ± 3
LDH	229 ± 13	235 ± 11
CK	84 ± 6	79 ± 7



CHAPTER 6**GENERAL SUMMARY**

More and more people in developing countries utilize traditional medicine for their major primary health care needs. Franch oil NH is an herbal medicine, which is a combination of cold pressed extraction of Ricinus Cummunis linn seed and root with Ocimum sanctum oil.

Franch oil NH is a multispectrum oil which is being used for the following ailments like Athletes foot, Corns, Cracked heels, Chill blain, Bums, lipcracks, skin disorders, pimples and black spot, stretch mark, menstrual pain, itches and facial oil.

To find out the efficacy of the Franch oil NH, the following study like Anti-Microbial, Anti-Fungal, Wound Healing, Stretch Mark, Analgesic and anti-inflammatory were carried out. The results obtained concludes that Franch oil NH is a potent ayurvedic medicine for external use.



Rayson Health Products Sdn. Bhd.

Tel : 03-6272 0553

Franch Herbs Technology Limited

Franch Herbs Technology Limited

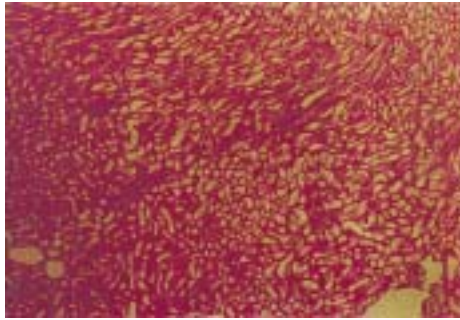


Plate 1a
Liver section of a control rat

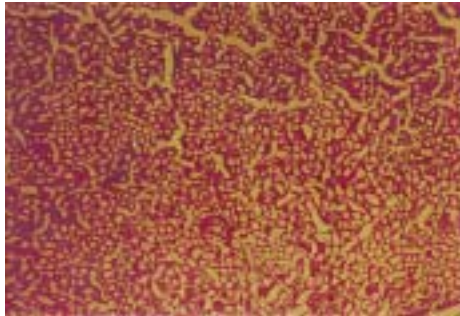


Plate 1b
Liver section of Franch Oil NH administered rat

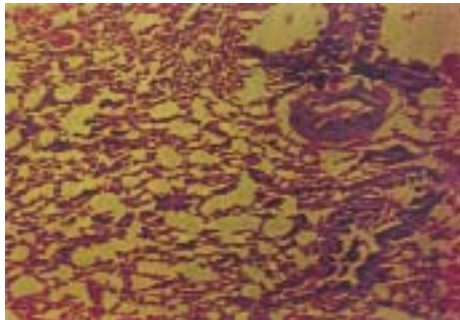


Plate 2 (a)
Kidney section of a control rat

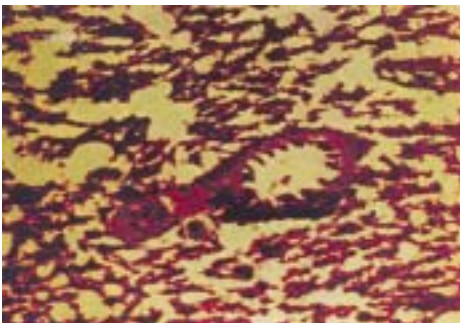


Plate 2 (b)
Kidney section of Franch Oil NH administered rat

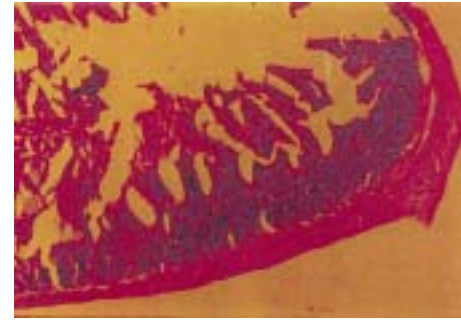


Plate 3 (a)
Intestine section of a control rat

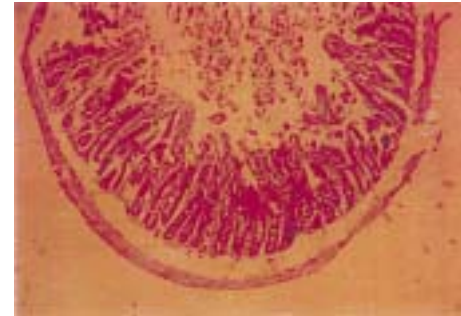


Plate 3 (b)
Intestine section of Franch Oil NH administered rat

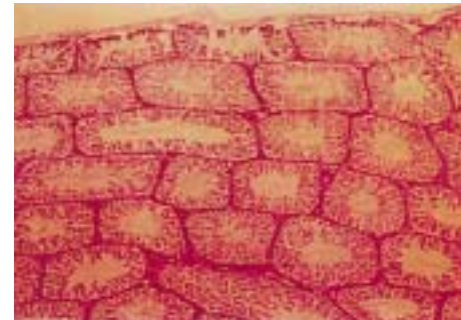


Plate 4 (a)
Testis section of a control rat

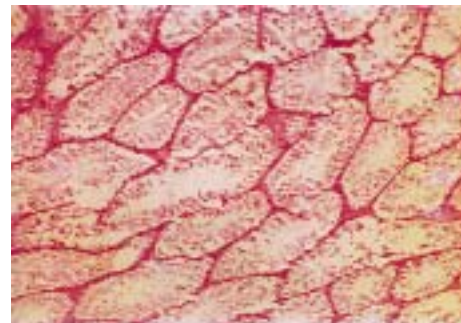


Plate 4 (b)
Testis section of Franch Oil NH administered rat