International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Hepatoprotective Effect of the *Santalum Album* Linn. Seeds against High Fat Diet Induced Non-Alcoholic Fatty Liver Disease in Wistar Rats

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become a significant global health concern, primarily due to poor dietary habits, such as high-fat diets, which contribute to liver dysfunction. This study investigates the hepatoprotective effects of *Santalum album Linn.* seeds in a high-fat diet-induced NAFLD model in Wistar rats. The rats were divided into several groups, including a normal control group, a high-fat diet-induced NAFLD group, and treatment groups administered with *Santalum album* seed extracts. Liver function was assessed through biochemical parameters such as serum liver enzymes (ALT, AST, ALP), lipid profiles, and histopathological analysis. Additionally, oxidative stress markers, including malondialdehyde (MDA) and antioxidant enzyme activities, were measured. Results showed that treatment with *Santalum album* seed extract significantly reduced serum liver enzyme levels, improved lipid profiles, and ameliorated liver histology. The antioxidant properties of *Santalum album* seeds were found to play a key role in mitigating oxidative stress, a major contributor to liver injury in NAFLD. These findings suggest that *Santalum album Linn.* seeds possess potential hepatoprotective effects and may be a promising natural therapeutic option for managing NAFLD induced by high-fat diets. Further clinical studies are recommended to validate these results in human populations.

Keywords: *Santalum album*, hepatoprotective, non-alcoholic fatty liver disease, high-fat diet, oxidative stress, Wistar rats.

1. INTRODUCTION

1.1. Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in clinic with a spectrum of disorders ranging from simple fatty liver, non-alcoholic steatohepatitis, and fibrosis/cirrhosis. Currently, NAFLD affects a quarter of the global population. Emerging evidence has indicated the dynamic alterations in bile acid (BA) profiles throughout NAFLD progression (Powell *et al*., 2021). Non - alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world. It is present in 30% of the general adult population and found in obese people with high-fat diets and inactive lifestyles. NAFLD comprises a spectrum of hepatic abnormalities that are observable in liver

histological slides, from a simple intrahepatic accumulation of fat (steatosis or non-alcoholic fatty liver, NAFL) to various degrees of necrotic inflammation (non-alcoholic steatohepatitis, NASH). Simple steatosis (i.e., NAFLD) rarely progresses to advanced disease whereas, in approximately 20% of patients with NASH, it progresses to fibrosis and cirrhosis and potentially to hepatocellular carcinoma over a 15-year time. Most patients with NAFLD are obese or even morbidly obese and have accompanying insulin resistance that plays a central role in the metabolic syndrome. Thus, NAFLD is also deemed to be hepatic manifestation of metabolic syndrome which is a cluster of complex conditions including central obesity, hypertension, hyperglycaemia, hyper tri glyceride, and low HDL (high density lipoprotein) that are predictive risk factors of cardiovascular disease, stroke, and diabetes (Jincheng *et al.*, 2016). NAFLD is characterized by hepatic steatosis, defined as accumulation of fat (triglyceride) in greater than 5% of hepatocytes in the absence of other causes of steatosis including excess alcohol intake and congenital errors of metabolism. While simple steatosis is characterized by liver lipid accumulation without inflammation and often carries a relatively favourable clinical course, NASH, which occurs in about 25%– 40% of NAFLD patients involves hepatocellular injury and liver inflammation and is a significant risk factor for cirrhosis and hepatocellular carcinoma (HCC). In the United States, NASH has been estimated to account for over 13% of HCC cases. Inside spectrum of NAFLD is considered especially worrisome as it signifies hepatocellular injury and liver inflammation, leading to other hepatic and extra hepatic complications. Day and James proposed the initial theory for NASH pathogenesis involving the two- hit hypothesis. According to this hypothesis, the first hit of NASH is simple steatosis resulting from insulin resistance and excessive fatty acids, sensitizing the liver to a second hit, likely involving oxidative stress, mitochondrial dysfunction, and lipid peroxidation and leading to inflammation and hepatic fibrosis However, it appears that steatosis resulting from insulin resistance and excessive fatty acids, sensitizing the liver to a second hit, likely involving oxidative stress, mitochondrial dysfunction, and lipid peroxidation and leading to inflammation and hepatic fibrosis. However, it appears that steatosis may not be a prerequisite for NASH and liver inflammation. Subsequently, the multiple parallel hit hypothesis by Tilg and Machen proposed that NASH results from a culmination of various factors in parallel, including disrupted lipid metabolism, lipo-toxicity, altered cytokines and adipokines, oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, gut-derived endotoxin, and genetic pre-disposition (Benini *et al.*, 2016)._Indian sandalwood, a member of the sandalwood family, has the oldest and most powerful medicinal powers. Ayurvedic, Siddha, and Unani medical systems employ essential oil extracted from the sandalwood heart for the treatment and prevention of a wide range of illnesses and ailments (Shiva tare *et al.*, 2018). It is 9,600 square kilometres in size and is primarily found in the deccan region of the Indian subcontinent's deciduous forests, 8,200 of which are spread throughout the states of Karnataka and Tamil Nadu. The region is in India. Because of its wood and essential oils, which are utilised in the incense, medicinal, cosmetic, and fragrance sectors, it has worldwide trade (Sutheesh *et al.*, 2016).

1.2. *Santalum album Linn*

In India's tropical and subtropical regions, it thrives in a variety of soil types and climates. Sandalwood oil is produced in large quantities in India for use in the pharmaceutical and perfume industries. According to the 1792 order of Tipu Sultan, the ruler of the Mysore Kingdom, this was referred to as the "Royal Tree". IUCN 2000 lists it as a vulnerable species in the wild. Countries including Australia, Indonesia, Japan, Belgium, China, Cambodia, Madagascar, Germany, Netherlands, Norway, Russia,

Switzerland, and the United States are among those where it is disseminated. Small to medium-sized, semi-parasitic trees with woody branches that develop slowly are known as sandalwoods (Polaiah *et al.*, 2020). Sandalwood essential oil is an effective treatment and preventive measure for a wide range of illnesses and ailments. It is derived from the Ayurvedic, Siddha, and Unani medical systems. A small to medium-sized evergreen plant, album can grow up to 18 metres high and 2.4 metres wide. It has robust branches. The leaves are hairless and have a diameter of 1.5-8 cm or 1.632 cm. The peel of the plant is dark red. A solitary seed resides within the drupe-like fruit, which becomes purple when fully ripe. During the months of April through May and September through October, sandalwood fruits are picked either directly off the tree or as soon as they fall to the ground (Patil *et al.*, 2019). East Indian Sandalwood oil is produced by steam distilling the heartwood and is widely regarded for its sweet aroma, tenacity, spicy, warm, woodsy undertones, stable composition, and fixative qualities. In addition to being a highly valued spice, it is also used as an expectorant, stimulant, diuretic, antiseptic, and treatment for bronchitis, gonorrhoea, urinary tract infections, and problems urinating (Goswami *et al.*, 2018).

PLANT PROFILE

- **Family:** Santalaceae, *Santalum album*, commonly known as sandalwood, is a small evergreen tree renowned for its aromatic heartwood. The tree can grow up to 9 meters (30 feet) tall and has a thin, smooth, greyish bark. Its leaves are simple, opposite, and lanceolate to elliptical in shape. The plant produces small, yellowish-green flowers arranged in spikes or panicles, and it yields a small, round fruit with a single seed.
- **Seed Characterization**

The seeds of *Santalum album*, commonly known as sandalwood, are relatively small and have a distinctive appearance that is important for identification and cultivation purposes. Each hundred seeds have a mass of 10-20 gram.

Colour	Brownish or reddish-brown colour
Odour	Emits a rich, sweet and woody aroma
Taste	Bitter or astringent flavour
Size	Seeds are relatively small, generally about 1or 2 cm (0.4 to 0.8 inches) in diameter
Shape	Small, round or oval drupe.

Table 1.1: The Morphology of *Santalum album Linn* seeds

Its heartwood is extensively utilised for wood carving as well as religious and therapeutic applications. It contains 1.5–5% of a strong, distinct oil scent. Its oil was thought to contain anti-melanoma chemicals and has been utilised as a raw material for cosmetics, a major ingredient in perfumes, and an aromatherapy technique. The price of sandalwood's heartwood increased dramatically to between USD 1.000 and USD 1.500 per kg25, making it a highly valuable and economical decorative wood. In the ten years between 1997 and 2007, there was even a tenfold increase in price, from 20,000 to 30,000 Rupees. Currently, most of this species' natural range in eastern Indonesia is thought to be gone (Ratnaningrum *et al.*, 2015).

Scientific Classification	
Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Santales
Family	Santalaceae
Genus	Santalum
Species	*Santalum al*
Botanical name	
Santalum album Linn	
Synonyms	
❖ *Santalum spicatum* ❖ *Santalum indicum* ❖ *Santalum austrocaledonicum*	

Table1.2: The Scientific classification of *Santalum album Linn*

Its heartwood is extensively utilised for wood carving as well as religious and therapeutic applications. It contains 1.5–5% of a strong, distinct oil scent. Its oil was thought to contain anti-melanoma chemicals and has been utilised as a raw material for cosmetics, a major ingredient in perfumes, and an aromatherapy technique. The price of sandalwood's heartwood increased dramatically to between USD 1.000 and USD 1.500 per kg 25, making it a highly valuable and economical decorative wood. In the ten years between 1997 and 2007, there was even a tenfold increase in price, from 20,000 to 20,000 Rupees. Currently, most of this species' natural range in eastern Indonesia is thought to be gone (Ratnaningrum *et al.*, 2015). Cendana has been used in Indonesian traditional medicine to treat a variety of conditions, including inflammation, gonorrhoea, stomach irritation, urinary tract infections, skin conditions, and chest pain. Numerous studies have been conducted worldwide on the pharmacological properties of Sandal wood, root, and essential oil. Their anti-ulcer, antibacterial, antifungal, antiviral, antioxidant, antipyretic, anti-inflammatory, anticancer, anti-hyperglycaemic, and anti-hyperlipidemic activities have been documented. Additionally, they have been shown to have metabolic, genitourinary, central nervous system, genotoxic, cardioprotective, insecticidal, and aromatherapy effects (Puspawati *et al.*, 2019). The Hindu and Buddhist faiths revere Indian sandalwood. Every part of human existence involves the usage of sandalwood, particularly in Indian culture and civilizations where it is necessary from birth to death. It is utilised in India to make attars that date back many centuries. Sandalwood oil and floral oils, like kewda, jasmine, and rose petals, are combined to create attar (Sandeep *et al.*, 2019). Berries that have

been air-dried and contain betulic acid as well as other phytoconstituents such as oleic, linoleic, palmitic, and santalbic acids. The berries are said to contain high levels of proanthocyanidins, ellagitannins, gallotannins, stilbenoids, and flavonoids. According to recent findings, berries provide significant nutritional and nutraceutical potential in addition to being a rich source of Cyanidin-3-glucoside (Umdale, Suraj, *et al.,* 2020). Little fragments of airborne sandal essential oil cause a reaction in the olfactory epithelium, or smell receptors, located in the roof of the nose, when someone senses the aroma. The limbic system of the brain, which controls motor functions, basic drives, emotions, and memories, is only a single nerve synapse away from here. The hypothalamus receives the impulses and uses them to control various body processes, including blood sugar regulation, growth, temperature regulation, appetite, thirst, and sexual desire. The pituitary is finally triggered, which triggers the endocrine system, which regulates all metabolic processes, emotional and sexual behaviour, digestion, and stress reactions (Goswami *et al.,* 2018). There is a long history of using sandalwood oil to treat skin issues. It nourishes all types of skin and is a fantastic moisturiser. Wounds, scars, and acne can all be effectively healed with sandalwood's astringent, anti-inflammatory, antibacterial, and pain-relieving qualities (Kumar *et al*., 2019). There is a lot of market demand for carved representations of gods and other mythological characters. Sandalwood is used to create a vast range of items, including picture frames, combs, jewel cases, boxes, hand fans, card cases, bookmarks, and letter openers. The core of the tree is called the heartwood, and it is prized for its aroma. However, the tree's other portions, including the bark and sapwood, are odourless. The plant has mostly been used for its sandalwood oil, which is produced by steam distilling the heartwood of the plant (Kumar *et al.,* 2015). A vast range of wild, underutilised fruits and their products are now in high demand on the international market as significant sources of essential phytoconstituents. A number of academics recently published their findings from the phytochemical examination of a number of underutilised wild fruits. Thus, we assessed the ripened sandalwood berries' nutritional potential, antioxidant efficiency, and wide range of phytoconstituents in the current study (Umdale *et al*., 2020)

1.3. Phytoconstituents of *Santalum album*

Santalum album L. rhizome essential oil, according to phytochemical studies includes santalol,santalene, santalal,curcumene, tricycloekasantal, and more. Afew pharmacological studies have shown that sandal wood oil has antiviralproperties, that α and βsantalols are neuroleptics, and that they are effectivein prev enting skin cancer in murine models of skin carcinogenesis. (Gu*etal*.,2014). For many years, Indian sandalwood has supplied 80–90% of the demand for sandal oil in the global market, with *Santalum album* having the greatest oil concentration in the genus Santalum (about 6%) (Sutheesh *et al*., 2016).

1.4. Producing regions

Native to the mountains of southern India, particularly Coorg, Chennai, and Mysore, is the plant known as *Santalum album*. At elevations of 2000–3000 feet it usually happens (Sindhu et al., 2010). But the sandalwood that was naturally found in Australia, S. spicatum, was of lower grade than Indian sandalwood (< 90%), with a low santalol concentration (George *et al.*, 2020). As part of the Hindustan Centre of Origin of Crops and Plant Diversity, India is one of the twelve centres where cultivated plants originated. Because of its diverse physiographic and climatic conditions, India has a very rich biological diversity. India is home to roughly 4-5 percent of all known plant species worldwide. All the world's

major ecosystems are included in the remarkable diversity of biodiversity found in India, which spans 25 different biotic provinces and 10 biogeography zones (Tewari *et al.*, 2014).

Fig1.1: The *Santalum album* tree

1.5. Botanical description

Evergreen in nature, sandalwood trees are indigenous to India and Indonesia and reach heights of 8 to 12 metres. Its girth increases by2.5cm. Leaves range in size from 3.8 to 6.3 by 1.6 to 3.2 cm. Leaves have an elliptic form. Compared to leaves, flowers are thinner and shorter. There is smooth bark. The little flowers have several short stalks. The bark is a grey-brown colour (Aftab *et al.*, 2023)

1.6. Utilised parts

Leaves, seeds, roots, stem, and bark.

1.7. Phytoconstituents active

Heartwood oil contains highly sought-after ingredients in the perfume industry, such as Santalic acid, Santalal, α and β santalol, and α and β santalene (Umdale *et al.*, 2020).Commercially available sandalwood oil contains sesquiterpene alcohols such as α- and β-santalols ($C_{15}H_{24}O$), bergamotols, and several of their stereoisomers as major constituents, while lanceol, nuciferol, bisabolol, and sesquiterpene hydrocarbons such as α-and β-santalenes ($C_{15}H_{24}$), bergamotenes, α-, β-, and γ-curcumenes, β-bisabolene, and phenylpropanoids are among the secondary constituents. A-santalol is more prevalent than β-santalol. Major components of essential oils, according to Verghese and colleagues, include sesquiterpene alcohols, cis-α and cis-β-santalol, α-transbergamotol, and epi-cis-β-santalol. Heterocyclic, hydrocarbon santene (C_9H_{14}), -santalene, β-santalene, -bergamotene, epi-β-santalene, -curcumene, β-curcumene, γ-curcumene, -bisabolene, and -bisabolol are among the minor ingredients. Other components found in the sandalwood oil include alcohol, santenol ($C_9H_{16}O$) and teresantalol ($C_{10}H_{16}O$); aldehydes, isovaleraldehyde and nor-tricycloekasantalal ($C_{11}H_{16}O$); ketones, l-santenone ($C_9H_{14}O$) and santalone ($C_{11}H_{16}O$); and acids, teresantalic acid ($C_{10}H_{14}O$), which occurs partially free and partly in relation to other substances (Pullaiah *et al.*, 2021). Following their extraction with petroleum ether, air-dried berries yield a viscous, greyish brown lipid that contains betulic acid, glucose, fructose, sucrose, and other phytoconstituents such as palmitic acid, oleic acid, linoleic acid,

and santalbic acid. Due to the berries' high nutrient content, many insect larvae attack ripened fruits, emptying their fleshy section (Harsha *et al.*, 2013)

Fig1.2: *Santalum album Linn* plant

1.8. Medicine uses and performance aspects

Mainly used as a cooling, sandalwood also has sedative and astringent properties that make it effective as a diuretic, expectorant, stimulant, and disinfectant in the genitourinary and bronchial tracts. Sandalwood oil is important in the perfume industry because of its strong, sweet scent. Additionally, the same is utilised as a blood purifier, memory enhancer, anti-poison, stomach and liver tonic, and heart tonic. The Ayurvedic system of medicine mentions using sandalwood for treating several additional conditions, including diarrhoea with bleeding internal haemorrhage bleeding piles, vomiting, poisoning, hiccoughs, the first stage of the pox, urticaria, eye infections, and inflammation of the umbilicus (Sindhu *et al.*, 2010). The oil derived from cendana finds extensive applications in the fragrance industry, cosmetics, culinary flavouring, and aromatherapy. Cendana has been used in traditional Indonesian medicine (Puspawati *et al*., 2019).

2. REVIEW OF LITERATURE

2.1 NAFLD

Non-alcoholic fatty liver disease (NAFLD) is a multifaceted condition that results from the interplay of dietary habits, lifestyle choices, genetic predispositions, and interactions among various organs and the gut microbiome. The development of NAFLD can be understood as a disruption in the balance between lipid accumulation and lipid removal. Fatty acids (FA) in the liver can originate from dietary sources (either from fats transported by chylomicrons or sugars converted into fats through de novo lipogenesis) or from the circulating non-esterified fatty acids (NEFA) pool. Normally, fatty acids are either oxidized to generate energy or converted into triglycerides (TAG) for export via very-low-density lipoprotein (VLDL) (Moore *et al.*, 2019).

2.2 ROS Production and Oxidative Stress in the Development of NAFLD

The liver acts as a primary centre for managing nutrients, balancing blood glucose and lipid levels between meals. Its triglyceride content changes with metabolic conditions. During fasting, fatty acids from adipose tissue are metabolized in liver mitochondria for energy production. After eating, when there is an excess of fatty acids and chylomicrons in the blood, the liver stores these lipids as lipid droplets for later use. Imbalances in this system, such as excessive fatty acid uptake, impaired fatty acid oxidation, and reduced lipid export, can disrupt liver lipid metabolism. Moreover, reactive oxygen species (ROS) are involved in these metabolic disruptions, leading to oxidative stress and tissue damage, which are significant contributors to the development of non-alcoholic fatty liver disease (NAFLD) (Ting Hong *et al*., 2021).

2.3. Insulin resistance in NAFLD

NAFLD is closely linked with insulin resistance in both the liver and adipose tissue, as well as decreased overall insulin sensitivity. Research shows a 45-50% decrease in glucose disposal, reflecting reduced whole-body insulin sensitivity, and a diminished capacity of insulin to inhibit endogenous glucose production, which points to hepatic insulin resistance. Furthermore, individuals with NAFLD often demonstrate impaired insulin suppression of free fatty acids (FFA), indicating insulin resistance at the adipocyte level (Kristina *et al*., 2006)

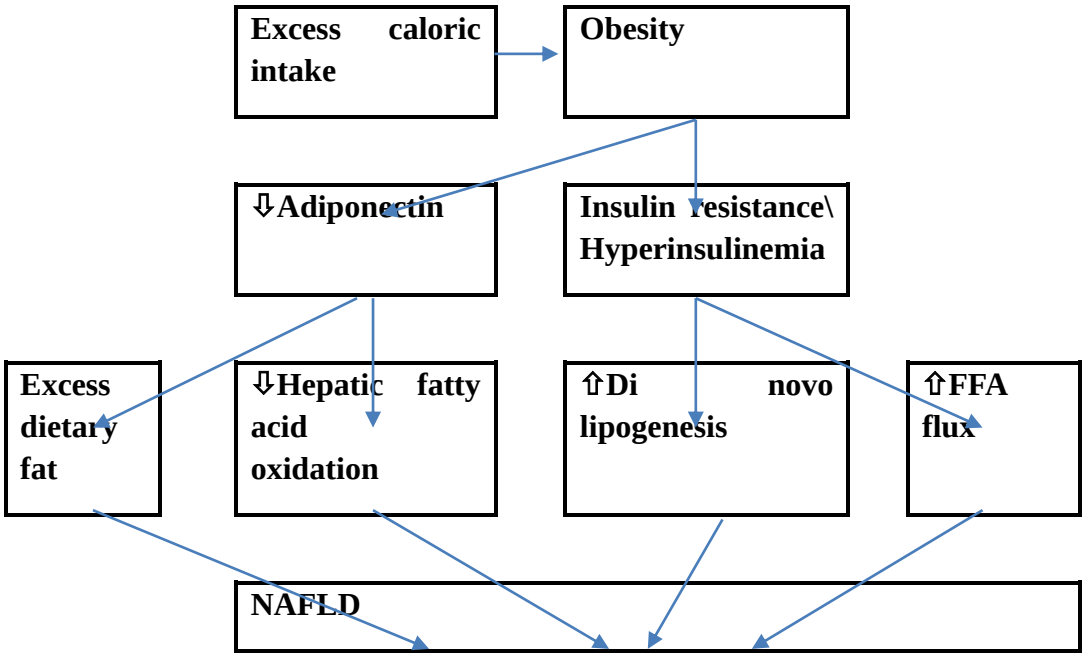

Fig. 2.1: The process of insulin resistance and fat accumulation in NAFLD (Kristina *et al.*, 2006).

2.4 Cholesterol and triglycerides homeostasis in NAFLD

Abnormal levels of lipoproteins in the plasma indicate imbalances in the major lipid components triglycerides, cholesterol, and cholesterol esters. An excessive build-up of triglycerides in the liver is a key characteristic of NAFLD. Factors contributing to hepatic steatosis include dietary fatty acids from

chylomicron remnants absorbed from the intestine, increased lipolysis of stored fat, and de novo lipogenesis. Research involving tracer studies in obese individuals with NASH has shown that approximately 60% of liver triglycerides come from free fatty acids, 25% from de novo lipogenesis, and 15% from dietary sources (Klementina *et al.*, 2011).

2.5 Plant Description

Santalum album is an evergreen tree that can grow up to 20 meters tall and attain a girth of up to 2.4 meters. The tree features slender, drooping branches. There are two primary commercial species: Indian Sandalwood and Australian Sandalwood. Australian Sandalwood trees are generally shorter in height. The bark of the tree varies in colour, including dark brown, reddish, dark grey, or nearly black, and is smooth on younger trees but becomes rough with deep vertical cracks as it ages. The sapwood is white and odourless, while the heartwood ranges from yellowish to dark brown and is notably fragrant. The leaves are thin, typically opposite, and either ovate or ovate-elliptical, measuring 3-8 cm in length and 3-5 cm in width. They are glossy green on the upper surface and glaucous, slightly paler on the underside. The leaf tips can be rounded or pointed, and the leaf stalk is grooved, ranging from 5-15 cm in length, with a noticeable reticulate venation. The flowers are small and may be purplish-brown, straw-coloured, reddish, green, or violet, about 4-6 mm long. They appear in clusters of up to 6 terminal or axillary panicles, which are unscented and arranged in paniculate cymes. The fruit is a globose, fleshy drupe that turns red, purple, or black when ripe, approximately 1 cm in diameter. It has a hard, ribbed endocarp and is almost stalkless, smooth, and contains a single seed. In India, flowering occurs from March to April, with fruit ripening in the cold season. In Australia, flowering happens from December to January and from June to August, with fruit maturing between June and September. The species spreads rapidly through seed dispersal, with viable seed production starting around the age of 5 years. Trees older than 30 years can have a circumference ranging from 18 to 38 inches (Kumar *et al.*, 2015).

Kingdom	Phylum
Class	Magnoliopsida
Plantae	Tracheophyte
Order	Santalales
Family	Santalaceous

Table 2.1: Taxonomy of *Santalum album* (Arun *et al.*, 2015)

2.6 Phytochemical constituents

The bark extract of *Santalum album* primarily consists of santalol (90%), along with other compounds such as exo-nor-bi-cycloekasantalal, β-santalic acid, teresantalic acid, nortricycloekasantalic acid, bicycloekasantalic acid, dihydro-β-santalic acid, urs-12-en-3β-palmitate, β-sitosterol, (+) epi-β-santalol, (-) β-santalol, (-) trans-β-santalol, and α-santalol (52%). Other components include β-santalol (23%), epi-β-santalene, cis-lanceol, cis-nuciferol, β- and epi-β-teresantalic acids, β- and epi-β-nor-ekasantalic acids, α-santalic acid, 11-keto-dihydro-α-santalic acid, and bias-bolenols A, B, C, D, and E. Additionally, the extract contains tri-cyclo-ekasantalol, α- and β-santalenes, trans-α-bergamottin, α-curcumin, and nuciferol. Other notable constituents of the bark extract include L-allo-hydroxyproline, botulinic acid

(0.05%), β-sitosterol, and various fatty acids. Although it has a small amount of trans-β-santalol, the extract is also rich in cis-lanceol, α-santalene, β-santalene, α-bergamottin, epi-β-santalene, α-cur cumene, β-cur cumene, γ-cur cumene, β-bisabolene, and α-bisabolene. The major components of sandalwood oil are cis-α-santalol (53%), cis-β-santalol (23%), and α-trans-bergamot oil (Sharifi *et al.*, 2023).

2.7 Traditional uses of *Santalum album* Linn.

Santalum album is mainly grown for its timber and fragrant oil. The timber weighing 870kg/cubic m. is durable and strong. Its close-grained heartwood is used for ornamental and carving work. The wood has been used as a fuel but is generally considered too valuable for this purpose. Sandalwood oil distilled from the heartwood is a pale yellow to yellow viscous liquid, with sweet, fragrant, persistent, spicy, warm, woody, animatic, milky and nutty notes. It is extensively and inflammation of umbilicus. Sandalwood oil, an active substance of agreeable odour employed in the treatment of sub-acute and chronic infections of mucous Tissues, particularly gonorrhoea after the active symptoms have been mitigated. Chronic bronchitis with fetid expectoration, chronic mucous diarrhoea, chronic inflammation of the bladder and pyelitic are also said to be benefited by it's used in perfumery, cosmetics, aromatherapy and pharmaceutical industry. Being good fixatives, it is highly valued in perfumery and toiletry industry, especially for certain delicate scents that are extremely rare and fragile. No composition of the heavy or oriental type of perfume is complete without an ample dose of sandalwood oil. Most Indian attars use Sandal oil as the base because of its inherent capacity to absorb most of the ethereal notes of other whole herbs or flowers, as it can enhance their perfumery status and stability. Sandalwood oil is used as a flavouring agent in a variety of food products, including frozen dairy desserts, candy, paan masala, baked goods, gelatine, puddings, and both alcoholic and non-alcoholic beverages. Regulatory bodies such as the US Food and Drug Administration, the Flavour and Extract Manufacturers Association, the council of Europe, and the Joint FAO/WHO Expert committee have approved sandalwood oil as a food additive. In traditional medicine, particularly among tribal healers, sandalwood, root, bark, and leaves are used to treat liver diseases like jaundice. It is also recognized for its benefits in alleviating gastric irritability, dysentery, tension, and confusion. Additionally, it serves as a tonic for the heart, stomach, liver, as an anti-poison, and for fever and memory enhancement. Ayurvedic medicine also highlights various uses of sandalwood, including treatments for diarrhoea with bleeding, internal haemorrhage, bleeding piles, vomiting, poisoning, hiccups, the early stages of pox, urticaria, eye infections, and inflammation of the umbilicus. Sandalwood oil is employed in the management of sub-acute and chronic infections of mucous tissues, particularly after the resolution of active symptoms of gonorrhoea. It is also used to benefit conditions like chronic bronchitis with foul expectoration, chronic mucous diarrhoea, chronic bladder inflammation, and pyelitic. (Kumar *et al*., 2015)

2.7.1 Hepatoprotective Activity

The hydro-alcoholic extract of *Santalum album* leaves demonstrate notable hepatoprotective effects. It counters liver damage induced by CCl_4 and paracetamol by reducing serum marker enzyme levels, bilirubin, and lipid peroxidation. Simultaneously, it significantly increases glutathione, superoxide dismutase, catalase, and protein levels in a dose-dependent manner. These results are supported by reductions in liver weight and positive histopathological findings.

2.7.2 Central nervous system Activity

Santalum album is noted for its potential to enhance memory. Research has shown that inhaling East Indian sandalwood oil reduces mouse motility by 40-78% compared to controls. The sedative properties of sandalwood oil and its aqueous extract have been established, with the oil having a calming effect on nerves, aiding in conditions like headaches, insomnia, and nervous tension. Santalols, the bioactive compounds in sandalwood, exhibit central nervous system depressant effects and may be beneficial for sleep disorders. Additionally, heartwood solvent extracts have shown neuroleptic properties in mice. Alpha- and β-santalol significantly increase levels of homo-vanillic acid, 3,4- dihydroxyphenylacetic acid, or 5-hydroxyindoleacetic acid in the mouse brain. Alpha-santalol acts as a potent antagonist of dopamine and serotonin 5-HT2A receptors, displaying antipsychotic effects like chlorpromazine. It induces significant physiological changes, including relaxation and sedation. Sandalwood oil, when absorbed trans dermally, leads to physiological deactivation but behavioural activation. Recent TLC254 bio-autographic assays have shown that alpha-santalol is a strong inhibitor of tyrosinase and cholinesterase, suggesting potential uses in treating Alzheimer's disease and in skincare applications.

2.7.3 Anti-ulcer activity

Oral administration of a hydroalcoholic extract from the stems of *Santalum album* has demonstrated significant gastric protective effects in rats. This extract effectively inhibits gastric ulceration induced by physical stress, chemical irritants, and NSAIDs.

2.7.4 Anti-bacterial activity

Numerous studies have investigated the antimicrobial properties of sandalwood oil, both East Indian and Australian varieties. A comparative study of 26 essential oils found that sandalwood oil, along with its synthetic analogues, exhibited the strongest antibacterial activities. Sandalwood oil is particularly effective against methicillin-resistant Staphylococcus aureus and antifungal-resistant Candida species. Both crude extracts and individual compounds such as α- and β-santalol from sandalwood oil show antibacterial activity against helicobacter pylori, a Gram-negative bacterium associated with ulcers in the duodenum, stomach, and other gastrointestinal areas. Additionally, sandalwood oil displays antiviral activity against Herpes simplex virus Type-1, while β-santalol has been shown to inhibit the H3N2 influenza virus. The oil also demonstrates significant inhibitory effects against bacillus mycoides and escherichia coli. Moreover, sandalwood oil has anti-dermatophyte properties against micros Porum canis, trichophyton rubru, and trichophyton mentagrophytes.

2.7.5 Anti-fungal Activity

Sandalwood oil has demonstrated antifungal properties against *microsporum canis, trichophyton mentagrophytes,* and *trichophyton rubrum.* However, it has shown limited effectiveness against *candida albicans, aspergillus niger,* and *aspergillus fumigatus*.

2.7.6 Anti-viral Activity

Sandalwood oil has demonstrated antiviral properties in various studies. It has been traditionally used in Ayurvedic and Chinese medicine to prevent and treat viral skin conditions such as warts and blemishes. Laboratory research has shown that sandalwood oil can inhibit the replication of Herpes simplex viruses (HSV-1 and HSV-2) in a dose-dependent manner. The oil is thought to protect cells by modulating liver

glutathione levels, S-transferase activity, and acid-soluble sulfhydryl compounds. Additionally, sandalwood oil has been found to inhibit HSV-2 in RC-37 cells, primarily by interfering with the virus ability to interact with host cells before entry. Sandalwood oil has been shown to have antiviral effects in multiple studies. Traditionally, it is used in Ayurvedic and Chinese medicine to address viral skin issues such as warts and blemishes. Research indicates that sandalwood oil can inhibit the replication of herpes simplex viruses (HSV-1 and HSV-2) in a dose-dependent manner. It is believed to offer cellular protection by influencing liver glutathione levels, S-transferase activity, and acid-soluble sulfhydryl compounds. Additionally, sandalwood oil has been observed to inhibit HSV-2 in RC-37 cells by disrupting the virus's ability to bind with host cells before it can enter them. The constituents of sandalwood oil, including α- and β-santalol and their derivatives, have been explored for their potential in treating various conditions. These include warts caused by HPV and DNA pox viruses, such as molluscum contagiosum, and are also being investigated for their possible effects against HIV and other RNA viruses. Additionally, sandalwood oil is considered beneficial for addressing skin issues such as dryness and flakiness associated with seborrheic dermatitis, psoriasis, and eczema. It is also used in treating acne, including pustular acne caused by staphylococcal and streptococcal infections. Moreover, sandalwood oil and its derivatives are employed in managing cold sores and herpes. Recently, research has shown that single-cell and somatic embryo suspension cultures of the Indian sandalwood tree could serve as a sustainable source of shikimic acid, a key precursor for the industrial production of Tamiflu, an antiviral drug used against Influenza A virus.

2.7.7 Antioxidant Activity

Phytochemical and pharmacological research has confirmed that *Santalum album* contains antioxidant compounds that support its traditional medicinal uses. In vitro studies have assessed the impact of *Santalum album* and other Indian medicinal plants on nitric oxide (NO) levels, using sodium nitroprusside as a NO donor. Many plant extracts, including those from S. album, showed significant, dose-dependent scavenging of NO. The plant is also known for its ability to scavenge nitrous oxide and exhibit DPPH antioxidant activity. *Santalum album* has been shown to protect cardiac tissue from oxidative stress-related damage and lipid peroxidation, and it modulates inflammatory and apoptotic responses in cardiac tissue induced by DOX (doxorubicin). Additionally, the anthocyanin pigment cyanidin-3-glucoside, derived from S. album, has been identified as an important antioxidant with nutritional value. In comparative studies, callus cells grown in vitro from *Santalum album* exhibited antioxidant activities like sandalwood oil across nine different antioxidant assays. Sandalwood oil was found to increase glutathione S-transferase (GST) activity and levels of acid-soluble sulfhydryl (SH) compounds in the livers of adult male swiss albino mice. These changes suggest a potential chemo preventive effect against cancer through blocking mechanisms. Furthermore, methanolic extracts of sandalwood demonstrated acetylcholinesterase inhibitory activity, along with DPPH and superoxide radical scavenging in albino mice, indicating its potential benefits in addressing dementia and memory loss related to Alzheimer's disease. Recent in vivo studies have also highlighted the anti-hyperglycaemic and antioxidant effects of sandalwood oil and its major constituent, α-santalol, in diabetic male swiss albino mice models induced by alloxan and D-galactose.

2.7.8 Anti-inflammatory activity

Santalols have demonstrated notable anti-inflammatory properties in various experimental models. Santalum album has shown effectiveness in reducing inflammation and ulcers, as evidenced by its ability to inhibit carrageenan-induced paw edema, cotton pellet-induced granuloma, and pylorus ligation-induced ulcers. These results support the use of this plant in traditional medicine for managing inflammatory conditions such as ulcers. Additionally, the methanolic extracts of the heartwood have been found to exhibit both in vitro antioxidant and in vivo analgesic and anti-inflammatory effects in mice (Rohini *et al*., 2016).

3. AIM AND OBJECTIVES

3.1 Aim of study

Hepatoprotective effect of *Santalum album* Linn. seeds extract against high fat diet induced non-alcoholic fatty liver disease.

3.2 Objective and Rational of study:

The primary objectives of the present study can be classified as follows:

- To experimentally evaluate the hepatoprotective activity of *Santalum album* seeds extract in NAFLD on Wistar rats.
- To profine variable effects of drug extract against NAFLD.
- To identify the *Santalum album* is effectively work as hepatoprotective.

a) Physical parameters

- Body weight
- Water intake
- Food intake
- Body mass index
- Rectal temperature

b) Biochemical Parameters:

- Liver function test (LFTs)
- Serum Glutamate oxaloacetate transaminase (SGOT)
- Alkaline Phosphate (ALP)
- Serum Total Bilirubin
- Total lipid
- Lipid profile

c) Estimation of In-Vitro Antioxidants

- DPPH radical scavenging assay

4. HYPOTHESIS

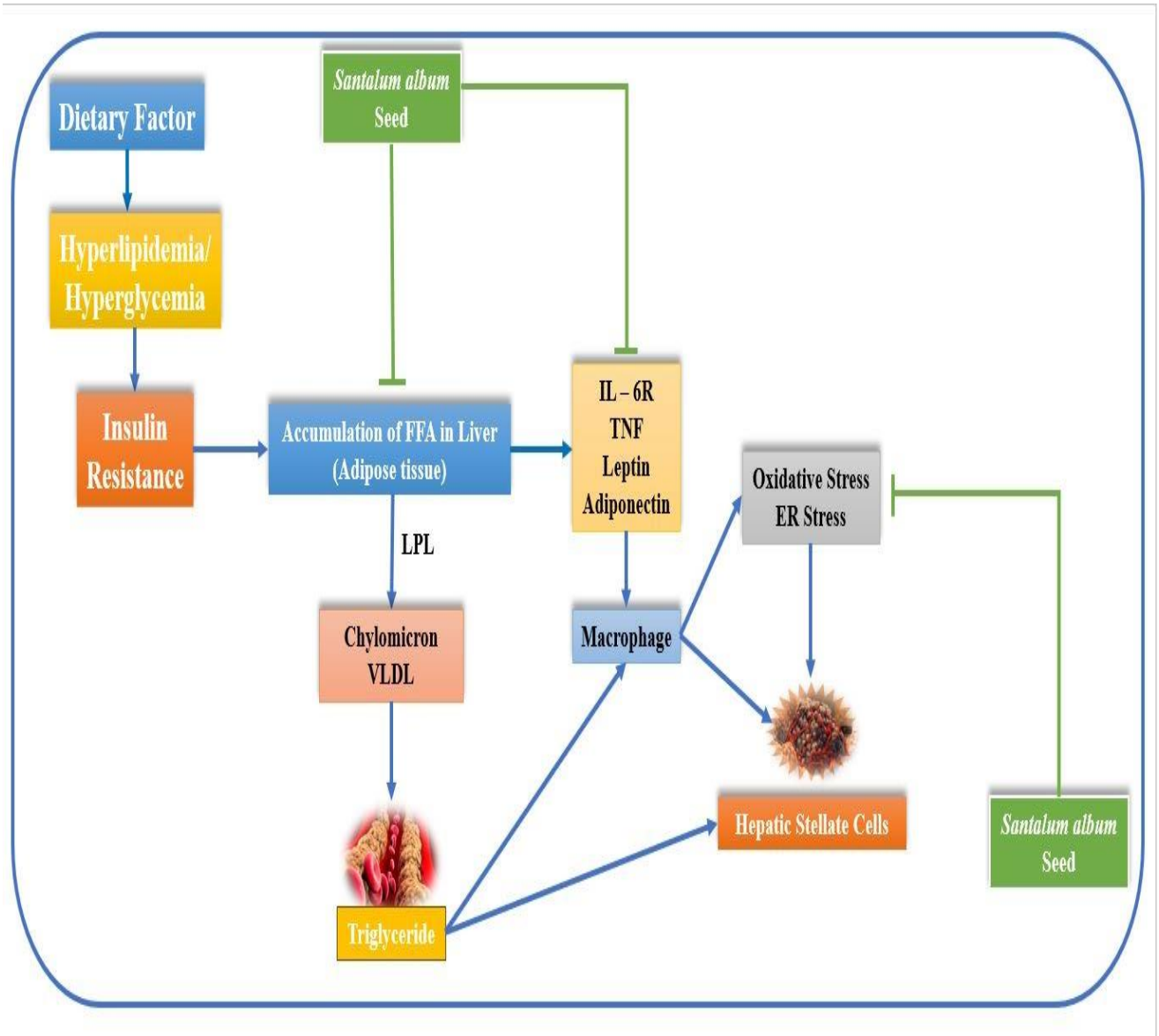

Fig.3.1: The possible mechanism involved by *Santalum album* Linn. against HFD induced NAFLD

5. MATERIALS AND METHODS

5.1 DRUGS AND CHEMICALS

The various chemicals, reagents and solvents used in present investigations are procured from well-known companies named S. D Fine Chemicals, Mumbai, India, E-Merck Ltd., Mumbai, India, Central Drug House Pvt. Ltd., Mumbai, India and Hi- media Laboratories Pvt. Ltd, Mumbai, India. High fat diet prepared for hepato-toxicity by using such ingredients that are shown in above table no.5.1. Diagnostic kits for the estimation of serum levels of various parameters were procured from Avecon Healthcare Pvt. Ltd. (Saha, Haryana). Diagnostic kits SGOT, SGPT, AST, ALT and ALP were procured from Avecon Healthcare Pvt. Ltd. (Saha, Haryana). It can be used to evaluate the serum total bilirubin, total cholesterol and lipid profile. Blood glucose is determined by standard glucometer. Glycated Hemoglobin

(Hb) was measured from whole blood by using ion exchange resin kit purchased from diagnostic laboratory. Insulin estimation using insulin Elisa kit. Total triglycerides, High density lipoprotein (HDL) and total cholesterol kit will be purchased from diagnostic lab, and very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) is calculated from total cholesterol and HDL cholesterol values.

S.no.	Chemicals	Manufacturer
1.	Di-methionine	Alp pure Life sciences Pvt.Ltd Sonipat, HR
2.	Sodium chloride	Vinipul Inorganics Pvt. Ltd. Mumbai, India
3.	Di-ethyl ether	Devlife Corporation Pvt. Ltd. Mumbai, India
4.	Casein	Nutra bay Retail Pvt. Ltd. New Delhi, India.

Table 5.1: Chemicals used for extraction\HFD preparation

The dried seeds of *Santalum album* were procured from RK seeds, Azadpur, Delhi-110033, India. The dried seeds were powdered by kitchen grinder. The healthy seeds were selected for authentication from pSRI Venkateshwara University Tirupati-517 502, A.P., India with Authentication no. 0990.

- **Preparation of Di-ethyl ether extract of *Santalum album* seeds**

The *Santalum album* plant seeds extract by using water bath system. 250 ml of di-ethyl ether was added to 50 grams seeds of *Santalum album* L. the mixture was kept on water bath at temperature 60ºC for 5 hrs. The extract was filtrated, dried then were dry extract stored in dark bottle at 4º C. The di-ethyl ether extract of *Santalum album* seeds material was subjected to preliminary phytochemical screening and treat non-alcoholic fatty liver disease in rats using different animal models.

- **Plant Authentication**

The seeds of *Santalum album* Linn. seeds were authenticated from Shri Venkateshwara University Tirupati-517 502, A.P, India with Authentication no.0990.

Sr. No.	Plant	Authentication No.
1.	*Santalum album* seeds	0990

Table.5.2: The *Santalum album* Linn. seeds authentication.

5.2 Phytochemical screening of extract

Phytochemical screening of *Santalum album* seeds extract showed the presence of various chemical constituents like anthraquinone, proteins, amino acids, alkaloids, flavonoids, carbohydrates, tannin, glycosides (Rohini *et al.*, 2016).

1. Test for Alkaloids.

- **Dragendroff's Reagent test:** The addition of 1ml of Dragendorff's reagent to 2mL of the extract

resulted in the formation of an orange-red precipitate, indicating the presence of alkaloids.

- **Mayer's Reagent test:** The addition of 1mL of Mayer's reagent to 2 mL of the extract resulted in the formation of white and pale-yellow precipitates, suggesting the presence of alkaloids.

2. Tests for Carbohydrates

- **Preparation of test solution:** The test extract was dissolved in water to create the test solution. After hydrolyzing it in 1 volume of 2N HCl, it was put through the subsequent chemical tests.
- **Molisch's test:** After adding a few drops of an alcohol-based a-naphthol solution to two to three milliliters of aqueous extract and shaking the mixture, add concentrated H_2SO_4 from the test tube's sides, and look for a violet ring where the two liquids meet.
- **Fehling's test:** 1ml Fehling's A and 1ml Fehling's B solutions were mixed and boiled for one minute. The test solution was added in the same volume. heated for five to ten minutes in a bath of boiling water watched for a precipitate that first turned yellow and then brick red**.**

3. Tests for Proteins

- **Million's test (for proteins):** In this test white precipitate was produced by mixing 3 milliliters of test sample with 5 milliliters of million's reagent. When a precipitate is heated,it gets brick red or dissolves and becomes red**.**

4. Test for Flavonoids

- **Lead acetate solution test:** A small amount of lead acetate solution (10%) added to the test solution produces yellow precipitates.
- **Ferric chloride test:** In this test add the sample solution in test tube with few drops of ferric chloride solution formed grass green color. (Misra *et al*., 2012).

6. Test for Tannins

- **Ferric test:** In this test add 0.2 g of drug extract was boiled and filtered. And add dropwise Ferric chloride solution in the test tube. Dark green colour formed in the test tube that indicate the presence of tannins.

7. Test for Glycosides

- **Fehling's test:** In the glycosides test add Fehling's solution (A: 1 mL, B: 2 ml) in little amounts to each extract that are present in the test tube (2 mL) and boiled with the help of boiling water bath. Each extract was dissolved by using hydrochloric acid and further neutralized with sodium hydroxide solution. The appearance of cherry red color precipitates that suggest the presence of glycosides.

8. Test for Anthraquinones

- **Brontranger's test:** Add 0.5 g of sample drug extract and add HCl (10%) then heat the solution for two to three minutes. After enough heating the heated solution was cooled and filtered and then add of chloroform in the sample. After filtration mixed the sample with ammonia solution (10%) and then heated again with the help of boiling water bath. The pink color is appearing that means the presence of anthraquinones (Dandge *et al*., 2016).

5.3 EXPERIMENTAL ANIMALS

Total 36 either male or female wistar rats weighing between 180 - 220g were used in this study obtained from Central Animal Facility National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab160062 (India) under controlled conditions of temperature (25±1°C) and humidity (50±5°C) with 12 h light/dark cycle) for one week before starting the experiments. The rats had free access to standard rodent food and water ad libitum. Rats will be acclimatized to environment for one week prior to commencement of the experiment. The research was carried out at the Rayat Institute of Pharmacy, Department of Pharmacology in Rail-majra, Distt, S.B.S. Nagar, Punjab. Experimental procedures were carried out in accordance with CPCSEA recommendations for the handling and use of laboratory animals. For conducting the studies, prior approval from the Institutional Animal Ethics Committee (IAEC) was acquired (CPCSEAApproval No. RIP/IAEC/2023-2024/02).

5.4 TOXICOLOGICAL EVALUATIONS

- **Acute oral toxicity study:**

By using OECD 423 to adhere the protocol (Acute Toxic Class Method). Each step of the step- by-step process involved three animals of the same sex. Average two to three stages may be required to allow judgement on the acute toxicity of the test drug, depending on the moral status of the animals. This method allows for an appropriate data-based scientific conclusion while using fewer animals overall. To classify and rank substances according to the Globally Harmonized System (GHS) for compounds that induce acute toxicity, the approach requires defined doses.

- **Experimental Procedure:**

Acute oral toxicity studies were performed according to OECD. Albino wistar rats (n = 6/each dose) selected by random sampling technique were used in this study. The animals were fasted for 12 hours with free access to water only. Following the period of fasting, animals were weighed and test extract was administrated orally at a dose of 100, 200, and 400 mg/kg. After the administration of test extract, food for animals were withheld for 2 hours. The mortality and clinical signs which included changes in skin, fur, eyes and mucous membranes were noted for the first 4 hours subsequently for 72 hours and after for 7 days of test drug administration. For complete 7 days, the gross behaviours like body positions, locomotion, rearing, tremors or gait were observed and the effect of plant extract on grip strength, pain response and righting reflex were noted. In addition, the intake of food or water behaviour was monitored (Subha *et al.*, 2017)

- **DOSE SELECTION**

Based on a literature review, the dosage of ppc (polyene phosphatidylcholine) (Cui, B *et al.*, 2011). As a hepatoprotective agent, rats were given at a dose of 88 mg/kg p.o. as hepato- protective drug. The dose of *Santalum album* seeds extract was chosen based on the guide lines of our laboratory acute toxicity test (OECD 423). Di-ethyl ether extract of *Santalum album* Linn. doses of (200 and 400mg/kg/p.o.) between were chosen.

- **Induction of Hepatotoxicity**:

Animal were fasted for 24 hours with free access to water, then high fat diet in a dose of 100mg/kg/p.o. by b.w. was given orally at 8th day for the induction of hepatotoxicity in wistar rats of either sex.

5.5 EXPERIMENTAL DESIGN

Total 36 either male or female wistar rats weighing between 180- 220g were used in this study. Animals will be allocated into six group (n=6).

Group-I (Normal control) will receive normal water 1ml/kg/day p.o. for 8 days.

Group-II (Drug perse) will receive normal diet+ *Santalum album Linn* seeds extract 200mg/kg/day p.o. for 8 days.

Group-III (Disease Group) will receive normal water for 8 days followed by HFD 100mg/kg p.o at the 8th day.

Group-IV (HFD+ *Santalum album* seeds extract 200mg\kg) will receive HFD 100 mg/kg/day orally for 8 daysfollowed by *Santalum album Linn* seeds extract 200mg/kg p.o. at the 8th day.

Group-V (HFD + *Santalum album seeds* extract 400mg\kg) will receive HFD 100 mg/kg/day p.o. followed by Santalum album Linn seeds Extract 400mg/kg p.o. at the 8th day.

Group-VI (Standard control HFD + Polyene Phosphatidylcholine) will receive HFD followed by polyene phosphatidylcholine 88mg/kg/day p.o. followed by the 8th day. Animals will be fasted for 24 h but have free access to water on the last day of the experiment.

Group (n=6)	Treatment (Dose and route of administration)
Group-I (Normal control)	Normal saline
Group-II (Drug Perse)	*Santalum album* Linn. seeds extract (200 mg/kg/day/p.o.)for 8 consecutive days**.**
Group-III (Disease Group)	High fat diet 100mg/kg/p.o. on the 8th day.
Group-IV (HFD + *Santalum album* seeds extract	HFD 100 mg/kg/day orally for 8 days followed by*Santalum album* Linn. seeds extract 200mg/kg p.o. at the 8th day.
Group-V (HFD + *Santalum album* seeds extract 400mg\kg)	HFD 100 mg/kg/day p.o. followed by *Santalum album* Linn. seeds extract 400mg/kg p.o. at the 8th day
Group-VI (Standard control HFD + Polyene Phosphatidylcholine	HFD followed by polyene-phosphatidyl choline 88mg/kg/day p.o. followed by the 8th day

Table 5.3: Dose selection and animal grouping in models of hepatotoxicity

Then, they will receive 100 mg/kg HFD for hepato-toxicity. Rats will be sacrificed after 6 hours of NSAID administration by ketamine-xylazine anaesthesia (50mg/kg-5mg/kg i.p) followed by cervical dislocation, and the Liver will be removed. They will be then washed with ice cold saline and examined macroscopically for mucosal lesions.

5.6 Total Antioxidant Activity

Santalum album seeds antioxidant activity, particularly its capacity to scavenge free radicals as determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) experiment, has been thoroughly investigated. This assay is frequently used to assess a natural component or extract's capacity to scavenge radicals. *Santalum album* seeds has a considerable antioxidant potential, as demonstrated by research, especially when it comes to scavenging DPPH radicals. By contributing hydrogen atoms or electrons to the stable DPPH radical, a material can neutralize itspurple colour and transform it into a colourless molecule. This is measured by the DPPH assay. The tested substance's antioxidant activity is correlated with the degree of discolouration. Compounds' ability to scavenge free radicals is determined using the DPPH test. The developed compounds' capacity to scavenge free radicals was determined using the DPPH (2,2-diphenyl-1- picrylhydrazyl) test (Shiva tare *et al.*, 2020). To create the DPPH control solution, 20 mg of DPPH were dissolved in 100 ml of methanol, which was subsequently diluted to achieve an absorbance of 0.70 (at 515 nm). This stock solution was then placed in a dark area for a whole day to produce free radicals. Five milligrams of each isolated component were dissolved in five millilitres of methanol to create sample solutions. Working standards: A dilution formula was used to prepare 62.5, 125, 250, 500, and1000 µg/ml. Each of them had about 2 ml that were carefully combined with 2 ml DPPH solutions and left in the dark for 15 min.

5.7 EVALUATION PARAMETER

- **Physical Parameter**

- **Measurement of Body weight:**

A weighing balance was used to measure daily weight changes starting the day before the first test was administered and continuing through the duration of the entire study period. The body weight gains from the day of initial test substance administration to 24 hours after High fat diet administration were computed as follows to minimize the individual difference between body weight gains (gm) over the course of 8 days = Body weight at 24 hours after High fat diet administration - Body weight on the day of initial test substance administration.

- **Measurement of Food intake:**

A weighing balance was used to measure daily food intake changes starting the day before the first test was administered and continuing through the duration of the entire study period. The food intake from the day of initial test substance administration to 7^{th} day followed by high fat diet administration after fasting of 24 hours were computed as follows to minimize the individual difference: Food intake (gm) over the course of 8 days = Food intake before 24 hours of fasting and HFD administration - Food intake on the day of initial test substance administration.

5.8 Histological Estimation

- **Liver weight**

In experiment, body weight of all experimental rats was recorded weekly. At the end of the experiment, body weight or liver weight of all rats from control and treated groups were measured or recorded. Relative liver weight was calculated following equation: Relative liver weight=Absolute liver weight (g) Body weight of rat on sacrifice day (g) ×100 (Maryam *et al.*, 2016).

5.9 Estimation of Serum Parameters

SGPT (ALT) catalysis the amino group transfer from L-alanine to alpha-ketoglutarate, producing pyruvate and L-glutamate. Pyruvate and NADH are then converted to lactate and NAD by lactate dehydrogenase. When NADH is converted to NAD, its absorption at 340 nm decreases. The rate of absorbance loss is evaluated and correlated to SGPT activity. (Reitman and Frankel method, 1957).

L- alanine + alpha – ketoglutarate ----------L-Glutamate + pyruvate

LDH

Pyruvate + NADH+ H+ ---------- Lactate + + NAD+

Alanine transaminase (ALT) is a protein-processing enzyme. (An enzyme is a protein that helps to accelerate chemical reactions. The body's cells contain several enzymes, and the liver cells create a substantial amount of ALT. ALT levels in the blood typically rise when the liver is harmed or irritated. The typical ALT range is 7-57IU/L. (EUSTICE, 2001). SGPT (ALT) activity in U/L blank0) = (Abs of test – Abs of control / Abs. of calibrator -Abs of blank) × conc. Calibrator (1070IU/L).

5.10 Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT)

When SGOT (AST) catalysis the transfer of an amino group from L-aspartate to α-ketoglutarate, L-glutamate, oxaloacetate, and NADH are transformed into malate and NAD by the enzyme malate dehydrogenase. SGOT activity is linked to the rate of NADH to NAD conversion (Reitman and Frankel, 1957; Henry, 1974).

GOT (AST)

L- Aspartate + alpha – ketoglutarate ---------- Oxaloacetate + L- glutamate

MDH

Oxaloacetate + NADH + H+ ---------- L- malate + NAD +

Another enzyme, known as aspartate aminotransferase (AST), is typically present inside of the liver cell. High level of this enzyme in the blood are typically indicative of liver damage when detected by blood test. however, the heart or skeleton muscles is injured, AST can also be released because of this, ALT is frequently thought to be more directly associated with liver issue decreases. The rate of absorbance loss is evaluated and correlated to SGPT activity. (Reitman and Frankel method, 1957).

GPT (ALT)

L- alanine + alpha – ketoglutarate ----------L-Glutamate + pyruvate

LDH

Pyruvate + NADH+ H+ ---------- Lactate + + NAD+

Alanine transaminase (ALT) is a protein-processing enzyme. (An enzyme is a protein that helps to accelerate chemical reactions. The body's cells contain several enzymes, and the liver cells create a substantial amount of ALT. ALT levels in the blood typically rise when the liver is harmed or irritated. The typical ALT range is 7-57IU/L. (EUSTICE, 2001). SGPT (ALT) Activity in U/L blank0) = (Abs of test – Abs of control / Abs. of calibrator -Abs of blank) × conc. Calibrator (1070IU/L) AST typically ranges between 5 and 47 UI/L (Akah *et al.*, 2009).

5.11 SGOT (ALT) activity in UI/L = (Abs. Estimation of Serum Total Bilirubin-Abs sample blank) C Standard \ Abs standard

Total bilirubin was quantified using a standard diagnostic kit (Avecon Healthcare Pvt. Ltd.) and the Jendrassik and Grof's method (1938).

Principle: The colour of the azo molecule, measured at 546 nm (530-560), is proportional to the blood bilirubin content and is produced when bilirubin and diazotized sulphate acid combine. The measuring unit for total bilirubin concentration in serum is milligrams per deciliter of control/Abs. of calibrator-Abs. of blank) X conc. of calibrator (160UI/L).

5.12 ESTIMATION OF TISSUE PARAMETERS

5.12.1 Estimation of Tissue Reduced Glutathione (GSH)-

The Butlers method was used for the quantitative determination of reduced glutathione (GSH) (Anderson *et al.,* 1985).

Preparation of Reagents

To prepare trichloroacetic acid with a 10% concentration, 10 g of the acid was dissolved in100 ml of distilled water.Disodium hydrogen phosphate was prepared by dissolving 4.26 grams of anhydrous disodium hydrogen phosphate in 100 milliliter's of distilled water.To prepare 5,5-dithiobis (2-nitrobenzoic acid) in 1 percent sodium citrate, 7.92 mg of thecompound were dissolved in 20 ml of the solution containing 1 percent w/v sodium citrate. Making 100 ml of reduced glutathione was accomplished by dissolving 6.14 mg of reducedglutathione in 200 ml of distilled water.

Procedure

The liver homogenate supernatant was diluted 1:1 with trichloroacetic acid (10% w/v). For 10 minutes, the tubes were centrifuged at 4 degrees Celsius and 10,000 revolutions per minute. 0.5 ml of the resulting supernatant was combined with 2ml of disodium hydrogen phosphate (0.3 M).The absorbance of 0.25 ml of newly prepared DTNB [5,5-dithiobis (2-nitrobenzoic acid) diluted in 1 percent w/v sodium citrate] was measured spectrophotometrically at 412 nm using a Shimadzu UV-1700, UV-VIS double-beam spectrophotometer. The results were given as micromoles of reduced glutathione per mg of protein, and a standard curve with 10-100 of the reduced form of glutathione was created.

5.12.2 HISTOPATHOLOGICAL STUDIES

The liver of animals was rapidly isolated. The liver was preserved in 10% buffer formaldehyde atroom temperature for 24 hours. The specimens were cleaned in tap water and dehydrated in rising ethanol concentrations; sections of these tissues were soaked in xylene and then embeddedin paraffin. Cut the tissue into fine sections (6 um thickness) and put them on a glass slide. The section was deparaffinized before being stained for 15 minutes with counter satin containing 0.6% w/v hematoxylins. Cosine (1% w/v) was counter-stained for 2 minutes before being studied under light microscopy.

5.12.3 STATISTICAL ANALYSIS

All values were expressed as mean ± S.D. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Turkey's multiple comparison Post hoc test. Graph Pad Prism version 8.0.1 software was used to statistically analyse the data. Value of $p<0.005$ was statistically significant.

6. RESULTS

6.1 PHYTOCHEMICAL SCREENING

The phytochemical screening of di-ethyl ether extract of *Santalum album* seeds extract revealed the presence of alkaloids, tannins, flavonoids, glycosides, steroids, phenolic compounds (Table 6.1). Alkaloids, tannins, flavonoids, glycosides, steroids, phenolic compounds have many pharmacological activities including anti-inflammatory and antioxidant activity, anti-cancer effects and anti-bacterial properties.

S.no.	Constituents	Present (+)/ Absent (-)
1	Alkaloids	+
2	Tannins	+
3	Saponins	-
4	Flavonoids	+
5	Phenols	+
6	Terpenoids	+
7	Amino acid	-
8	Carbohydrate	-
9	Glycoside	+
10	Reducing sugars	-
11	Steroids	+
12	Amino acid	-

Table 6.1.1: Phytochemical screening of *DE-SASE*, Here (-) indicates the absence of chemical constituents/ (+) indicates the presence of chemical constituents in *(DE-SASE).*

6.2 ACUTE ORAL TOXICITY

The di-ethyl ether extract of *Santalum album* seeds extract was deemed safe for additional pharmacological testing because it showed no toxicity or morality symptoms in rats when administered up to 200-400mg/kg body weight orally. Rats body weights before and after the medication was administered were recorded. No changes in the animals skin, hair, eyes, mucus membranes, respiratory, circulatory, autonomic nervous system, motor activity, or behavioral pattern were noticed, nor were there any indications of tremors, convulsions, salivation, diarrhea, sleep, or coma. Throughout, study there was no toxicity and death were observed at these levels table.

Rat No.	Treatment	Dose mg/kg	Weight of animals		Signs of Toxicity	Onset of toxicity	Duration of study
			Before Test	After Test			
1	Drug perse	200	155	250	No signs of toxicity	Nil	8 Days
2	Low Dose	200	165	255	No signs of toxicity	Nil	8 Days
3	High Dose	400	150	225	No signs of toxicity	Nil	8 Days

Table 6.2.1: Acute toxicity study

6.3 PHYSICAL PARAMETER

6.3.1 Body Weight

Significant changes in body weight were seen in the disease group, standard and *Santalum album* seeds extract groups, as shown in the table. All the groups saw an increase in final body weight. Rats body weight of the animals given *Santalum album* seeds extract for 8 days at doses of 200mg\kg and 400 mg\kg between increased Fig.6.1.

S. No.	Experimental Groups	Initial body weight (gm)	Final Body Weight (gm)
1	**Normal Control**	195±0.01	200.8±0.01
2	**Disease Control**	211.7±0.02	243.3±0.01
3	**Drug Perse**	198.3±0.03	220±0.01
4	**High Dose**	256.7±0.10	245±0.01
5	**Low Dose**	190.0±0.10	241.7±0.01
6	**STD**	219.2±0.10	240.8±0.02

Table 6.3.1: Effect of body weight changes in rats

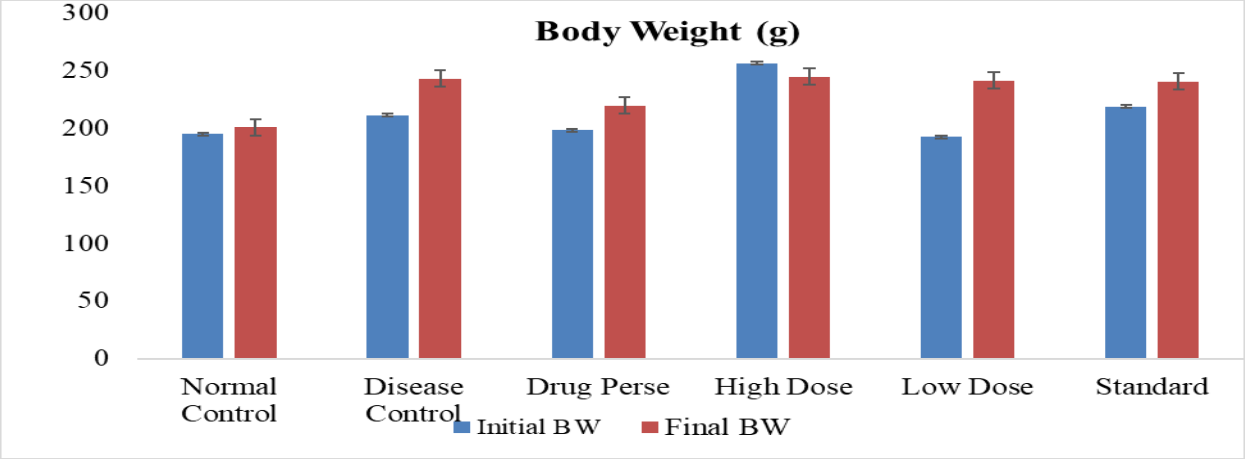

Fig.6.3.2: Graphical representation of body weight changes in rats

Data were analyzed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, $p<0.05$ $p<0.01$ and $p < 0.0001$ when compared with normal control group.

6.4 Food Intake

Significant changes in food intake were seen in the disease group, standard and high fat diet groups, as shown in the table. All the groups saw an increase in final food intake. Rats food intake of the animals given *Santalum album* seeds extract for 8 days at doses of 200 mg/kg, 400 mg/kg between increased (Fig.6.2).

S. No.	Experimental groups	Initial food intake (gm)	Final food intake (gm)
1	Normal Control	15.5±0.04	14.33±0.05
2	Drug Perse	14.7±0.06	16.4±0.06
3	Disease Group	16.33±0.02	16.5±0.07
4	Standard Drug	18.33±0.05	19.67±0.01
5	Low dose (200mg/kg)	17.17±0.02	18.17±0.03
6	High dose (400mg/kg)	13.83±0.02	17.67±0.04

Table 6.4.1: Effect of food intake changes in rats

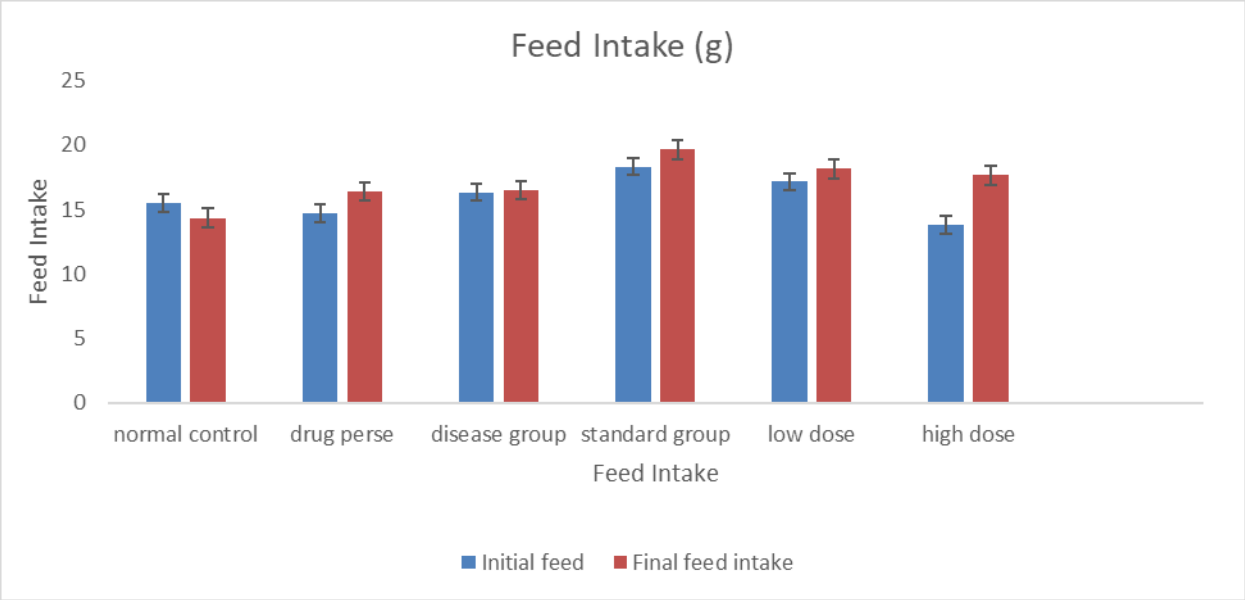

Fig.6.4.2: Graphical representation of feed intake changes in rats

Data were analyzed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, $p<0.05$ $p<0.01$ and $p < 0.0001$ when compared with normal control group.

6.5 EFFECT OF LOW DOSE ON HIGH FAT DIET INDUCED NAFLD

6.5.1 Effect of *Santalum album* seeds extract on triglycerides level in triglycerides estimation

Disease group showed significantly increased in triglycerides levels ($p< 0.001$) in the blood serum as compared to a normal control group, which indicates an elevation in lipid level. After the treatment with *Santalum album Linn* seeds extract (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (# $p< 0.001$) dose-dependent effect that decreased the level of triglycerides in blood serum as compared to the disease control group.

S.No.	Groups	Triglycerides
1	Normal control	70±0.013
2	Drug perse	54±0.016
3	Disease group	170±0.09
4	Standard group	55±0.054
5	Low dose	80±0.01
6	High dose	60±0.09

Table 6.5.1: Effect of *DE-SASE*on triglycerides level

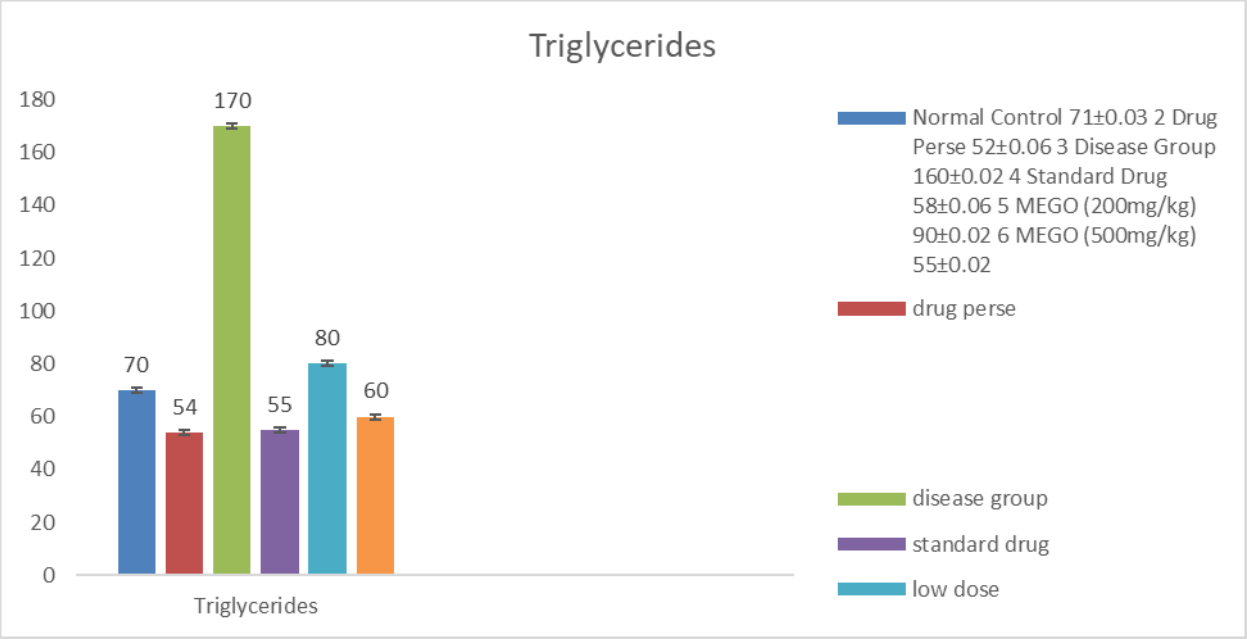

Fig. 6.5.2 : Graphical representation of *DE-SASE* on triglycerides level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (Mean ± SEM), n=6, p<0.05 p<0.01when compared with the normal control group.

6.6 Effect of *Santalum album* seeds extract on SGPT level

In SGPT estimation, Disease group showed significantly increased in SGPT levels (p< 0.001) in the blood serum as compared to a normal control group, which indicates an elevation in liver injury. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (# p< 0.001) dose-dependent effect that decreased the level of SGPT in blood serum as compared to the disease control group.

S.no.	Experimental Groups	SGPT level (U\L)
1	Normal Control	25±0.03
2	Drug perse	30±0.04
3	Disease group	95±0.01
4	Standard Drug	25±0.04
5	Low dose	45±0.04

Table 6.6.1: Effect of DE-SASE on SGPT level

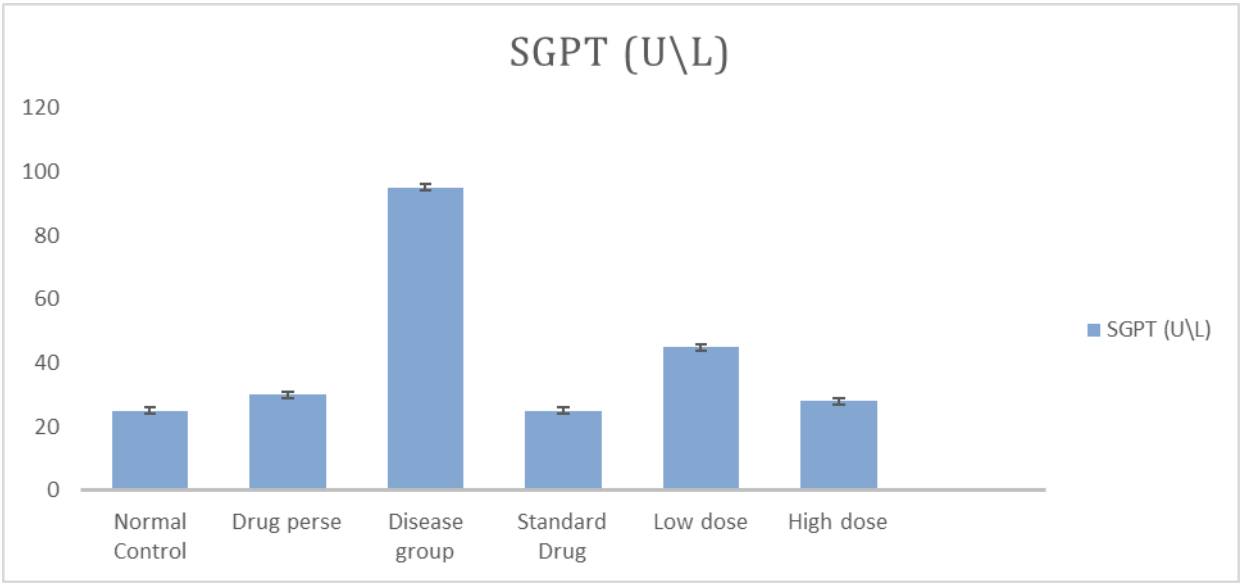

Fig. 6.6.2: Graphical representation of *Santalum album* seeds extract on SGPT level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p<0.05 p<0.01when compared with the normal control group.

6.7 Effect of *Santalum album* seeds extract on SGOT level

In SGOT estimation, Disease group showed significantly increased in SGOT levels (p< 0.001) in the blood serum as compared to a normal control group, which indicates an elevation in liver injury. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (p< 0.001) dose-dependent effect that decreased the level of SGOT in blood serum as compared to the disease control group.

S.No.	Experimental groups	SGOT (U\L)
1	Normal Control	44±0.01
2	Drug Perse	50±0.02
3	Disease Group	125±0.03
4	Standard drug	35±0.01
5	Low dose	70±0.05
6	High dose	30±0.02

Table 6.7.1: Effect of *Santalum album* seeds extract on SGOT level

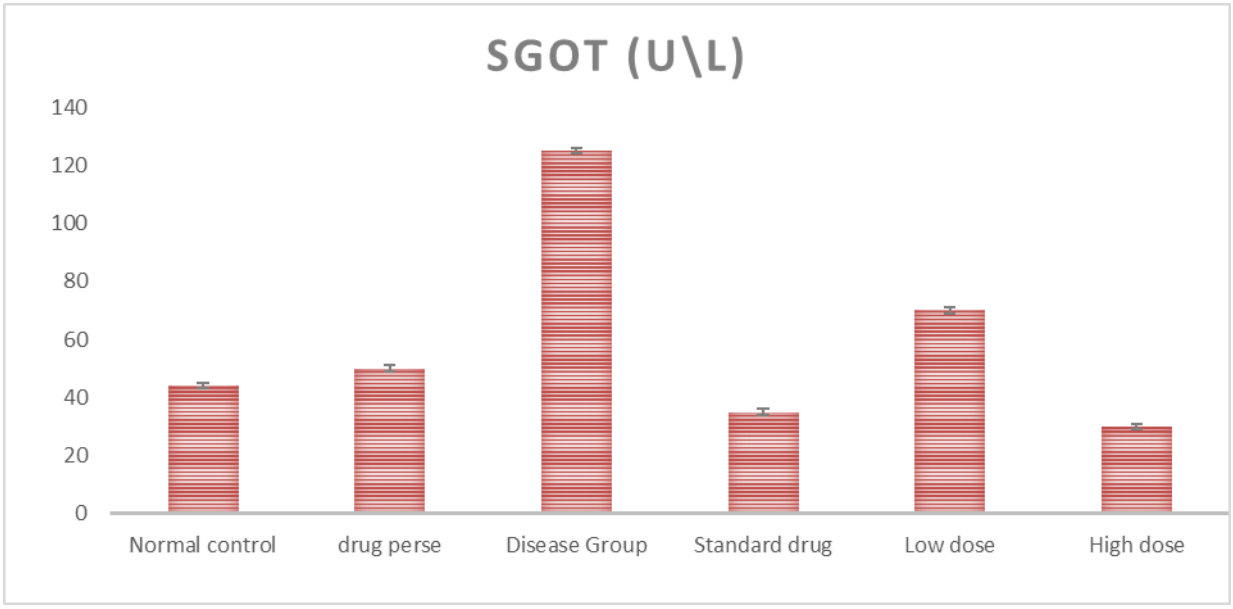

Fig.6.7.2: Graphical representation of *Santalum album* seeds extract on SGOT level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p<0.05 p<0.01when compared with the normal control group.

6.8 Effect of *Santalum album* seeds extract on HDL level

In HDL estimation, disease group showed significantly decreased in HDL levels (p< 0.001) in the blood serum as compared to a normal control group, which indicates an elevation in HDL level. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (p< 0.001) dose-dependent effect that increased the level of HDL in blood serum as compared to the disease control group.

S.No.	Experimental animals	HDL
1	Normal Control	55±0.02
2	Drug perse	53±0.062
3	Disease Group	27±0.012
4	Standard Group	65±0.03
5	Low dose(200mg\kg)	47±0.01
6	High dose (400mg\kg)	75±0.04

Table 6.8.1: Effect of *DE- SASE* on HDL level

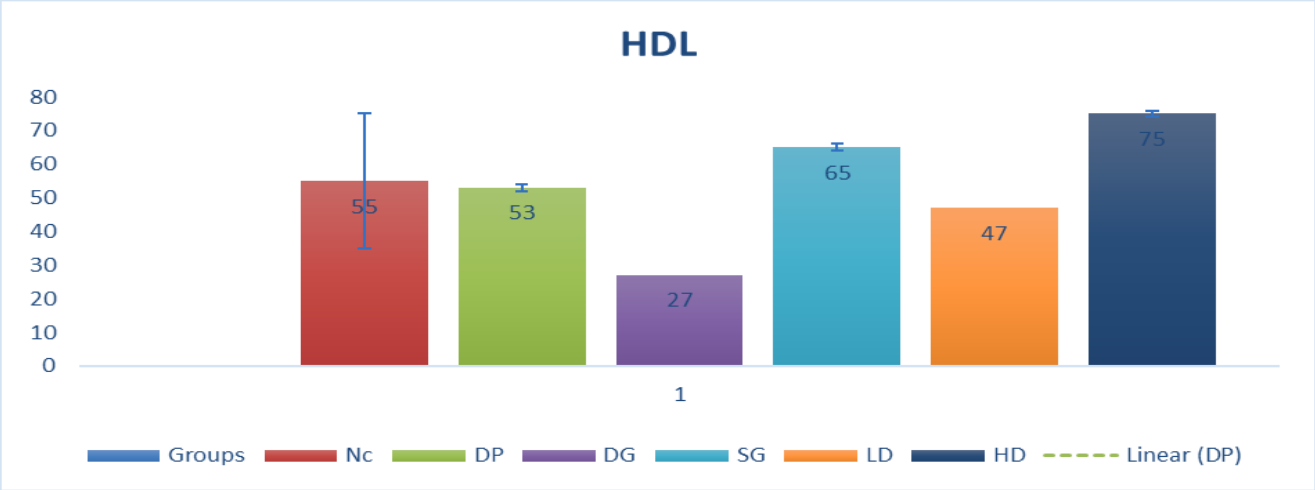

Fig.6.8.2: Graphical representation of *DE-SASE* on HDL level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p<0.05 p<0.01when compared with the normal control group.

6.9 Effect of *Santalum album* seeds extract on total cholesterol level

In cholesterol estimation, disease group showed significantly increased in cholesterol levels (p< 0.001) in the blood serum as compared to a normal control group, which indicates an elevation in cholesterol level. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (p< 0.001) dose-dependent effect that decreased the level of cholesterol in blood serum as compared to the disease control group.

S. No.	Experimental animals	Total Cholesterol
1	Normal control	149±0.01
2	Drug perse	158±0.032
3	Disease control	225±0.01
4	Standard drug	120±0.03
5	Low dose	178±0.02
6	High dose	135±0.01

Table 6.9.1: Effect of *DE-SASE* on total cholesterol level

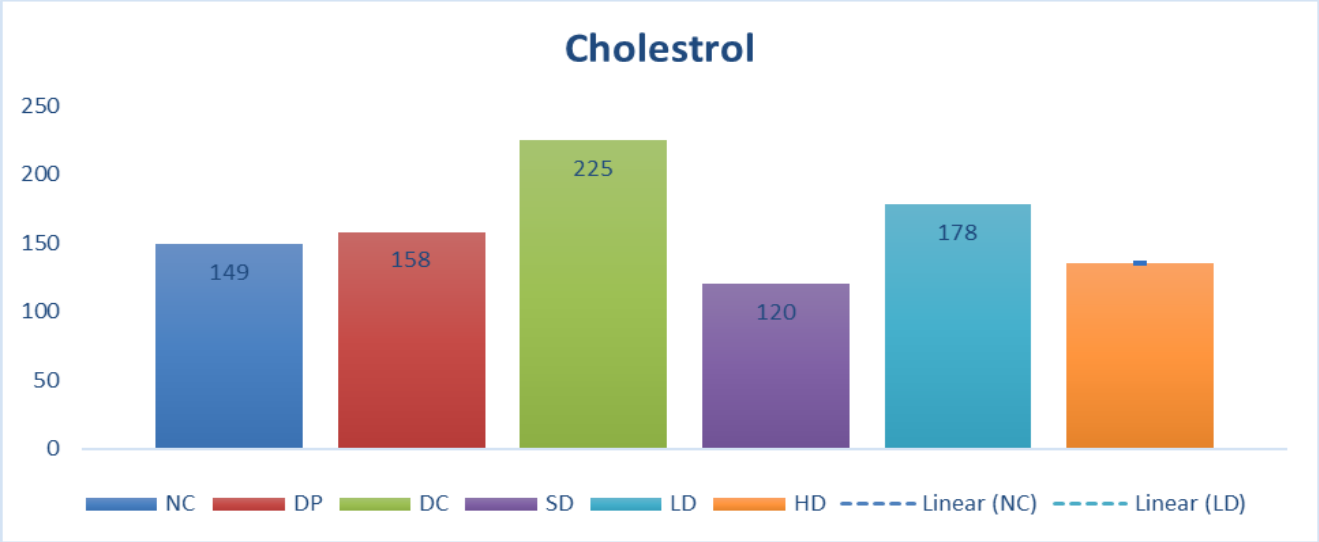

Fig.6.9.2: Graphical representation of *DE-SASE* on total cholesterol level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p p<0.05 p<0.01when compared with the normal control group.

6.10 Effect of *Santalum album* seeds extract on bilirubin level

In bilirubin estimation, disease group showed significantly increased in bilirubin levels (p< 0.001) in the blood serum as compared to a normal control group, which indicates an elevation in bilirubin level. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (p< 0.001) dose-dependent effect that decreased the level of bilirubin in blood serum as compared to the disease control group.

S. no.	Experimental animals	Bilirubin level
1	Normal Control	0.7±0.03
2	Drug Perse	2.1±0.03
3	Disease Group	3.4±0.021
4	Standard Group	0.6±0.05
5	Low Dose (200mg\kg)	0.8±0.02
6	High Dose (400mg\kg)	0.7±0.01

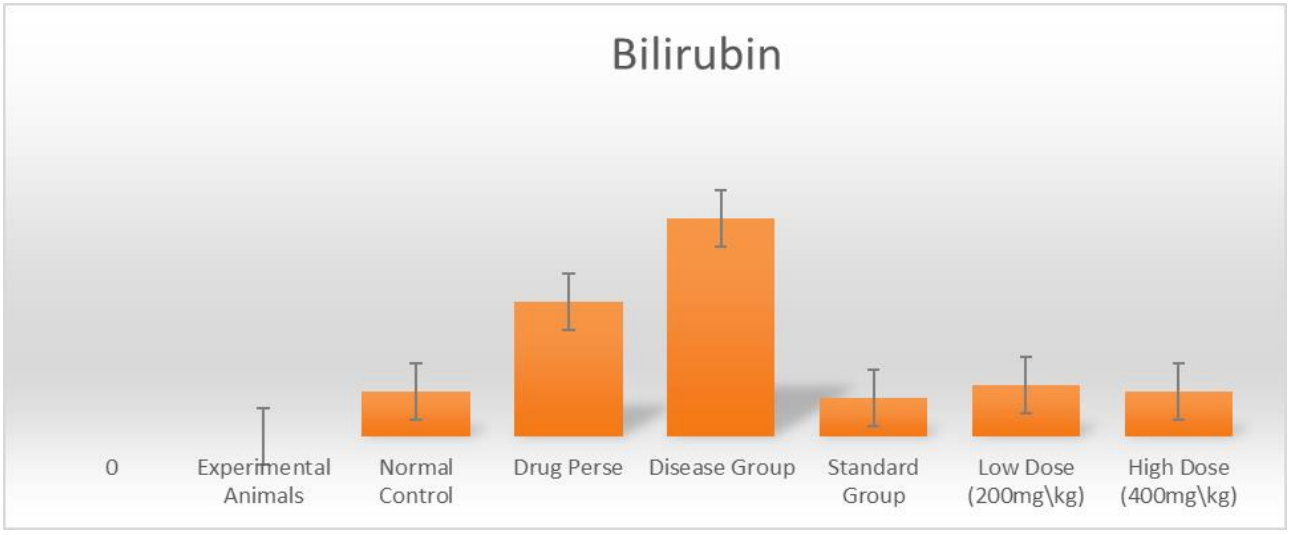

Fig.6.10.2: Graphical representation of *DE-SASE* on bilirubin level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p p<0.05 p<0.01when compared with the normal control group.

6.11 Effect of *Santalum album* seeds extract on GSH level

In GSH estimation, disease group showed significantly decreased in GSH levels (p< 0.001) in the liver homogenate as compared to a normal control group, which indicates an elevation in oxidative stress. After the treatment *Santalum album* seeds extract with (200, and 400 mg/kg, p.o.) for 2 weeks, showed a significantly (p< 0.001) dose-dependent effect that increased the level of GSH in high dose treated as compared to the disease control group.

S.No.	Experimental animals	GSH Level
1	Normal control	2.51±0.013
2	Drug perse	2.29±0.011
3	Disease control	1.88±0.012
4	Standard drug	1.44±0.015
5	Low dose	3.22±0.026
6	High dose	2.11±0.011

Table 6.11.1: Effect of *DE-SASE* extract on GSH level

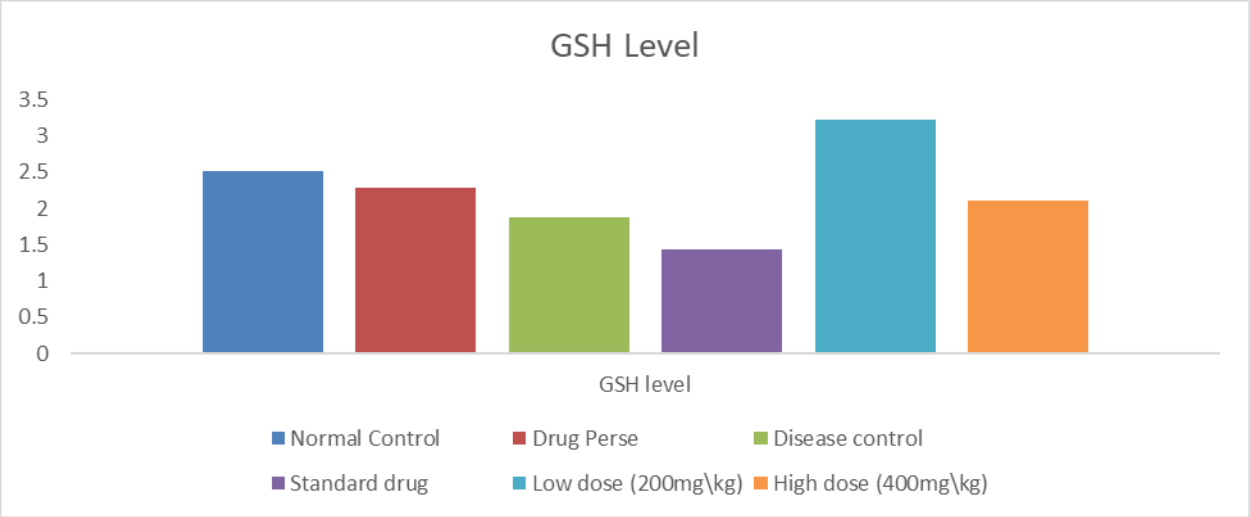

Fig.6.11.2: Graphical representation of *DE-SASE* on GSH level

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p p<0.05 p<0.01when compared with the normal control group.

6.12 Antioxidant activity of di-ethyl ether extract of *Santalum album* seeds extract (*Di-SASE*) by 1,1-Diphenyl-2-Picrylhydrazyl method.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging method is a rapid, simple and inexpensive method to measure antioxidant capacity of extracts. DPPH is widely used to test the ability of compounds to act as free radical scavengers of hydrogen donors, and to evaluate their antioxidant activity. 150 It has also been used to quantify antioxidants in complex biological systems in recent years. The 1,1- Diphenyl-2-picrylhydrazyl method can be used for solid or liquid samples and is not specific to any antioxidant component, but applies to the overall antioxidant capacity of the sample. 1, 1-Diphenyl-2-picrylhydrazyl is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or an unknown plant extract. In the present study, di-ethyl ether extract of *Santalum album* seeds showed potential free-radical scavenging activity. The antioxidant activities of the individual compounds, present in the extracts may depend on structural factors, such as the number of phenolic hydroxyls of methoxy groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features. 151 The reduction capacity of 1, 1- diphenyl-2-picrylhydrazyl radical was determined by decrease in absorbance at 517 nm induced by antioxidant. Due to hydrogen donating capacity of 1, 1-diphenyl-2- picrylhydrazyl it gets converted into 1, 1 Diphenyl-2- picrylhydrazine and hence shows decrease in absorbance. The maximum % age inhibition was found to be 40.7% at 300µg/ml by 1, 1-diphenyl-2-picrylhydrazyl.

S. No.	Concentration (mg/ml)	Mean Abs.(*DE-SASE*)	Mean Abs. (STD)
1	100	26.21±0.02	41.79±0.23
2	200	35.23±0.03	59.44±0.23
3	300	45.54±0.01	76.91±0.11

4	400	56.23±0.04	86.61±0.41
5	500	70.88±0.01	92.34±0.24

Table 6.12.1: DPPH scavenging activity of *(DE-SASE)* and standard ascorbic acid.

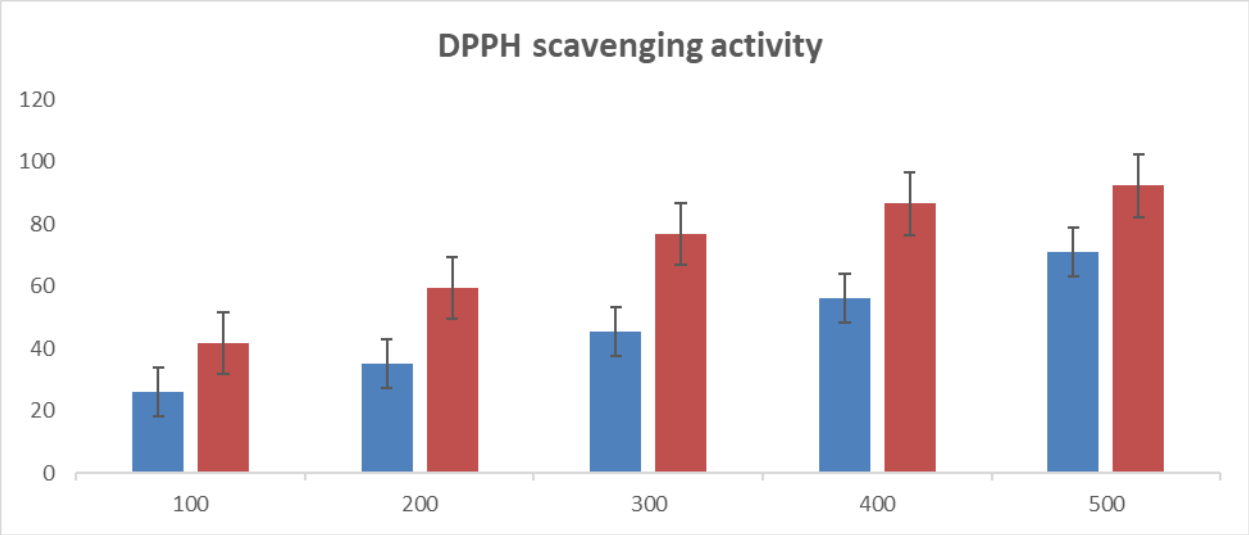

Fig.6.12.2: Graphical representation of DPPH scavenging activity

Di-ethyl ether extract of *Santalum album* seeds (*DE-SASE*) exhibited DPPH scavenging activity. With the increase in concentration of seeds extract, the antioxidant activity increased proportionally with the maximum activity of 70.88±0.01% at 500µg/mL. The absorption decreased proportionally with the increase in the concentration of extract. The maximum absorption was found to be of control sample excluding drug.

6.13 Effect of *Santalum album* seeds extract on wet liver weight

The weight of the liver is measured after the isolation of the tissue of individual rat in each group. This parameter confirms physically indication of fatty liver, if the weight is significantly more compared with normal individual.

S.no.	Experimental group	Wet Liver wt(g)	Avg. Wet Liver wt(g)	STD
1	Normal Control	5.56	5.78	0.04
2	Drug perse	5.22	5.89	0.06
3	Disease control	6.51	6.22	0.01
4	Standard	5.63	5.42	0.12

5	Low dose (200mg\kg)	5.6	5.88	0.10
6	High dose (400mg\kg)	5.5	5.33	0.01

Table 6.13.1: Effect of *DE-SASE* on wet liver weight

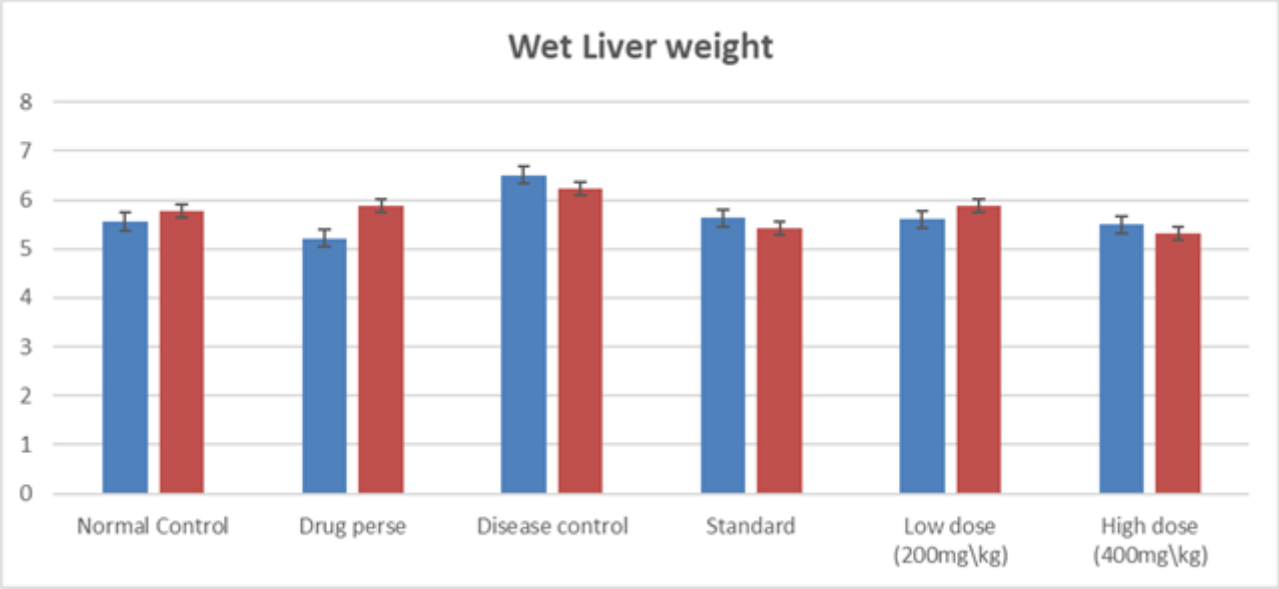

Fig.6.13.2: Graphical representation of *DE-SASE* on wet Liver weight

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p p<0.05 p<0.01when compared with the normal control group.

6.14 Effect of *Santalum album* seeds extract on liver index

The liver index is a measurement used to assess liver health, often indicated by liver weight relative to body weight. Studies have shown that treatment with *Santalum album* seeds extract can significantly affect the liver index, suggesting a positive impact on liver health.

S.NO.	Experimental Animal groups	Liver Index	Average Liver index	Standard deviation
1	Normal Control	2.36	2.44	0.04
2	Drug Perse	2.82	2.88	0.08
3	Disease control	3.09	3.12	0.02
4	Standard control	2.24	2.12	0.03
5	Low dose(200mg\kg)	2.92	2.66	0.023
6	High	2.88	2.72	0.012

	dose(400mg\kg)			

Table 6.14.1: Effect of *DE-SASE* on liver index

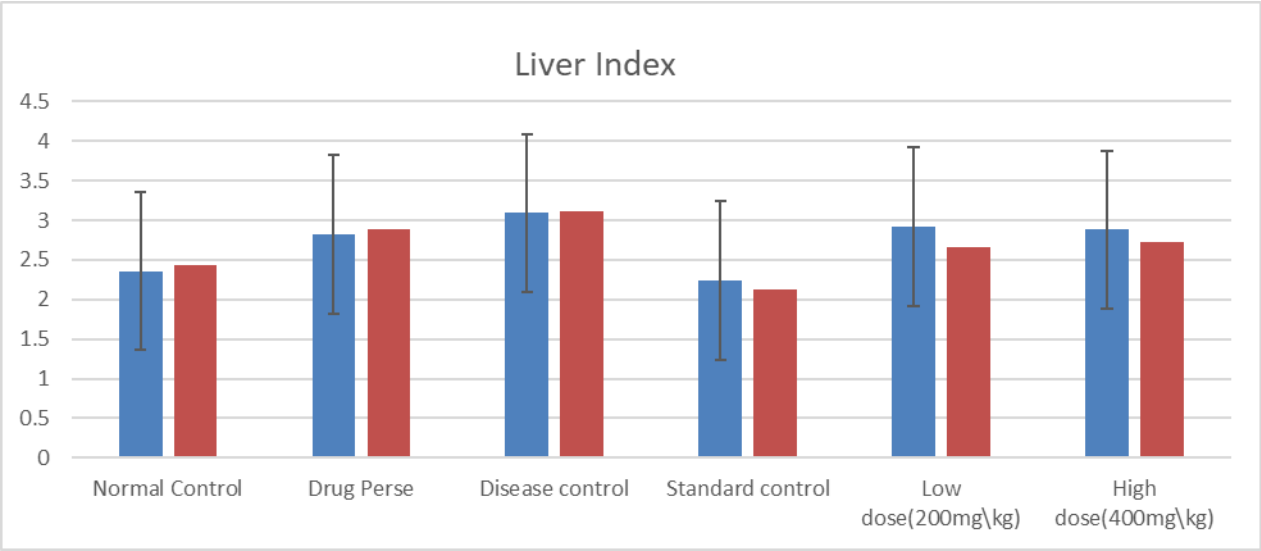

Fig.: 6.14.2 Graphical representation of *DE-SASE* on liver index

Data were analysed by one-way ANOVA followed by multiple comparison post hoc test and values are expressed as (mean ± SEM), n=6, p p<0.05 p<0.01when compared with the normal control group.

7. DISCUSSION

The hepatoprotective effects of *Santalum album* seeds extract observed in this study offer significant insights into potential therapeutic strategies for managing non-alcoholic fatty liver disease (NAFLD). Our findings indicate that *Santalum album* seeds extract effectively mitigates the adverse impacts of a high-fat diet on liver health in wistar rats, reflecting its promising role as a natural hepatoprotective agent.

Mechanisms of Action: The hepatoprotective effects of *Santalum album* seeds extract can be attributed to several mechanisms. Firstly, the reduction in liver fat accumulation observed in the treated rats suggests that the extract might influence lipid metabolism and fatty acid oxidation. This effect is crucial given that NAFLD is characterized by excessive fat buildup in the liver due to disturbances in lipid metabolism. The extract could potentially enhance the liver's capacity to process and eliminate excess lipids, thereby reducing fat deposition.

Secondly, the significant reduction in serum liver enzymes, such as ALT and AST, in the extract-treated groups indicates that *Santalum album* seeds extract may help preserve liver cell integrity and function. Elevated levels of these enzymes are commonly associated with liver damage and inflammation. By lowering these enzyme levels, the extract seems to mitigate liver injury and inflammation, which are central features of NAFLD progression.

Antioxidant Activity: Another key aspect of the hepatoprotective effect is the reduction in oxidative stress observed with the extract. Oxidative stress plays a pivotal role in NAFLD development and

progression, often leading to cellular damage and inflammation. The ability of *Santalum album* seeds extract to lower markers of oxidative stress suggests that it possesses antioxidant properties that can counteract the harmful effects of reactive oxygen species (ROS) in the liver.

Histopathological Findings: Histological analysis further supports the hepatoprotective role of *Santalum album seeds* extract. The improved liver morphology in treated rats, characterized by reduced hepatic steatosis and preserved tissue architecture, reinforces the biochemical and physiological findings. These results align with the extract's capacity to counteract the pathological changes induced by a high-fat diet.

Limitations and future aspects: While the study provides valuable insights, it is important to acknowledge its limitations. The research was conducted in an animal model, and results may not directly translate to human physiology. Moreover, the study did not investigate the long-term effects of *Santalum album* seeds extract or its potential interactions with other medications or dietary factors.

8. SUMMARY

The study investigated the hepatoprotective effects of *Santalum album* (Sandalwood) seeds di-ethyl ether seeds extract against non-alcoholic fatty liver disease (NAFLD) induced by a high-fat diet in Wistar rats. NAFLD is a growing concern due to its association with obesity, metabolic syndrome, and liver dysfunction. In this experiment, Wistar rats were divided into groups, with one group receiving a high-fat diet to induce NAFLD, while the others were given *Santalum album* seeds extract in varying doses 200mg\kg or 400mg\kg alongside the high-fat diet. Key findings included a significant reduction in liver fat accumulation, as evidenced by histological examination and biochemical assays measuring liver enzymes and lipid profiles. The extract demonstrated notable hepatoprotective effects, including decreased levels of alanine aminotransferase & aspartate aminotransferase (AST), reduced oxidative stress, and improved overall liver morphology compared to the control group on a high-fat diet alone. These outcomes suggest that *Santalum album* seeds extract possesses significant potential in mitigating liver damage associated with NAFLD. Furthermore, the extract significantly increased endogenous antioxidant levels, such as Glutathione (GSH) levels, indicating decreasing oxidative stress.

CONCLUSION

The results of this study provide compelling evidence supporting the hepatoprotective effects of *Santalum album* seeds extract against NAFLD induced by a high-fat diet in wistar rats. The extract's ability to reduce liver fat accumulation, improve liver enzyme levels, and mitigate oxidative stress underscores its therapeutic potential. This study highlights the potential of *Santalum album* as a natural intervention for NAFLD & suggests further investigation into its mechanisms of action and possible clinical applications. Given the rising prevalence of NAFLD and its complications, incorporating *Santalum album* into dietary or therapeutic regimens could offer a promising strategy for liver health management. Its ability to reduce liver fat accumulation, decrease oxidative stress, and improve liver enzyme levels positions it as a promising natural treatment option for NAFLD. Future research should focus on validating these findings in human studies and elucidating the mechanisms through which *Santalum album* exerts its beneficial effects.

9. REFERENCES

1. Abbasi, Ebrahim, *et al.* “Protective effects of cerium oxide nanoparticles in non-alcoholic fatty liver disease (NAFLD) and carbon tetrachloride-induced liver damage in rats: Study on intestine and liver.” Metabolism Open 12 (2021): 100151.
2. Ahmad, Figar, Syed S. Ali, and Abdul R. Shakoori. "Sublethal effects of Danitol (Fenpropathrin), a synthetic pyrethroid, on Chinese grass carp, Ctenopharyngodon idella." *FOLIA BIOLOGICA-KRAKOW*- 43 (1995): 151-160.
3. Akah, P. A.,John Lucy-Africa, and *Nworu. C. S.* "Gastroprotective properties of the leaf extracts of *Ocimum gratissimum* L. against experimental ulcers in rats." *Int J Pharmacol* 3 (2007): 461-7.
4. Anderson, Mary E. "Glutathione and glutathione delivery compounds." *Advances in pharmacology* 38 (1996): 65-78.
5. Akhtar, *Shoaib.M et al*. “Evaluation of antioxidant and hepatoprotective effects of Khamira Gonzaba Ambri Jad war Ood Saleeb Wala (KGA).” Bangladesh Journal of Pharmacology 8.1 (2013): 44-48.
6. Anderson, Mary E. "Tissue glutathione." Handbook of methods for oxygen radical research (1985): 317-323.
7. Kumar A, Dhyani A.N, & Joshi G.2019. *Santalum album*. The IUCN Red List of Threatened Species 2019.
8. Aftab, Arooj "*Santalum album*: Medicinal and Pharmacological Properties." Journal of Medicine and Health Research 8.1 (2023): 9-14.
9. Benini B.A., & Sanyal A.J. “Non-alcoholic Fatty Liver Disease: Epidemiology, Pathogenesis, Natural History, Diagnosis, and Current Treatment Options.” Clinical Medicine Insights: Therapeutics 8 (2016).
10. Bisht S. S., and Kumar R. "Phytochemicals from Indian sandalwood (Santalum album L.) and their adulterants: an overview." Trop. Plant Res.8 (2021): 6-12.
11. Chen G. Ni, Y., Nagata, N., Xu, L., & Ota, T. “Micronutrient Antioxidants and Nonalcoholic Fatty Liver Disease.” Int. J. Mol. Sci. 17.1379 (2016).
12. Cui, B., Liu, S., Lin, X., Wang, J., Li, S., Wang, Q., & Li, S.“Effects of Lycium Barbarum Aqueous and Ethanol Extracts on High-Fat-Diet Induced Oxidative Stress in Rat Liver Tissue.” Molecules 16.9116-9128 (2011).
13. Davoodi, Ida, “Promising effect of Rosa damascene extract on high-fat diet-induced non-alcoholic fatty liver.” Journal of Traditional and Complementary Medicine 7.4 (2017): 508-514.
14. Dandge, Babulal P. "Phytochemical Screening with LC-HRMS Profiling and In-Vitro Biological Activities of A. Cuneata (L.) & A. Setosa (L.)."
15. George, Alex K.,"Progress and future research trends on Santalum album: A bibliometric and science mapping approach." Industrial Crops and Products 158 (2020): 112972.

16. Goswami, Bhattachryya .N, and Tah.J "White sandal (Santalum album L.), a precious medicinal and timber yielding plant: a short review." (2018): 1048-1056.

17. Guo, Huimin," Anti-diarrhoeal activity of methanol extract of Santalum album L. in mice and gastrointestinal effect on the contraction of isolated jejunum in rats." Journal of SrEthnopharmacology 154.3 (2014): 704-710.

18. Harsha, Sri P.S.C., et al."Cyanidin-3-glucoside, nutritionally important constituents and in vitro antioxidant activities of Santalum album L. berries." Food Research International 50.1 (2013): 275-281.

19. Hüske, Christin, "Towards optimized anesthesia protocols for stereotactic surgery in rats: Analgesic, stress and general health effects of injectable anesthetics. A comparison of a recommended complete reversal anesthesia with traditional chloral hydrate monoanesthesia." *Brain research* 1642 (2016): 364-375

20. Jin sheng Yu, Sharon Marsh, Junbo Hu, Wenke Feng, Choong Wu. "The Pathogenesis of Non-alcoholic Fatty Liver Disease: Interplay between Diet, Gut Microbiota, and Genetic Background." Gastroenterology Research and Practice vol. 2016, Article ID 2862173, 13 pages, 2016.

21. Khalaf, Abdel Azeem A., "Comparative study on the protective effect of some antioxidants against CCl4 hepatotoxicity in rats." Egyptian Journal of Natural Toxins 6.1 (2009): 59-82.

22. Klementina Fon Tecer, Rozman. D "Non-alcoholic Fatty Liver Disease: Focus on Lipoprotein and Lipid Deregulation." Journal of Lipids vol. 2011, Article ID 783976, 14 pages, 2011.

23. Kumar, Ravi G. "Pharmaceutical importance, physio-chemical analysis and utilisation of Indian sandalwood (*Santalum album* Linn.) seed oil." Journal of Pharmacognosy and Phytochemistry 8.1 (2019): 2587-2592.

24. Kumar. R, Anjum. N, and Tripathi. Y.C "Phytochemistry and pharmacology of *Santalum album L.*: a review." World Journal of Pharmaceutical Research 4.10 (2015): 1842-1876.

25. Kulkarni, Chaitanya R., "Antihyperglycemic and antihyperlipidemic effect of *Santalum album* in streptozotocin induced diabetic rats." Pharmaceutical Biology 50.3 (2012): 360-365.

26. Li X., Wang H. "Multiple organs involved in the pathogenesis of non-alcoholic fatty liver disease." Cell Boski 10 (2020): 140.

27. Maryam Mash maul, "Protective effects of saffron extract and crocin supplementation on fatty liver tissue of high-fat diet-induced obese rats." BMC Complementary and Alternative Medicine 16 (2016): 1-7.

28. Misra, Biswapriya Biswavas, and Satyahari Dey. "Comparative phytochemical analysis and antibacterial efficacy of in vitro and in vivo extracts from East Indian sandalwood tree (Santalum album L.)." *Letters in applied microbiology* 55.6 (2012): 476-486.

29. Moore J. (2019). "From sugar to liver fat and public health: Systems biology driven studies in understanding non-alcoholic fatty liver disease pathogenesis." Proceedings of the Nutrition Society 78.3 (2019): 290-304.

30. Patil, Dharmaraj P., "Development, validation and application of RP-HPLC method for estimation of Xymenynic acid in *Santalum album* seeds extract." Journal of Pharmacognosy and Phytochemistry 8.4 (2019): 1955-1962.

31. Polaiah, A. C., "Effect of presowing seed treatments on seed germination and seedling growth of sandalwood (*Santalum album L.*)." (2020).

32. Pullaiah, Thammineni, "Phytochemistry and Pharmacological Properties of *Santalum album L.*" Sandalwood: Silviculture, Conservation and Applications (2021): 67-96.

33. Puspawati, N. M., and Rita. W.S"Topical anti-inflammatory activity of n-hexane extract of *Santalum album Linn* leaves on rat ear oedema induced by croton oil." Journal of Physics: Conference Series. Vol. 1321. No. 2. IOP Publishing, 2019.

34. Ratnaningrum, Yeni W.N, and Sapto Indrioko."Response of flowering and seed production of sandalwood (*Santalum album Linn*., Santalaceae) to climate changes." Procedia Environmental Sciences 28 (2015): 665-675.

35. Rohini, Vishwa. M, and Padmini.E "Preliminary phytochemical screening of selected medicinal plants of polyherbal formulation." Journal of Pharmacognosy and Phytochemistry 5.5 (2016): 277-282.

36. Sandeep, C., and Manohara. T.N "Sandalwood in India: Historical and cultural significance of *Santalum album L*. as a basis for its." NeBIO 10 (2019): 235-242.

37. Saleem, Mohammad, "Hepatoprotective potential and chemical characterization of *Artocarpus lakoocha* fruit extract." Bangladesh Journal of Pharmacology 13.1 (2018): 90-97.

38. Sharifi-Rad, Javad, "Santalum Genus: phytochemical constituents, biological activities and health promoting effects." Zeitschrift für Naturforschung C vol. 78, no. 1-2 (2023): 9-25.

39. Subha, K., and S. Kother Mohideen. "A Study of Segmentation and Glaucoma Diagnosis through Fundus Images using Deep Convolution Neural Network with Transfer Learning Techniques." *International Journal of Mechanical Engineering* 7.1 (2022): 5395-5407.

40. Shivatare, Rakesh S. "In vitro Antioxidant Activity and Stability Indicating High-performance Thin-layer Chromatographic Method for Ximenynic Acid in Santalum album Seed Extract." *Asian Journal of Pharmaceutics (AJP)* 14.1 (2020).

41. Shivatare, Rakesh S. "In vitro Antioxidant Activity and Stability Indicating High-performance Thin-layer Chromatographic Method for Ximenynic Acid in *Santalum album* Seed Extract." Asian Journal of Pharmaceutics (AJP) 14.1 (2020).

42. Shivatare, Rakesh S., and Dheeraj H. Nagore. "Extraction, Characterization and Antioxidant Activities of Various Solvent Extracts of *Santalum album L*. Seeds."

43. Sindhu, Rakesh K., Kumar A. U, and Arora. S"*Santalum album Linn:* a review on morphology, phytochemistry and pharmacological aspects." International Journal of PharmTech Research 2.1 (2010): 914-919.

44. Sutheesh, V. K., C. M. Jijeesh, and Divya T.P "Evaluation of organic and inorganic pretreatments for better seed germination and seedling vigour in *Santalum album L."* Plant Archives 16.1 (2016): 143-150.

45. Tewari, V., "Distribution, growth & yield and demand & supply of *Santalum album*: survey & review." Proceedings of the National Seminar on Conservation, Improvement (2014),

46. Sindhu, Rakesh K., Kumar A.U, and Arora S. "*Santalum album Linn*: a review on morphology, phytochemistry and pharmacological aspects." International Journal of PharmTech Research 2.1 (2010): 914-919.

47. Ratnaningrum, Yeni WN, and Sapto Indrioko. "Response of flowering and seed production of sandalwood (*Santalum album Linn*., Santalaceae) to climate changes." Procedia Environmental Sciences 28 (2015): 665-675,

48. Umdale, Suraj, "Phytochemical investigation and antioxidant efficacy of wild, underutilized berries of economically important Indian Sandalwood (*Santalum album L.)."* Bio catalysis and Agricultural Biotechnology 27 (2020): 101705.

49. Vengal Rao, P., "Evaluation of Hepatoprotective Activity of *Santalum Album* (Stem) Against Paracetamol Induced Hepatotoxicity in Albino Wistar Rats." (2014).

50. Yang, Ting, "Genotype-guided dosing versus conventional dosing of warfarin: a meta-analysis of 15 randomized controlled trials." *Journal of clinical pharmacy and therapeutics* 44.2 (2019): 197-208.