ORIGINAL RESEARCH PAPER

Volume - 13 | Issue - 04 | April - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A SYSTEMATIC SUMMARY OF S. NIGRUM PHYTOCHEMISTRY, WIDESPREAD USE AS A FOOD IN MANY COUNTRIES AROUND THE WORLD, EXTENSIVE USES IN TRADITIONAL MEDICINES, PHARMACOLOGICAL ACTIVITY, AND APPLICATION AND SAFETY AS A FOOD SUPPLEMENT.

Nutritional Science

Michael Wakeman Faculty of Health and Wellbeing, University of Sunderland, Sunderland, SR1 3SD, UK

KEYWORDS

INTRODUCTION

Within the genus Solanum (Solanaceae family) over 2,000 species exist which are distributed throughout the world and includes foods such as tomatoes, potatoes, and aubergines (1,2). The family member, *Solanum nigrum* (S. nigrum) the European blackberry nightshade or black nightshade is native originally to Eurasia and was later established in the Americas, Africa and Australasia. Whereas the cooked leaves and ripe berries of edible strains are frequently used as ingredients in foods, traditional medicine and as food supplements the plant is sometimes confused with the toxic deadly nightshade (*Atropa belladonna*) a member of another Solanaceae genus. The entire plant of S. nigrum has beneficial detoxification effects of detoxification and has been commonly used for thousands of years (3). Recently, research has identified that the plant is rich in in steroidal alkaloids and saponins as well as organic acids, flavonoids, polysaccharides, and other compounds of interest which have been demonstrated to deliver numerous health benefits including likely immunomodulatory, antitumor, anti-inflammatory, antioxidative, neuroprotective and hepatoprotective effects.

As a result of the increased interest in S. nigrum and its demonstrable pharmacological effects, this systematic summary reviews its phytochemistry, widespread use as a food in many countries around the world, extensive uses in traditional medicines, its pharmacological activity, and application as a food supplement and safety based upon sources of databases of the scientific literature.

Botanical Description and Taxonomy

S. nigrum is an annual herb. It is erect, 25-100 cm high, with bifurcated branches and ovate/oblong/lanceolate, sinuate or toothless leaves of a colour that is dull to dark green. It produces 3-8 white flowers and small ripe berries that are typically reddish brown or black brown around 10 mm in diameter (4,5). It has numerous other descriptive names based upon the characteristics of its leaves, flowers and fruits that differentiate it from deadly nightshade(6,7).

Culinary usage

Since early times *S. nigrum* has been extensively used as a food, with records showing the berries eaten during famines in the 15th-century in China (8). Although the boiled leaves have a slightly bitter and strong flavor, they are used like spinach in quiches and pies in certain European countries, whilst the black ripe berries taste both salty and sweet, somewhat like melon or liquorice (9). Since ancient times, both the cooked young leaves and berries have been consumed on every continent (10, 11) with remains of seeds excavated in York in Viking layers (12) as well as other similar sites in Germany, Denmark, Russia, Sweden and Ireland. These excavated seeds like those still used nowadays highlight their widespread use throughout different areas and cultures dating back to the migration from Africa of early humans during in mid-Pleistocene (13)

The UK Medicines and Healthcare Regulatory Authority recognizes that the fresh fruit and berries are reported to have culinary use (14). EFSA/EU menu guidance appendix 5.2.2 suggests a maximum daily intake of 500g of black nightshade (A00NL) with a maximum of 300g at a single eating event (15). In Turkey and Greece, the leaves are known as *istifno*, whilst in Crete they are called *stifno*. They are an ingredient in a salad dish of boiled greens called *horta* (16,17).

Cultivated species are grown in Africa, where the leaves are used as vegetables which are high in Vitamin C, flavonoids, folate and antioxidants (18-20) and to make them less bitter often cooked in milk. A delicacy in Kenya, it is either blanched or boiled and then sauteed and salted and eaten with the corn meal dish Ugali (21). It is also popular as a vegetable in Tanzania where it is cooked in a similar way (22). The ripe berries are eaten in Ethiopia, especially during famines, whilst the leaves are cooked in salty water as a vegetable (23). Because the plant matures before maize harvesting can begin, it is often used as a source of food until other crops become available (24) In Ghana, leaves are used in preparing stews and soups (25). The very ripe fruit is prepared as a purple runny jam in South Africa (26).

In Indonesia, the fruit is cooked as a fried dish with *oncom* or as part of a traditional salad known as *lalapan*, where the young leaves and fruits are eaten raw (27). The small white flowers, young shoots, berries and leaves are reported to have been eaten historically in Hawaii, where the leaves would be cooked with other greens (28, 29).

In the Chinese treatise *Jinhuang Bencao* of the the 13th century, *S. nigrum* leaves and berries are reported to be used both as a food and medicine (30). Today in southern China the leaves are still consumed as a food. In Asia, especially on the subcontinent of Indian the leaves are used as a vegetable (31,32), for its fruits (33) and in medicine (34). In the South of India, the berries and leaves are commonly cooked and eaten with onion, tamarind and cumin seeds (35, 36). In Kerala, Tamil Nadu South Pradesh and Karnataka the fruits are eaten both fresh and dried.

Traditional Medicinal Uses

As a result of its wide range of pharmacological effects S. nigrum has been extensively employed as traditional medicine throughout the world.

The first known record of its use as a medicine appears in the texts from the Yao Xing Lun Tang Dynasty in China (37). Here, the plant has numerous names in various localities, such as “Yehaijiao”, “Yelahu”, “Heitiantian”, “Heixingxing”, “Kucai”, “Kukui”, “Yesanzi” and “Heidoudou” (38). It is considered to be of cold property, and is designated to be of use in the kidney and lung meridians. In traditional Chinese medicine (TCM) as well as folk medicine, the effects of the whole plant are described to have the whole plant, the effects are described to have benefits that clear away heat, disperse stasis of the blood, detoxify the body, as well as in the treatment of eczema, canker sores, prostate issues, bacterial dysentery, urinary tract infections, and chronic bronchitis (39).

In Oriental medicine S. nigrum is extensively used for its anti-inflammatory, antitumorigenic, hepatoprotective, antipyretic and diuretic effects effects (40). Infusions are used in traditional medicine in India to treat stomach conditions, fevers and dysentery (41) and the juice in skin diseases and on ulcers. The fruits are employed as a laxative, appetite stimulant, tonic, and to relieve asthma (42). In the North of India, boiled extracts of berries and leaves are also used to treat liver-related conditions such as jaundice. Juice extracted from the roots is utilised to treat whooping cough and asthma (43). However, typically in the Ayurvedic system it is the berries and leaves and berries that are used therapeutically (44). For example, in the winter in Tamil Nadu, the leaves are used to treat mouth ulcers (45). The leaves are also used to relieve toothache (46), whilst the juice from the berries has been used to treat liver and eye diseases as well as dysentery (47). Fruits have been found of benefit in skin diseases and inflammation (48).

The plant has similarly been historically used to treat many complains and diseases in many other areas of the ancient world (49-51). In Europe its uses are recorded in numerous national Pharmacopoeias (52). S. nigrum appears to be the most commonly utilised European species of *Solanum* and Dunal (53) reports use dating back to Dioscorides, Theophrastus and Hippocrates. Commonly, its anti-inflammatory, antiseptic, diuretic and expectorant properties are cited as well as being used to treat stomach problems and eye conditions (54).

In order to distinguish black nightshade from poisonous species, deadly nightshade-known as “great morel”- S. nigrum was called “petit (small) morel”. In his herbal, Culpepper notes: 'Do not mistake the deadly nightshade for this,' cautiously adding, 'if you know it not, you may then let them both alone.' In the 14th century, the plant also known as Petty Morel was used to treat cankers and taken with wine for dropsy (55). In the first English herbal John Gerard (56) extensively discussed the use of what he described as the Garden Nightshade (“Solanum Hortense” = *S. nigrum*). However, by the middle of the 15th century, as a result of black nightshade becoming commonly confused with the toxic deadly nightshade (*Atropa belladonna*) a reputation for all nightshades' as being poisonous was developing which has resulted in its use being limited in succeeding centuries throughout Europe. This despite its widespread use in local cultures throughout the region.

In Africa medicinal uses of *S. nigrum* include treatment of diarrhoea and as an antiseptic for the skin and eyes as well as a tonic for general good health (57,58). The seeds which are a source of fatty acids are also utilised in certain areas (59). In Libya, it has a reputation as a folk medicine, where the berries are used as an emetic, antispasmodic and diuretic as well as to relieve fever, diarrhoea, and eye conditions (60). The leaves are employed externally to relieve itching and treat wounds. In Yemen, it is used as an expectorant and to staunch bleeding and relieve diarrhoea (61,620 The juice of the roots is used to treat whooping cough and asthma in Assam (63). In Jordan, the fruit is employed as an antirheumatic and antispasmodic (64). The juice is used as a laxative by locals in Hawaii and the boiled young leaves used to coughs and relieve sore throats (65).

Phytochemical Constituents

To date, over 180 phytochemical compounds have been identified in different parts of *S. nigrum.* These include alkaloids, steroidal molecules, flavonoids, organic acids, glycosides of phenylpropanoids along with other groups of compounds such as polysaccharides (66). Steroidal alkaloids and steroidal saponins are considered to be the main bioactive compounds with extensive pharmacological activity such as anti-inflammatory, antitumour, antiviral and hepatoprotective effects. The polysaccharide components are thought to be responsible for the plant's activity as an immunomodulator.

Steroidal Saponins

To date, 76 compounds in this class of secondary metabolites have been isolated and characterised and found to be responsible for a number of pharmacological activities (67).

Alkaloids

Although other types of alkaloids have been identified, the main alkaloids present in *S. nigrum* consist of steroidal alkaloids such as solanine, which are present as glycosides in the leaves, fruits, roots and stems (68). The immature fruit has concentration of these compounds reaching levels as high as 4.2% content. However, as the plant grows the content gradually decreases. Respectively, solasonine and solamargine make up to 0.2% and 0.25% and after alkaline hydrolysis the glycoside of both compounds is solasodine (69). Solamargine has been identified to have inhibitory activity against cancers of the cervix, liver, lung, larynx, oesophagus, as well as cholangiocarcinoma cancer (70-72).

As the leaf grows, the total amount of alkaloid in it increases, but the concentration becomes diminised. The small green fruit contains high levels of solasodine, and as with the leaf, as it matures, the absolute amount and concentration of solasodine in each fruit decreases (73). Throughout most of the plant, the glycoalkaloid, solanine is present as a phytoalexin making up some 95% of its' total alkaloid content. When raw, the fruit has the highest level of solanine, but as the plant matures, the concentration eventually becomes lower (74-76).

Phenylpropanoids

These include phenylpropionic acids and their esters, coumarin, and lignans, which have been identified in the whole plant (77).

Flavonoids

Flavonoids have various effects which are typically anti-inflammatory and antioxidant. (78). The latter activity is closely aligned to the flavonoid content of the plant.

Phenolics

Gallic acid, caffeic acid, quercetin, catechins, rutin, protocatechuic acid (PCA), epicatechins and naringenin and flavonoids act primarily as antioxidants scavenging free radicals. Total flavonoid and phenolic concentrations were respectively estimated to be 2262.81 mg/g quercetin equivalents and 3222.66 mg/g gallic acid equivalents (79-81).

Benzoic Acids

These include 2, 4-dihydroxybenzoic acid, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid and salicylic (82,83) which have antioxidant, anti-inflammatory, antiviral and antibacterial activities.

Polysaccharides

At present, 12 different types of polysaccharides have been identified, that are reported to have immunomodulatory, antitumor, and hepatoprotective effects (84,85).

Other Compounds

These include citric acid, ursolic acid, malic acid, tartaric acid and acetic acid as well as mineral such as Ca, K, Mg, Na, Fe, Zn and Mn. The root and fruit also contain vitamin C with the highest concentration in the latter (86) and as well as containing lipids, the seeds also have a moderate amount of protein.

Pharmacological Activities

Various types of extraction techniques have isolated numerous bioactive compounds with extensive pharmacological activity including hepatoprotective, anti-inflammatory, antitumor, immunomodulatory, antioxidant, antimicrobial, antiviral and antihypertensive effects (87-89).

Hepatoprotective Activity

Quantitative and qualitative histopathological investigations identified S. nigrum water extract (SNWE) administered to rats for six weeks at dosages of 0.2, 0.5, and 1.0 g/kg in CCl4- induced chronic hepatotoxicity, reversed organ and body weight loss and fatty degeneration. Furthermore, the extracts also lowered serum liver enzyme marker levels (GPT, GOT, bilirubin and ALP), hydroxyl and superoxide radicals and restored SOD and GSH the normal especially at the two higher doses (90). When rats with liver damage induced by AAF were supplemented with 1% and 2% SNWE, the liver/body weight ratios were respectively 3.1- and 2.9-fold of those of a control group. Decreased levels of the serum biomarkers APF, GOT GPT, and γ-GT were observed as well as an induced the expression and activation of GST-μ and GST-α, which metabolise a wide range of carcinogens and xenobiotics. In addition, the extract regulated levels of Nrf2 as well as downstream antioxidant enzymes including catalase, SOD-1 and GPx (92). A different hydroalcoholic extract containing 250 mg/kg caused a significant change tissue antioxidant status in rats with D-galactosamine-induced hepatic fibrosi and also demonstrated a hepatoprotective effect on the liver (93). Polysaccharides extracts have been shown to alleviate liver swelling, increase levels of CAT, GSH, and SOD and reduced MDA concentrations (94).

The saponins (nigrumnins I and II) have also been demonstrated to deliver hepatoprotective effects against CCl_4-induced liver damage (95,96). Protection of DNA against oxidative damage suggests that these hepatoprotective effects might also be the result of the extract's effectiveness in suppressing any oxidative degradation of DNA in tissue debris (97). Other researchers report elevated activity of uridine diphosphate aminopyrine N-demethylase, glucuronyltransferase, and glutathione S-transferase, without any changes in serum levels of aspartate aminotransferase, gamma-glutamyltransferase and alkaline phosphatase (98). S. nigrum has been identified to be a potent scavenger of DPPH and hydroxyl radicals (99). In vitro cytoprotective assays of extracts show significant inhibition of cytotoxicity, along with hydroxyl radical scavenging activity (100). Similarly, gastric mucosal cytoprotection has been identified against aspirin-induced gastric ulcers (101)

Antitumor Activity

Crude Extract

Various solvent extracts deliver significant inhibition in the growth of a number of cancer cell lines including those of the breast, kidney, oesophagus, liver, stomach, colon and lung (102). SNWE at concentrations of 1% and 2% significantly respectively reduced carcinogenesis to 40% and 20% as well as delivering an increased survival rate of 90% and 100% in rats with hepatoma induced by AAF/NaNO2 (103).In the human breast cancer cell line (MCF-7), the same extract at a concentration of 10 g/L also produced 43%

cytotoxicity and inhibited cell migration (104). In-vitro antitumor activity has been also reported with other different solvent extracts (water, chloroform, n-Butanol and ethanol) across a broad spectrum of cancers (105,106).

Isolated Compounds

α-solanine has displayed multiple antitumor effects (107) and it has been established that solamargine can induce autophagy and apoptosis in cancer of the liver (108). In renal cancer degalactotigonin inhibits invasion, proliferation, tumorigenicity and migration of cells (109). In vitro work suggests Solanine can inhibit pancreatic and breast cancer cell proliferation by inducing autophagy and apoptosis and inhibiting metastasis (110).

Immunomodulatory Activity

In macrophages, S. nigrum polysaccharides displayed significant immunoregulatory activity by inducing NO release and enhancing IL-6 and TNF-α cytokine secretion of cytokines and improving expression of TLR4 (111). One study identified that glycoalkaloid compounds such as quercetin, apigenin and kaempferol interact with SARS-CoV-2 protease (112) suggesting these immunostimulant effects might help in the regulation of Covid-19 (113).

Anti-Inflammatory Activity

A chloroform lower case extract demonstrated an 80% inhibition of the production of iNOS and NO stimulated by LPS (114). Other experiments of hydroalcoholic extracts in subacute and acute rat models showed more deposition of collagen fibres and less in macrophages as well as protective actions in the stomach, liver and kidney (115).

Antibacterial and Larvicidal Activity

Different extracts of different parts of the plant have exhibited antifungal activities against five fungal strains (116,117).

Antioxidant Activity

In vitro and studies have focussed on the antioxidant activity of S. nigrum extracts (118). In cultures of primary rat astroglial cells an extract of the leaf decreased intracellular ROS levels and increased GSH suggesting that it could restore the oxidative status (119). In a hydroxyl radical scavenging assay, an alcoholic extract demonstrated significant DPPH scavenging activity (120).

Neuroprotective Activity

An oscopolamine-induced cognitive impairment model was used to evaluate the effect of a S. nigrum extract in rats and showed significant restoration of impaired memory function as well as a decrease in the MDA content, AChE activity, and additionally the BChE activity and GSH content of the brain was increased (121).

Anti-cholesterol Activity

It has been reported that polyphenols present in S. nigrum might have anti-obesogenic effects, and promote decreases in serum triglycerides, hepatic lipolysis, low-density lipoprotein (LDL)-cholesterol and total cholesterol and inhibit lipogenesis (122). Additionally, in hyperlipidaemic rats, oral administration of an extract reversed the increases of serum levels of total cholesterol, LDL cholesterol, triglycerides and the reduction of HDL cholesterol (123).

Cardioprotective activity

Results from a model using global in vitro ischemia-reperfusion injury where an alcoholic extract of S. nigrum was administered for 6 days a week over 30 days suggest significant cardioprotective effects (124).

Clinical Effectiveness in Humans

Given its widespread use of S. nigrum around the world as a traditional medicine, not surprisingly it has attracted researchers interest in clinical investigations (125).

Hepatoprotection

S. nigrum efficacy in the treatment of liver cancer was assessed in a prospective open label, randomized study carried out between 2012 to 2015. Eighty-two liver cancer patients were allocated to either a control or observation group. Those in the former group received sorafenib, whilst S. nigrum tablets were used to treat the observation group. The rates of complete remission and total effectiveness in those receiving S. nigrum observation group were 14.63% and 43.90% whilst in the control group these outcomes were reported as 2.44% and 14.63% respectively. At 3, and 12 months significantly higher numbers of patients in the S. nigrum group were recorded with liver function recovery than in the control group. Upon completion of treatment, levels of TNF-α, IL-1, IL-6 were significantly lower in those treated with S. nigrum. Similarly, those in the observation group experienced significantly higher one and two year survival rates than their counterparts in the control group (126). A further study identified S. nigrum treatment in advanced primary liver cancer patients effectively improved clinical symptoms, immune and liver function as well as their quality of life (127).

Antidiabetic Effects

An aqueous extract of was assessed for hypolipidemic and hypoglycaemic effects in hyperlipidaemic and diabetic male patients aged 35-60 over 90 days. Levels of HbA1c, LDL- cholesterol, total cholesterol and triglycerides, were reduced respectively by 19%, 10%, 8%, and 17% at the end of treatment (128)

Gastric Ulcer

In a placebo-controlled investigation, 200mg of S.nigrum extract in the treatment of gastric ulcers over 7 days significantly reduced the ulcer index (129). A subsequent higher daily dose of 4 g/kg of extract resulted in no detectable changes in counts of red or white blood cells, haemoglobin, haematocrit or mean corpuscular volume.

Use as a food supplement

Solanum nigrum whole plant has two on-hold claims in the EFSA Article 13.1 claims-claim 4127 (Supports the health of the kidneys and urinary function) and 4128 (Supports the immune system and has an anti-inflammatory effect) (130)

However, the most widely purchased supplement containing Solanum nigrum is the preparation Liv 52 which is registered as a nutritional supplement in those European countries where this process is mandatory-Latvia, Slovakia, Romania, Italy, Bosnia, Portugal, Bulgaria, Serbia, Spain, as well as in Canada where the product is a Licensed Natural Health Product in Canada (NPN 80042427)

Liv.52

The first introduction of Liv. 52 was by The Himalaya Drug Company in 1955. It is used as a liver tonic in India as a treatment for conditions such alcoholic liver disease-ALD (130) and contains herbal ingredients including Solanum nigrum (Kakamachi), Capparis spinosa (Himsara), Mandur bhasma, Terminalia arjuna (Arjuna), Cichorium intybus (Kasani), Achillea millefolium (Biranjasipha), Cassia occidentalis (Kasamarda), and Tamarix gallica (Jhavaka) that have been demonstrated to deliver hepatoprotective effects in hepatotoxicity which has been induced chemically (131). The preparation is commonly used in the treatment or prevention liver conditions such as ALD, pre and early liver cirrhosis, viral hepatitis, loss of appetite, anorexia and radiation induced liver damage (132). As well, it used alongside medications with known hepatotoxicity such as chemotherapeutic agents, anti-tubercular drugs and antiretrovirals (133). The recommended dosage is 2–3 tablets taken twice a day and that of syrup is 10-15ml two or three times a day (134). The formulation is based on Ayurvedic principles (135) around hepatoprotection:

Cichorium intybus and Capparis spinosa contain p-methoxybenzoic acid and esculetin which in animal models has been shown to have effects that are hepatoprotective effects and antioxidant (136,137). Arjuna contains flavonoids and arjunolic acid which elevate levels of glutathione levels, while Solanum has been demonstrated to significantly protect hepatocytes from free radical induced DNA damage (138,139). Achillea millefolium and Cassia occidentalis also have hepatoprotective and antioxidative effects (140,141).

Preclinical Studies of Liv.52

Alcoholic liver disease

Liv 52 prevented increases in gamma-glutamyl transpeptidase (GGT) typically induced by ethanol in rats (142). It also decreased liver lipid peroxidation. Following consumption of alcohol, the levels of antioxidant enzymes such as glutathione, glutathione peroxidase and superoxide dismutase enzyme levels are known to reduce, however Liv 52 has been shown to have a protective action on their activity (143). In HepG2 cells, Liv.52 both suppressed any alcohol-induced increased levels of tumor necrosis factor-alpha (TNFα) induced by alcohol and upregulated expression of peroxisome proliferator activator receptor gamma (PPAR) indicating hepatoprotective effects (144). In hepatic organelles Liv.52 has be shown to reduce hepatic damage by significantly inhibiting the accumulation of acetaldehyde (145). In a related study of chronic administration of alcohol, Liv.52 normalized the elevated levels of blood acetaldehyde and ethanol in a dose-related way (146). Liv.52 is reported to reduce the deleterious effects of ethanol administered to pregnant rats and reduce the levels of acetaldehyde present in amniotic fluid (147).

Clinical Studies of Liv.52

Alcohol Absorption

Here, 8 social drinkers consumed 30 mL whisky in 5 minutes. The half-life absorption time of alcohol was significantly less-3.62 minutes-in those consuming one dose of Liv 52 tablets compared to placebo-6.29 minutes. The maximum concentration of alcohol was also significantly increased in those taking Liv.52 compared to placebo (49.9 mg/100 mL versus 40.5 mg/100 mL), however it was noted that those in the former group had significantly reduced levels of acetaldehyde at 3 and 4 hours. In the Liv52 group excretion of aldehyde in the urine was increased by a factor of four. Hence, it is suggested Liv.52 minimises the formation of liver acetaldehyde protein adducts and lowers levels of acetaldehyde in the blood of social drinkers of alcohol thereby explaining some of its effects as a hepatoprotective (148,149).

Alcoholic Liver Disease

A retrospective observational, in-patient, study in a tertiary care teaching hospital setting in India of hepatoprotective drug prescribing in ALD, reported that Liv.52 was the most commonly used medication (40% of prescriptions) (150).

A 6 month prospective open-label study in 50 patients diagnosed with early onset alcoholic cirrhosis taking one Liv.52 tablet twice daily showed a highly significant reduction in symptoms of tiredness, easy fatigability, aesthenia, anorexia, nausea, abdominal pain and discomfort, muscle cramps and abdominal pain as early as the first month. Similarly, at the end of the study, scores of physical signs were reduced significantly, these included-jaundice, muscle wasting, anaemia, hepatomegaly, ascites, and oedema as well as improvements in parameters of liver function (ALP, AST, ALT, total bilirubin, prothrombin time and albumin) (151). These observations have been corroborated in a study of treatment of patients with liver damage taking Liv.52 tablets for a year without side effects (152).

In a prospective, randomised, phase 3 study of twenty five patients with alcoholic hepatitis confirmed by ultrasound, improvements for echogenicity and clearance of ascites were observed following the eight week treatment period of two Liv.52 taken twice daily. Significant improvements in liver function test scores were also observed at the end of the study (153)

Alcoholic Hepatitis

An eight week study of Liv.52 in 50 patients with alcoholic hepatitis demonstrated treatment reduced lipid peroxidation and increased the antioxidant levels of hepatic intracellular and cellular membranes, thereby likely reducing free radical damage in the liver as well as increasing Vitamin C and E levels suggesting liver cells may be also be regenerating after treatment. (154).

Meta-analysis and systematic review

Three reviews of Liv 52 are reported in the peer reviewed literature. The first meta-analysis examined a total of fifty studies carried out over a period of thirty years with a mean duration of 6.62 months (155). Of these, there were three placebo-controlled, double-blind studies, twenty-one placebo-controlled studies, twenty-two non-comparative and four case studies. Of the population of 4490 patients, 233 were children. Cumulative analysis of data identified a significant reduction in mean levels of SGOT, SB, AP, PT and SGPT, and mean time necessary for required for total recovery using clinical, symptomatic, and biochemical assessments. In all studies, when compared to the pre-treatment values SG and SA were also significantly increased. No significant adverse events were reported in any trials and medication compliance was considered to be excellent. Hence, this meta-analysis concluded that “Liv.52 tablets and syrup are effective and safe in the management of hepatitis”.

A more recent systematic review assessed more than 35 studies investigating the clinical effectiveness of Liv.52 in a number of chronic liver diseases (156). Its hepatoprotective effects was evaluated using both clinical symptoms together with parameters of liver function in: viral hepatitis (nine studies), tuberculosis (six studies), non-alcoholic fatty liver disease (six studies), alcoholic liver disease (five studies), liver cirrhosis (three studies), and one study in each of the following non-infectious chronic liver diseases, hepatomegaly syndrome and liver function in pregnancy. Despite differences in the trials around dose, duration, and product formulation, results suggested a positive trend for Liv.52 treatment. Even though many studies were placebo-controlled and randomized, the authors noted some were of a limited sample size. However, in most an improvement in liver parameters and clinical symptoms in various hepatic disorders were noted often within four to twelve weeks, likely as a result of reduced inflammation and oxidative stress in hepatocytes.

It concluded that overall, the systemic review indicates that in a number of liver diseases, for example: non-alcoholic fatty liver disease, liver dysfunction in pregnancy, drug-induced hepatotoxicity, hepatitis and alcoholic liver disease, Liv.52 may deliver a beneficial improvement in both symptoms as well as hepatic parameters. It also demonstrates that Liv.52 has potential effects as a hepatoprotective treatment and delivers significant improvement in function of the liver and quality of life. It is well-tolerated, with no drug-related or serious side effects identified in any of the studies.

A further systematic review of clinical and preclinical and clinical studies identified that Liv.52 reduces inflammation, modulates the lipotropic activity of hepatocytes, protects the parenchyma of the liver by restoring hepatocyte antioxidant levels, and enhances acetaldehyde and alcohol metabolism (157). It concluded these studies provide evidence that Liv.52 delivers both improvements in test parameters of hepatic function as well as delivering improvement in subjective patient symptoms. It suggests Liv.52 is well tolerated with no reported side effects.

Safety of LIV 52

An unpublished communication (158) from Himalaya Drug Company provides a detailed assessment of the safety of Liv 52 tablets and concludes:-

Liv.52 tablets safety in relevance to glycoalkaloid (GA) content: Total GA in Liv.52 tablets was calculated as equivalent to solasodine. Repeated batches of Liv.52 tablets were analysed and the solasodine content were in the range of below quantification limit (0.05 ppm) to 0.082 µg/tablet. Based on the maximum daily recommended human dose (2 tablets twice daily) it corresponds to 0.328 µg of glycoalkaloid/day which is 3048.78 times lesser than the accepted daily intake-ADI- for GA. Based on the outcome it is explicit that the concentration of GA is negligible and is very unlikely to cause any adverse effects. This is in turn reaffirmed by the long-standing safety (launched in the year 1955) of the product.

Inferences on the safety of Solanum nigrum in Liv.52 tablets

- Repeated dose 21-day oral toxicity studies in rats with *Solanum nigrum* and glycoalkaloids was observed at 4000 mg/kg and 100 mg/kg respectively.
- In vivo genotoxicity study with *Solanum nigrum* extract indicated that it is non-mutagenic at 1000mg/kg.
- Repeated dose 270-day (Chronic) toxicity study as well as developmental and reproductive toxicity studies performed with Liv.52 (based on the dose conversion in terms of *Solanum nigrum* equivalence) the margin of safety with respect to the maximum daily recommended human dose (2 tablets twice daily) is higher by 14.66 to 21.99 times, thereby indicating wide margin of safety.
- Extensive preclinical safety studies with Liv.52 revealed that it is safe and devoid of any adverse effects. A repeated dose 270-day (Chronic) toxicity study as well as developmental and reproductive toxicity studies performed with Liv.52 revealed NOAEL of 3000 and 2000 mg/kg, which is above the limit test dose of 1000 mg/kg as recommended by OECD guidelines.
- Dietary studies in human volunteers consuming 1 mg/kg of GA in diet for 1 week reported no signs of toxicity.

Pharmacokinetic study performed in human volunteers with GA dose of 0.95-1.10 mg/kg body weight revealed no adverse effects.

- Maximum daily recommended human dose of Liv.52 (2 tablets twice daily), solasodine content present in Liv.52 tablets corresponds to 0.328 µg of glycoalkaloid/day which is 3048.78

times lesser than the ADI for GA.

Based on the literature-based weight of evidence, extensive toxicological screening, studies in human volunteers, proposed ADI and GA content present in tablets, it could be concluded that *Solanum nigrum* in Liv.52 tablets do not pose any safety concerns.

Toxicity of solanum nigrum

When administered to mice by continuous gavage, a dose of 21.5 g/kg of S. nigrum juice over fourteen days resulted in no reports of death or toxicity (159). The results of evaluations of genotoxicity and short-term mutagenicity using the mouse sperm deformation, Ames and micronucleus tests were all negative. A further study in mice of an aqueous preparation of S. nigrum identified 494.4 g/kg as a maximum dose. There are reports of healthy individuals consuming 30g to 60g rarely experiencing side effects or toxicity (160).

A study examining the oral toxicity and anticonvulsant effect of S. nigrum berries acutely, reported the oral median lethal dose of the extract to be 3129 mg/kg body weight and allocated it to category 5 on the basis of this result and hence considered safe (161). Other researchers consider doses of 5 g/kg body weight to be safe (162). When evaluating hepatic and haematological parameters oral S. nigrum extract was found to be safe at a dose of 4 gm/kg over a period of 21 days, however toxicity of the glycoalkaloid fraction was found at a dose of 200 and 400 mg/kg (163).

Other investigators evaluated the genotoxic and acute toxic effects of oral administration of an alcoholic extract in three groups of 5 mice at doses of 300, 2000 and 5000 mg/kgBW over seven days and found no lethal effects, even at the highest dose (164).

A further acute toxicity study performed to determine the safe dose as per OECD guideline 425 with aqueous leaf extract of *Solanum nigrum* indicated that the median lethal dose (LD50) was greater than 2000 mg/kg and no signs and symptoms of toxicity was noticed during the 14 days post-dose observation period (165). Acute toxicity study performed as per OECD guideline 423 with hydroalcoholic whole plant extract of *Solanum nigrum* showed no mortality and behavioural changes during the 14 days observation period. The median lethal dose (LD50) was greater than 2000 mg/kg (166). Acute oral toxicity studies performed with whole plant powder of *Solanum nigrum* and glycoalkaloids in mice indicated that the median lethal dose (LD50) was greater than 5000 mg/kg and 2000 mg/kg, respectively (167).

Crude extract of *Solanum nigrum* was evaluated in a repeated dose oral toxicity study in mice for 10 days at doses ranging from 250 – 2000 mg/kg. The observations indicated that it was safe as indicated by increase in body weight and devoid of any overt signs and symptoms of toxicity throughout the study period (168). In a 21 day repeated dose oral toxicity study in rats *Solanum nigrum* (dose range - 1000 to 4000 mg/kg b.wt.) and glycoalkaloids (dose range - 100 to 400 mg/kg b.wt.), it was observed that the hematological and clinical chemistry parameters were within the normal range and the NOAEL for *Solanum nigrum* and glycoalkaloids was observed at 4000 mg/kg and 100 mg/kg respectively (167).

Based on the literature based weight of evidence outcome on oral administration of *Solanum nigrum* in acute, repeated dose and *in vivo* genotoxicity studies it is inferred that

- Median lethal dose (LD50) 2000 – 5000 mg/kg
- NOAEL 4000 mg/kg/day
- Non-mutagenic at 1000 mg/kg

Safety of glycoalkaloids (GA) in Human Volunteers:

Dietary studies in human volunteers consuming 1 mg/kg of GA in diet for 1 week reported no signs of toxicity (170). A pilot study on the pharmacokinetics of potato glycoalkaloids was performed in human volunteers in the form of a solution containing 0.20-0.70 mg/kg body weight of GA or a portion of mashed potatoes containing 0.80-1.25 mg/kg body weight of GA. No adverse effects were reported after administration of GA doses 0.95 and 1.10 mg/kg body weight (171). Dose of 1 mg GA/kg body weight is considered to be the acceptable daily intake (ADI) for an adult (172).

Suggested limits on consumption of solanine

Since potatoes can also contain high levels of solanine, the concentration of this glycoalkaloid in this commonly consumed food can act as a useful reference. In the US, typical average consumption of potatoes is calculated at around 167 g/person/day (173). Although levels of this glycoalkaloid vary in different varieties farmers try to maintain solanine concentrations at no more than 0.2 mg/g, which results in a daily consumption of no more than 33.4mg of solanine (174). Given, a potato typically contains 0.075 mg/g of solanine, based on the above consumption this equates to a daily intake of around 0.18 mg/kg. It has been calculated that an intake of 2-5 mg/kg of glycoalkaloids such as solanine is likely to induce toxic effect in humans (175).

Glycosylation is the main metabolic reaction of steroidal alkaloids during the maturation of Solanaceae plants. Glycosylation can reduce the toxicity of aglycones. For example, the steroidal alkaloid aglycone tomatine in tomatoes is toxic to a variety of organisms, including bacteria, fungi, animals, and even plants themselves, while the toxic effect of the glycosidic alkaloid α-tomatine formed after glycosylation on tomato fruits and leaves is almost negligible (176).. Glycosyltransferases in *Solanum nigrum* fruit are now thought to result in glycosylation products of steroidal alkaloids and were observed in a recent study (177).

CONCLUSIONS

Solanum Nigrum has been used through history as a food, traditional medicine and more recently as a food supplement. The similarity between black nightshade (Solanum nigrum) and deadly night shade (Atropa belladonna) rightly requires caution in selecting the correct species for these purposes due to the high toxicity of the latter. There is concern regarding the levels of potentially problematic glycoalkaloids, such as solanine, solasonine and solamargine, however, the concentrations and any potential toxicity around these compounds significantly decreases in the fruit as the plant ripens as exemplified by the situation of other members of the Solanaceae family such as tomatoes and potatoes.

Given the acceptable daily intake for glycoalkaloids is considered to be 1mg/kg body weight, clearly, the safest way to obtain the well described physiological benefits from solanum nigrum is to consume it in the form of a regulated food supplement manufactured to the highest standards of quality assurance, which has undergone extensive safety testing and also been demonstrated to be safe and effective in a clinical setting. One of the few products of this type is Liv 52 which delivers 32mg solanum nigrum extract per tablet. At the recommended dose of two tablets per day-or equivalent depending on dosage format-consumption of Liv 52 is likely to deliver only 0.328mcg of glycoalkaloids per day which is 3048.78 time less than the acceptable daily intake for this family of compounds. Hence the explicit concentration of glycoalkaloids is insignificant and unlikely to cause any adverse effects, an observation reinforced by the long-standing safety of the product over the past 70 years.

REFERENCES

1. Ganaie MM, Raja V, Reshi ZA, Verma V. Family Solanaceae: Taxonomy and modern trends. Annals of plant science. 2018;7(9):2403-14.
2. Samuels J. Biodiversity of food species of the Solanaceae family: a preliminary taxonomic inventory of subfamily Solanoideae. Resources. 2015 May 12;4(2):277-322.
3. Jain R, Sharma A, Gupta S, Sarethy IP, Gabrani R. Solanum nigrum: current perspectives on therapeutic properties. Altern Med Rev. 2011 Mar 1;16(1):78-85.
4. Poczai P, Hyvönen J. On the origin of Solanum nigrum: can networks help?. Molecular biology reports. 2011 Feb;38:1171-85.
5. Edmonds JM, Chweya JA. Black nightshades: Solanum nigrum L. and related species. Bioversity International; 1997.
6. Schilling EE, Ma QS, Andersen RN. Common names and species identification in black nightshades, Solanum sect. Solanum (Solanaceae). Economic Botany. 1992 Apr 1:223-5.
7. Edmonds JM. Nomenclatural notes on some species of Solanum L. found in Europe. Botanical Journal of the Linnean Society. 1979 Apr 1;78(3):213-33.
8. Sarma H, Sarma A. Solanum nigrum L., a nutraceutical enriched herb or invasive weed?. InInternational Conference on Environment and BioScience IPCBEE 2011 (Vol. 21, pp. 105-109).
9. Irving, M (2009). The Forager Handbook — A Guide to the Edible plants of Britain. Ebury Press. ISBN 978-0-09191-363-2.
10. Saleem TM, Chetty C, Ramkanth S, Alagusundaram M, Gnanaprakash K, Rajan VT, Angalaparameswari S. Solanum nigrum Linn.-A review. Pharmacognosy reviews. 2009 Jul 1;3(6):342.
11. Odukoya JO, Oshodi AA. Evaluation of the nutritional qualities of the leaves of Parquetina nigrescens, Launaea taraxacifolia and Solanum nigrum. European Journal of Pure and Applied Chemistry Vol. 2018;5(1).
12. Kenward H, Hall A. Enhancing bioarchaeological interpretation using indicator groups: stable manure as a paradigm. Journal of archaeological science. 1997 Jul 1;24(7):663-73.
13. Särkinen, Tiina, et al. "A revision of the Old World black nightshades (Morelloid clade of Solanum L., Solanaceae)." PhytoKeys 106 (2018): 1.
14. https://assets.publishing.service.gov.uk/media/5a806f79ed915d74e622e6f6/List-of-herbal-products.pdf

15. https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/3944A-5-2-2.pdf
16. Forbes MH. Gathering in the argolid: a subsistence subsystem in a Greek agricultural community: gathering in the Argolid. Annals of the New York Academy of Sciences. 1976 Feb;268(1):251-64.
17. Dogan Y. Traditionally used wild edible greens in the Aegean Region of Turkey. Acta Societatis Botanicorum Poloniae. 2012;81(4).
18. Sangija F, Martin H, Matemu A. African nightshades (Solanum nigrum complex): The potential contribution to human nutrition and livelihoods in sub Saharan Africa. Comprehensive Reviews in Food Science and Food Safety. 2021 Jul;20(4):3284-318.
19. Moyo SM, Kayitesi E. African nightshade (Solanum nigrum complex species). InHandbook of Phytonutrients in Indigenous Fruits and Vegetables 2022 Oct 31 (pp. 97-117). GB: CABI.
20. Mohyuddin A, Kurniawan TA, Khan ZU, Nadeem S, Javed M, Dera AA, Iqbal S, Awwad NS, Ibrahium HA, Abourehab MA, Rabea S. Comparative insights into the antimicrobial, antioxidant, and nutritional potential of the Solanum Nigrum complex. Processes. 2022 Jul 25;10(8):1455.
21. WAGIO RS, RUNO S, MUCHUGI A. Genetic diversity of Solanum nigrum cultivated in Kenya. Asian Journal of Agriculture. 2019 Nov 21;3(2).
22. Sangija F, Kazosi M, Martin M, Matemu A. Trends and constraints in the utilization of African nightshade (Solanum nigrum complex) in Tanzania: A case study of Kilimanjaro and Morogoro regions. African Journal of Food, Agriculture, Nutrition and Development. 2022;22(6):20623-45.
23. Yimer A, Forsido SF, Addis G, Ayelign A. Phytochemical profile and antioxidant capacity of some wild edible plants consumed in Southwest Ethiopia. Heliyon. 2023 Apr 1;9(4).
24. Jibat M, Getachew W, Getu A, Kifelew H. Survey and identification of major weeds of seeds spice in Ethiopia. Journal of Plant Pathology & Microbiology. 2019;10(4):477.
25. Glew RS, Amoako-Atta B, Ankar-Brewoo G, Presley J, Chuang LT, Millson M, Smith BR, Glew RH. Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. Food. 2009;3(1):39-42.
26. Viljoen E. Morphology and genetic relationships in members of the Solanum nigrum L. complex used for jam production in the Highveld of South Africa. University of Pretoria (South Africa); 2011.
27. MULYANTO D, ISKANDAR J, ABDOELLAH OS, ISKANDAR BS, RIAWANTI S, PARTASASMITA R. Leunca (Solanum americanum Mill.): the uses as vegetable in two villages in upper Citarum area, Bandung, West Java, Indonesia. Biodiversitas Journal of Biological Diversity. 2018 Sep 21;19(5):1941-54.
28. https://wehewehe.org/gsdl2.85/cgi-bin/hdict?a=q&r=1&hs=1&m=-1&o=-1&e=q-11000-00---off-0hdict--00-1----0-10-0---0---0direct-10-ED--4-------0-1lpm--1 1-haw-Zz-1---Zz-1-home-papolo--00-3-1-00-0--4----0-0-11-00-0utfZz-8-00&q=popolo&j=pm&af=1&fqf=ED
29. Defelice MS. The black nightshades, Solanum nigrum L. et al.—Poison, poultice, and pie1. Weed Technology. 2003 Jun;17(2):421-7.
30. Zhu SZ. Jinhuang bencao, or treatise on wild food plants used for saving famime (translation and annotation by Wang JX, Tang YC; in Chinese). Shanghai gu ji chu ban she, Shanghai. 2015.
31. Sarma H, Sarma A. Solanum nigrum L., a nutraceutical enriched herb or invasive weed?. InInternational Conference on Environment and BioScience IPCBEE 2011 (Vol. 21, pp. 105-109).
32. Schilling EE, Ma QS, Andersen RN. Common names and species identification in black nightshades, Solanum sect. Solanum (Solanaceae). Economic Botany. 1992 Apr 1:223-5.
33. Kumar P, Kumar J, Kumar R, Dubey RC. Studies on phytochemical constituents and antimicrobial activities of leaves, fruits and stems of Solanum nigrum L. Asian Journal of Plant Science and Research. 2016;6(4):57-68.
34. Edmonds, Jennifer M., and James A. Chweya. Black nightshades: Solanum nigrum L. and related species. Vol. 15. Bioversity International, 1997.
35. Nandhini DU, Sakthinathan B, Kumaresan S. Ethanobotany of medicinal herbs in indigenous folklore of siruvani region of Tamil Nadu. Journal of Pharmacognosy and Phytochemistry. 2017;6(5):2078-81.
36. Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity of two traditional Indian vegetables: Solanum nigrum L. and Solanum torvum L. Food Science and Biotechnology. 2010 Feb;19:121-7.
37. Editorial Committee of State Administration of traditional Chinese Medicine (1999). Zhonghua Bencao, 7. Shanghai: Science and Technology Press, 309–311.
38. Editorial Committee of Nanjing University of Chinese Medicine (2006). Dictionary Of Chinese Materia Medica. second ed. Shanghai: Science & Technology Press, 873–874.
39. Editorial Committee of Flora of China (1979). Chinese Academy of Sciences. Flora of China, 67. Beijing: Science Press, 76.
40. Jain R, Sharma A, Gupta S, Sarethy IP, Gabrani R. Solanum nigrum: current perspectives on therapeutic properties. Altern Med Rev. 2011 Mar 1;16(1):78-85.
41. Jain, SK (1968). Medicinal Plants. Thomson Press (India) Ltd. pp. 133–134.
42. Chauhan R, Ruby KM, Shori A, Dwivedi J. Solanum nigrum with dynamic therapeutic role: A review. International Journal of Pharmaceutical Sciences Review and Research. 2012;15(1):65-71.
43. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
44. Potawale SE, Sinha SD, Shroff KK, Dhalawat HJ, Boraste SS, Gandhi SP, Tondare AD. Solanum nigrum Linn: A phytopharmacological review. Pharmacologyonline. 2008;3:140-63.
45. Nyeem MA, Rashid AM, Nowrose M, Hossain MA. Solanum nigrum (Maku): A review of pharmacological activities and clinical effects. IJAR. 2017;3(1):12-7.
46. Parveen S, Ahmed K, Siddiqui MA, Quamri MA, Doni M, Baig S. Solanum nigrum (MAKO) with Dynamic Therapeutic Role and Pharmacological Actions: A Review. Res. Rev. J. Unani Siddha Homeopath. 2019;6:18-23.
47. Rani YS, Reddy VJ, Basha SJ, Koshma M, Hanumanthu G, Swaroopa P. A review on Solanum nigrum. World J. Pharm. Pharm. Sci. 2017 Oct 1;6:293-303.
48. Kaushik D, Jogpal V, Kaushik P, Lal S, Saneja A, Sharma C, Aneja KR. Evaluation of activities of Solanum nigrum fruit extract. Archives of Applied Science Research. 2009;1(1):43-50.
49. Atanu, F. O., U. G. Ebiloma, and E. I. Ajayi. "A review of the pharmacological aspects of Solanum nigrum Linn." Biotechnol Mol Biol Rev 6.1 (2011): 1-7.
50. Kaunda JS, Zhang YJ. The genus solanum: an ethnopharmacological, phytochemical and biological properties review. Natural products and bioprospecting. 2019 Apr;9(2):77-137.
51. Daunay MC, Laterrot H, Janick J. Iconography of the Solanaceae from antiquity to the XVIIth century: A rich source of information on genetic diversity and uses. InVI International Solanaceae Conference: Genomics Meets Biodiversity 745 2006 Jul 23 (pp. 59-88).
52. Santhosh Sivan V, Kumar S. AN UPDATED REVIEW ON SOLANUM NIGRUM WITH DYNAMIC ROLE.
53. Dunal MF (1813) Histoire naturelle, médicale et économique des Solanum et des genres qui ont été confundus avec eux. Renaud, Montpellier.
54. Medha K, Patki PS. Solanum nigrum-a review. Biomed. 2009;4(2):99-108.
55. Culpeper N. The English Physician Enlarged with Three Hundred and Sixty-nine Medicines, Made of English Herbs... By Nich. Culpepper.. John Binns; 1799.
56. Gerard J. The herbal or general history of plants: the complete 1633 edition as revised and enlarged by Thomas Johnson. Courier Dover Publications; 2015 Sep 16.
57. Ontita EG, Onyango CM, Nyamongo D. Indigenous Knowledge on the Uses of African Nightshades Solanum Nigram L. Species Among Three Kenyan Communities.
58. Matasyoh LG, M Murigi H, C Matasyoh J. Antimicrobial assay and phyto-chemical analysis of Solanum nigrum complex growing in Kenya. MICROBIOLOGY RESEARCH. 2014 Dec 10.
59. Singh BK, Kumar A. Chemical examination of Solanum nigrum Linn. Part I. The component fatty acids and the probable glyceride structure of the fatty oil from seeds. InProceedings of the Indian Academy of Sciences-Section A 1945 Nov (Vol. 22, No. 5, p. 310). New Delhi: Springer India.
60. Younes LS, Shibli RA, Tahtamouni RW, Al-Qudah TS, Al Hawmdeh F. Micropropagation of Black Nightshade (Solanum nigrum L.): A promising medicinal plant in Libya. The Libyan Journal of Agriculture. 2020 Mar 14;24(2).
61. Hussein S, Dhabe A. Ethnobotanical study of folk medicinal plants used by villagers in Hajjah district-Republic of Yemen. Journal of Medicinal Plants Studies. 2018 Sep;6(5):24-30.
62. Al-Fatimi M, Wurster M, Schröder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of ethnopharmacology. 2007 May 22;111(3):657-66.
63. Gogoi P, Islam M. Phytochemical screening of Solanum nigrum L and S. myriacanthus Dunal from districts of upper Assam, India. IOSR J Pharm. 2012 Jun;2(3):455-9.
64. Al-Qura'n S. Ethnobotanical survey of folk plants in southern part of Jordan. Toxicon. 2005 Aug 1;46(2):119-29.
65. Judd NL. Laau Lapaau: herbal healing among contemporary Hawai'ian healers. Pacific Health Dialog. 1998;5:239-45.
66. Chen X, He X. Solanum nigrum Linn.: an insight into current research on traditional uses, phytochemistry, and pharmacology. Frontiers in Pharmacology. 2022 Aug 16;13:918071.
67. Wang, Y., Xiang, L., Yi, X., and He, X. (2017). Potential anti-Inflammatory steroidal saponins from the berries of solanum nigrum L. (European black nightshade). J. Agric. Food Chem. 65 (21), 4262–4272. doi:10.1021/acs.jafc. 7b00985
68. He, J., Zhou, C. D.,Ma, B. Z., Liu, F., Liu, X., and Zhao, T. (2015). Research Progress on Chemical Constituents and Antitumor Pharmacological Activities of Solanum nigrum. China Pharm. 26 (31), 4433–4436. doi:10.6039/j.issn.1001- 0408.2015.31.37
69. Wang, L. Y. (2007). Continue study on cytotoxic active constituents and study on quality control of Solanum nigrum L. Liaoning: Master, Shenyang Pharmaceutical University.
70. Sun, L., Zhao, Y., Li, X., Yuan, H., Cheng, A., and Lou, H. (2010). A lysosomalmitochondrial death pathway is induced by solamargine in human K562 leukemia cells. Toxicol vitro 24, 1504–1511. doi:10.1016/j.tiv.2010. 07.013
71. Ding, X., Zhu, F. S., Li, M., and Gao, S. G. (2012a). Induction of Apoptosis in Human Hepatoma SMMC-7721 Cells by Solamargine From Solanum nigrum L. J. Ethnopharmacol. 139, 599–604. doi:10.1016/j.jep.2011.11.058
72. Xiang, L., Wang, Y., Yi, X., and He, X. (2019). Steroidal alkaloid glycosides and phenolics from the immature fruits of Solanum nigrum. Fitoterapia 137, 104268. doi:10.1016/j.fitote.2019.104268
73. Eltayeb EA, Al-Ansari AS, Roddick JG. Changes in the steroidal alkaloid solasodine during development of Solanum nigrum and Solanum incanum. Phytochemistry. 1997 Oct 1;46(3):489-94.
74. Jabamalairaj A, Priatama RA, Heo J, Park SJ. Medicinal metabolites with common biosynthetic pathways in Solanum nigrum. Plant Biotechnology Reports. 2019 Aug 12;13:315-27.
75. Zhang Q, Liu D, Cui Y, Xu T, Lu T, Liu X, Liu K, Wang Q, Li A, Zhao P, Cheng Z. Bioactivities and chemical profiling comparison and metabolomic variations of polyphenolics and steroidal glycoalkaloids in different parts of Solanum nigrum L. Phytochemical Analysis. 2023 Oct 18.
76. Shibli RA, Tahtamouni RW, AlQudah TS, Abu-Iramaileh BB. Improving solanine production in in vitro cultures of Solanum nigrum L. using different chemical and physical factors. Journal of Agricultural and Marine Sciences [JAMS]. 2019;24:51-62.
77. Chu, Z. X., Lu, M., and Xiong, S. (2019). Research Advances on Pharmacological Activities and Pharmacokinetics of Scopoletin. Chem. Res. 30 (14), 434–440. doi:10.14002/j.hxya.2019.04.017
78. Sabudak, T., Ozturk, M., and Alpay, E. (2017). New bioflavonoids from Solanum nigrum L. by anticholinesterase and anti-tyrosinase activities-guided fractionation. Rec. Nat. Prod. 11 (2), 130–140.
79. Alam MN, Roy S, Anisuzzaman SM, Rafiquzzaman M. Antioxidant activity of the ethanolic extracts of leaves, stems and fruits of Solanum nigrum. Pharmacogn. Commun. 2012 Dec 19;2:67-71.
80. Veerapagu M, Jeya KR, Sankaranarayanan A, Rathika A. In vitro antioxidant properties of methanolic extract of Solanum nigrum L. fruit. The Pharma Innovation. 2018 May 1;7(5, Part F):371.
81. Campisi A, Acquaviva R, Raciti G, Duro A, Rizzo M, Santagati NA. Antioxidant activities of Solanum nigrum L. leaf extracts determined in in vitro cellular models. Foods. 2019 Feb 8;8(2):63.
82. Wang, L. Y., Wang, N. L., and Yao, X. S. (2007). Non-Saponins from Solanum nigrum L. Zhong Yao Cai 30 (7), 792–794. doi:10.13863/j.issn1001-4454
83. Gao, S, H., Su, Z. Z., Yang, L. J., and Li, Z. Y. (2021). Chemical Components From Stems of Solanum nigrum by LC-MS and NMR. Chin. Tradit. Herb. Drugs. 52 (5), 1263–1273. doi:10.7501/J.issn.0253-2670.2021.05.006
84. Liu, Y. (2003). Study on the composition of polysaccharices from Solanum Nigrum L. J. Chang. Univ. Sci. Technol. Nat. Sci. 15 (4), 20–21.
85. Liu, T. (2005). The studies on the extraction, purification and determination of active compositions in solanum nigrum L. Hunan: Master, Central South University
86. Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE. Mineral and phytochemical contents in leaves of Amaranthus hybridus L and Solanum nigrum L. subjected to different processing methods. African Journal of Biochemistry Research. 2008 Feb;2(2):040-4.
87. Yang, M. Y., Hung, C. H., Chang, C. H., Tseng, T. H., and Wang, C. J. (2016). Solanum nigrum suppress angiogenesis-mediated tumor growth through inhibition of the AKT/mTOR pathway. Am. J. Chin. Med. 44 (16), 1273–1288. doi:10.1142/S0192415X16500713
88. Tian, H. L., Yu, S. W., Pei, Y., Shen, Z. D., Dong, R. J., and Kang, D. Z. (2019). Effects of crude polysaccharide from Solanum nigrum L. on immune system of mice. J. Yanbian Med. Coll. 42 (1), 8–11. doi:10.16068/j.1000-1824.2019.01.003
89. Sivaraj, C., Yamini, S., Yahavi, A., Kumar, R. P., Arumugam, P., and Manimaaran, A. (2020). Antioxidant, antimicrobial activities and GCMS analysis of fruit extract of Solanum nigrum L. J. Pharmacogn. Phytochem. 9 (4), 1114–1121.
90. Lin, H. M., Tseng, H. C., Wang, C. J., Lin, J. J., Lo, C. W., and Chou, F. P. (2008). Hepatoprotective effects of Solanum nigrum Linn extract against CCl(4)- induced oxidative damage in rats. Chem. Biol. Interact. 171, 283–293.

doi:10.1016/j.cbi.2007.08.008
91. Hsu, J. D., Kao, S. H., Tu, C. C., Li, Y. J., and Wang, C. J. (2009). Solanum nigrum l. Extract Inhibits 2-Acetylaminofluorene-Induced Hepatocarcinogenesis Through Overexpression of Glutathione s-Transferase and Antioxidant enzymes. J. Agric. Food Chem. 57, 8628–8634. doi:10.1021/jf9017788
92. Chester, K., Zahiruddin, S., Ahmad, A., Khan, W., Paliwal, S., and Ahmad, S. (2019). Bioautography-Based Identification of Antioxidant Metabolites of Solanum nigrum L. and Exploration its Hepatoprotective Potential Against D-Galactosamine-Induced Hepatic Fibrosis in Rats. Pharmacogn. Mag. 15, 104–110. doi:10.4103/pm.pm_359_18
93. Yang, Y., Hu, X. X., Zhou, L. L., Gao, S. L., and Ding, X. (2014). Protective effect of Solanum nigrum polysaccharide on CCl4 induced acute liver injury in mice. Chin. Tradit. Pat. Med. 36 (12), 2602–2605. doi:10.3969/j.issn.1001-1528.2014. 12.036
94. Gupta AK, Ganguly P, Majumder UK, Ghosal S. Hepatoprotective and antioxidant effects of total extracts and stereoidal saponins of Solanum xanthocarpum and Solanum nigrum in paracetamol induced hepatotoxicity in rats. Pharmacologyonline. 2009;1(27):757-68.
95. Sankaran M. Protective effect of Solanum nigrum fruit extract on the functional status of liver and kidney against ethanol induced toxicity. Journal of Biochemical Technology. 2012 Feb 20;3(4):339-43.
96. Sultana S, Perwaiz S, Iqbal M, Athar M. Crude extracts of hepatoprotective plants, Solanum nigrum and Cichorium intybus inhibit free radical-mediated DNA damage. Journal of ethnopharmacology. 1995 Mar 1;45(3):189-92.
97. Elshater AE, Salman MM, Mohamed SA. The hepato-ameliorating effect of Solanum nigrum against CCL4 induced liver toxicity in Albino rats. Egyptian academic journal of biological sciences. C, physiology and molecular biology. 2013 Jun 1;5(1):59-66.
98. Elhag RA, El Badwi SM, Bakhiet AO, Galal M. Hepatoprotective activity of Solanum nigrum extracts on chemically induced liver damage in rats. Journal of Veterinary Medicine and Animal Health. 2011 Aug;3(4):45-50.
99. Guediri I, Boubekri C, Smara O, Lanez T. Total phenolic contents and determination of Antioxidant activity by DPPH, FRAP, and cyclic voltammetry of the fruit of Solanum nigrum (black nightshade) growing in the south of Algeria.2021:1-9
100. Lin HM, Tseng HC, Wang CJ, Chyau CC, Liao KK, Peng PL, Chou FP. Induction of autophagy and apoptosis by the extract of Solanum nigrum Linn in HepG2 cells. Journal of agricultural and food chemistry. 2007 May 2;55(9):3620-8.
101. Jainu M, Devi CS. Antioxidant effect of methanolic extract of Solanum nigrum berries on aspirin induced gastric mucosal injury. Indian journal of clinical biochemistry. 2004 Jan;19:57-61.
102. He, J., Zhou, C. D.,Ma, B. Z., Liu, F., Liu, X., and Zhao, T. (2015). Research Progress on Chemical Constituents and Antitumor Pharmacological Activities of Solanum nigrum. China Pharm. 26 (31), 4433–4436. doi:10.6039/j.issn.1001- 0408.2015.31.37
103. An, H. J., Kwon, K. B., Cho, H. I., Seo, E. A., Ryu, D. G., Hwang,W. J., et al. (2005). Solanum nigrum Produces Nitric Oxide via Nuclear Factor-kappaB Activation in Mouse Peritoneal Macrophages. Eur. J. Cancer Prev. 14 (4), 345–350.
104. Hsu, J. D., Kao, S. H., Tu, C. C., Li, Y. J., and Wang, C. J. (2009). Solanum nigrum l. Extract Inhibits 2-Acetylaminofluorene-Induced Hepatocarcinogenesis Through Overexpression of Glutathione s-Transferase and Antioxidant enzymes. J. Agric. Food Chem. 57, 8628–8634. doi:10.1021/jf9017788
105. Churiyah, C., Ningsih, S., and Firdayani, F. (2020). The Cytotoxic, Apoptotic Induction, aand Cell Cycle Arrest Activities of Solanum nigrum L. Ethanolic Extract on MCF-7 Human Breast Cancer Cell. Asian Pac J. Cancer Prev. 21 (12), 3735–3741.
106. Ye, L. F., and Gao, Z. W. (2019). Study of Solanum nigrum n-butanol extract on the proliferation of human colorectal cancer SW480 and its mechanism. World J. Integr. Tradit. West Med. 14 (3), 356–358+363.
107. Huang, M. M., Liu, M. Y., Li, B. H., and Li, K. (2020). Solanine Regulates Proliferation and Apoptosis of Gastric Cancer Cells By Targeting miR-140/ MACC1 pathway. Chin. J. Clin. Pharmacol. 36 (16), 2440–2443.
108. Yin, S., Jin, W., Qiu, Y., Fu, L., Wang, T., and Yu, H. (2022). Solamargine induces hepatocellular carcinoma cell apoptosis and autophagy via inhibiting LIF/miR- 192-5p/CYR61/Akt signaling pathways and eliciting immunostimulatory tumor microenvironment. J. Hematol. Oncol. 15 (32), 1–6.
109. Wang, Y., Hong, T., Chen, L., Chu, C., Zhu, J., Zhang, J., et al. (2020). The natural extract degalactotigonin exerts antitumor effects on renal cell carcinoma cells through repressing YAP. Transl. Cancer Res. 9 (12), 7550–7561.
110. Wen Z, Huang C, Xu Y, Xiao Y, Tang L, Dai J, Sun H, Chen B, Zhou M. α-Solanine inhibits vascular endothelial growth factor expression by down-regulating the ERK1/2-HIF-1α and STAT3 signaling pathways. European journal of pharmacology. 2016 Jan 15;771:93-8.
111. Ding, X., Zhu, F., and Gao, S. (2012). Purification, Antitumour and Immunomodulatory Activity of Water-Extractable and Alkali-Extractable Polysaccharides From Solanum nigrum L. Food Chem. 131, 677–684.
112. Nallusamy S, Mannu J, Ravikumar C, Angamuthu K, Nathan B, Nachimuthu K, Ramasamy G, Muthurajan R, Subbarayalu M, Neelakandan K. Shortlisting phytochemicals exhibiting inhibitory activity against major proteins of SARS-CoV-2 through virtual screening.
113. Sharma D, Joshi M, Apparsundaram S, Goyal RK, Patel B, Dhobi M. Solanum nigrum L. in COVID-19 and post-COVID complications: a propitious candidate. Molecular and Cellular Biochemistry. 2023 Oct;478(10):2221-40.
114. Pu, Y. W. (2020). Extraction and isolation of Solanum nigrum polysaccharide and its immunomodulatory mechanism. Chongqing: Master, Chongqin Medical University
115. Tian, H. L., Yu, S. W., Pei, Y., Shen, Z. D., Dong, R. J., and Kang, D. Z. (2019). Effects of crude polysaccharide from Solanum nigrum L. on immune system of mice. J. Yanbian Med. Coll. 42 (1), 8–11.
116. Mazher, M., Anjum, M., Mushtaq, W., Noshad, Q., and Malik, Z. N. (2017). Antifungal assay of Solanum nigrum Linn. fruit, leaves and stems extracts in different solvents. Int. J. Biosci. 10 (4), 380–385.
117. Ge, J. Q. (2019). Inhibitory Effects of Plants Extract of Trapa pinosa and Solanum Nigrum on Pathogenic Bacteria. J. Jingling, Tech. 35 (1), 88–92 Sivaraj, C., Yamini, S., Yahavi, A., Kumar, R. P., Arumugam, P., and Manimaaran, A. (2020). Antioxidant, antimicrobial activities and GCMS analysis of fruit extract of Solanum nigrum L. J. Pharmacogn. Phytochem. 9 (4), 1114–1121.
118. Campisi, A., Acquaviva, R., Raciti, G., Duro, A., Rizzo, M., and Santagati, N. A. (2019). Antioxidant Activities of Solanum nigrum L. Leaf Extracts Determined in In Vitro Cellular Models. Foods 8 (2), 63. doi:10.3390/foods8020063
119. Sivaraj, C., Yamini, S., Yahavi, A., Kumar, R. P., Arumugam, P., and Manimaaran, A. (2020). Antioxidant, antimicrobial activities and GCMS analysis of fruit extract of Solanum nigrum L. J. Pharmacogn. Phytochem. 9 (4), 1114–1121.
120. Teng, F., Yuan, C. P., and Wang, P. (2014). Study on antioxidant activity of extracts from Solanum nigrum L.berries and analysis of the active ingredients. J. Anhui Agric. Sci. 42 (19), 6217–6219.
121. Ogunsuyi, O. B., (2018). Green leafy vegetables from two Solanum spp. (Solanum nigrum L and Solanum macrocarpon L) ameliorate scopolamine-induced cognitive and neurochemical impairments in rats. Food Sci. Nutr. 6, 860–870.
122. Peng, C. H.,Cheng, J. J.,Yu,M.H., Chung, D. J.,Huang,C.N., andWang, C. J. (2020). Solanum nigrum polyphenols reduce body weight and body fat by affecting adipocyte and lipid metabolism. Food Funct. 11, 483–492.
123. Sharma, B. K., Iyer, D., and Patil, U. K. (2012). Bioactivity guided fractionation in experimentally induced hyperlipidemia in rats and characterization of phytoconstituent from Solanum nigrum. J. Herbs, Spices Med. Plants 18, 257–267.
124. Varshney P, Vishwakarma P, Sharma M, Saini M, Bhatt S, Singh G, Saxena KK. Cardioprotective effect of Solanum nigrum against doxorubicin induced cardiotoxicity-an experimental study. Int J Basic Clin Pharmacol. 2016 May;5(3):748-53.
125. Mei, Q. X., Zhang, Z. Q., Lin, H., Guan, J., Jiang, Q. M., and Li, H. N. (2011). Research progress on the pharmacological effects and clinical application of Solanum nigrum. China Pharm. 23 (39), 3735–3737.
126. Yang, X. F., Wen, Q. X., and Zhang, J. R. (2017). To investigate the effect of morel on prevention and treatment of liver cancer. Chin. J. Integr. Tradit. West. Med. Liver Dis. 27 (06), 353–355
127. Huang, D. B., and Guan, J. (2013). Clinical Study of Solanum nigrum Mixture on Quality of Life and Immune Function in Patients with Advanced Liver Cancer. Lishizhen Med. Mater. Med. Res. 24 (07), 1676–1678.
128. Sugunabai J, Jayaraj M, Karpagam T, Varalakshmi BJ. Antidiabetic efficiency of Moringa oleifera and Solanum nigrum. Int J Pharm Pharm Sci. 2014;6(Suppl 1):40-2.
129. Shree GK, Parvathi S, Ramkumar PS, Priya SS. Pharmacological and phytochemical evaluation of anti-ulcerogenic potential of Solanum nigrum. International Journal of Pharmaceutical Sciences and Research. 2012 Aug 1;3(8):2837.
130. https://www.efsa.europa.eu/sites/default/files/topic/ndaart13ref04.pdf
131. Kaláb, M.; Krechler, T. The effect of the heptoprotective agent LIV 52 on liver damage. Cas. Lek. Ceskych **1997**, 136, 758–760.
132. Siregar, G.; Paramesh, R.; Kumawat, R.; Palaniyamma, D.; Srikrishna, H.; Company, M.T.H.D. A prospective, interventional clinical study to evaluate the safety and efficacy of Liv.52 DS in the management of non-alcoholic fatty liver disease. Eur. J. Clin. Exp. Med. **2021**, 19, 129–136.
133. Girish, C.; Koner, B.C.; Jayanthi, S.; Rao, K.R.; Rajesh, B.; Pradhan, S.C. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian J. Med. Res. **2009**, 129, 569.
134. Himalaya Liv.52, 100 Tablets—Uses, Ingredients, Side Effects—Himalaya Wellness (India).https: //himalayawellness.in/products/liv-52.
135. Karandikar, S.M.; Joglekar, G.V.; Chitale, G.K.; Balwani, J.H. Protection by indigenous drugs against hepatotoxic effects of carbon tetrachloride—A long term study. Acta Pharmacol. Toxicol. **1963**, 20, 274–280.
136. Germano, M.P.; De Pasquale, R.; D'Angelo, V.; Catania, S.; Silvari, V.; Costa, C. Evaluation of extracts and isolated fraction from Capparis spinosa L. buds as an antioxidant source. J. Agric Food Chem. **2002**, 50, 1168–1171.
137. Gilani, A.; Janbaz, K.; Shah, B. ESCULETIN PREVENTS LIVER DAMAGE INDUCED BY PARACETAMOL AND CCL4. Pharmacol. Res. **1998**, 37, 31–35.
138. Sumitra, M.; Manikandan, P.; Kumar, D.A.; Arutselvan, N.; Balakrishna, K.; Manohar, B.M.; Puvanakrishnan, R. Experimental myocardial necrosis in rats: Role of arjunolic acid on platelet aggregation, coagulation and antioxidant status. Mol. Cell. Biochem. **2001**, 224, 135–142.
139. Sultana, S.; Perwaiz, S.; Iqbal, M.; Athar, M. Crude extracts of hepatoprotective plants, Solanum nigrum and Cichorium intybus inhibit free radical-mediated DNA damage. J. Ethnopharmacol. **1995**, 45, 189–192.
140. Jafri, M.; Subhani, M.; Javed, K.; Singh, S. Hepatoprotective activity of leaves of Cassia occidentalis against paracetamol and ethyl alcohol intoxication in rats. J. Ethnopharmacol. **1999**, 66, 355–361.
141. Candan, F.; Unlu, M.; Tepe, B.; Daferera, D.; Polissiou, M.; Sökmen, A.; Akpulat, H. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). J. Ethnopharmacol. **2003**, 87, 215–220.
142. Sandhir, R.; Gill, K.D. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian J. Exp. Biol. **1999**, 37, 762–766.
143. Sandhir, R.; Gill, K.D. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian J. Exp. Biol. **1999**, 37,762–766.
144. Mitra, S.K.; Varma, S.R.; Godavarthi, A.; Nandakumar, K.S. Liv.52 regulates ethanol induced PPARgamma and TNF alpha expression in HepG2 cells. Mol. Cell. Biochem. **2008**, 315, 9–15.
145. Singh, B.; Dhawan, D. Role of Liv. 52–A Herbal Formulation on 14C-Ethanol Metabolism and 14C-Acetaldehyde Accumulation in Rat Liver. Indian J. Nucl. Medicine. **2000**, 15, 27–29
146. Chauhan, B.; Mohan, A.; Kulkarni, R.; Mitra, S. Bioassay for evaluation of the hepatoprotective effect of Liv.52, a Polyherbal formulation, on ethanol metabolism in chronic alcohol- Exposed rats. Indian J. Pharmacol. **2022**, 26, 117.
147. Gopumadhavan, S.; Jagadeesh, S.; Chauhan, B.L.; Kulkarni, R.D. Protective effect of Liv.52 on alcohol-induced fetotoxicity. Alcohol Clin. Exp. Res. **1993**, 17, 1089–1092.
148. Chauhan, B.L.; Kulkarni, R.D. Effect of Liv.52, a herbal preparation, on absorption and metabolism of ethanol in humans. Eur. J. Clin. Pharmacol. **1991**, 40, 189–191.
149. Kulkarni, R.D.; Chauhan, B.L. Blood, urine ethanol and acetaldehyde levels from six different alcoholic beverages and effect of liv.52. Eur. J. Pharmacol. **1990**, 183, 1865–1866.
150. Prakash Kolasani, B.; Sasidharan, P.; Divyashanthi, C.M.; Jayabal, P.; Rajaseharan, A. Prescribing pattern of drugs in patients with alcoholic liver disease in a tertiary care teaching hospital. Natl. J. Physiol. Pharm Pharmacol. **2017**, 7, 538.
151. Agal, S.; Prasad, S. Liv.52 DS Tablets Evaluation of Efficacy and Safety in Alcoholic Liver Cirrhosis. Med. Update **2007**, 15, 25–32.
152. Mahto, A.; Sailal, M. Study of the efficacy and safety of Liv.52 DS tablets in alcoholic hepatitis: Clinical, biochemical and ultrasonographic evaluation. Indian Med. J. **2009**, 103, 150–158.
153. De Silva, H.A.; Saparamadu, P.A.M.; Thabrew, M.I.; Pathmeswaran, A.; Fonseka, M.M.D.; De Silva, H.J. Liv.52 in alcoholic liver disease: A prospective, controlled trial. J. Ethnopharmacol. **2003**, 84, 47–50.
154. Nikam, P.S.; Nikam, S.V.; Sontakke, A. Pharmacie globale international journal of comprehensive pharmacy liv.52 in alcoholic hepatitis. Pharm Glob. **2011**, 2011, 10.
155. Kolhapure SA, Mitra SK. Meta-analysis of 50 phase III clinical trials in evaluation of efficacy and safety of Liv. 52 in infective hepatitis. Med update. 2004;12(2):51.
156. Kantharia C, Kumar M, Jain MK, Sharma L, Jain L, Desai A. Hepatoprotective Effects of Liv. 52 in Chronic Liver Disease Preclinical, Clinical, and Safety Evidence: A Review. Gastroenterology Insights. 2023 Jul 31;14(3):293-308.
157. Ganesh S, Joshi N, Jain MK, Sharma L, Desai A, Rafiq M, Babu UV, Kumawat R. Clinical and Safety Evaluation of Liv. 52 in Alcoholic Liver Disease: A Review. Gastroenterology Insights. 2022 Nov 13;13(4):377-86.
158. Personal communication 21.12.2023 Dr Gopumadhavan General manager-Preclinical Toxicology Himalaya Wellness Company
159. Lai, Y. H., Ma, Z. C., Yan, H. Y., and Mao, H. X. (2005). Acute Toxicity and Genetic Toxicity Tests of Solanum Nigrum L Juice. Carcinog. Teratog. Mutagen. 17 (3), 54–58
160. Mo, L. J., He, D., Zhou, C. R., and Ling, L. L. (2014). Experimental study on acute toxicity of raw Solanum nigrum and Solanum nigrum juice. China health care Nutr. 5, 2889–2890.
161. Le Son H, Yen PT. Preliminary Phytochemical, Acute Oral Toxicity and Anticonvulsant

Activity of the Leaves of Solanum Nigrum Linn. Life Science Journal. 2014;11(2):204-8.
162. Sharma A, Mehta D, Ahmed S, Sharma S. International Journal of Modern Pharmaceutical Research.
163. Mukhopadhyay G, Sarkar S, Kundu S, Kundu S, Sarkar P, Sarkar S, Sengupta R, Kumar C, Mitra S, Jain D, Sodani A. Ethno-pharmacological activity of Solanum nigrum. Pharma Innov Journey. 2018;7(10):692-8.
164. Rumiyati R, Muna LN, Hidayati DN, Jenie RI. Acute toxicity and genotoxic activity of leunca (Solanum nigrum L.) herb ethanolic extract. Indonesian Journal of Cancer Chemoprevention. 2015 Mar 1;6(1):30-4.
165. Patel A, Biswas S, Haneefa Shoja M, Venkata Ramalingaya G, Nandakumar K. Protective effects of aqueous extract of Solanum nigrum Linn. Leaves in rat models of oral mucositis. The Scientific World Journal 2014; 1-10.
166. Aryaa A and Viswanathswamy AH. Effect of Solanum nigrum Linn on acute and subacute inflammation. Journal of Young Pharmacists 2017; 9(4): 566-570.
167. Ganguly P, Gupta AK, Majumder UK, Ghosal S. The chemistry behind the toxicity of Black Nightshade, - Solanum nigrum and the remedy. Pharmacologyonline 2009; 1: 705-723.
168. Kibichiy SE, Ngure RM, Chome J, Korir SC, Mdachi RE, Mburu JN. Effect of long-term administration of Solanum nigrum extracts in female Swiss White mice infected with Trypanosoma Brucei Rhodesiense. Science Journal of Medicine and Clinical Trials 2013; 1-8.
169. WHO Food additive series 30, 764. Solanine and chacocine. http://www.inchem.org /documents/jecfa/jecmono/v30je19.htm
170. Rompelberg CJM, Sips AJAM, van Twillert K, Mensinga Tj, van den Top HJ, Meulenbelt J, van Egmond HP. RIVM Report (National Institute of Public Health and Environment); 2001: 1-77
171. Slanina P. Solanine (glycoalkaloids) in potatoes: Toxicological evaluation. Food and Chemical Toxicology 1990; 28(11): 759-761.
172. Friedman M, McDonald GM, Filadelfi-Keszi M (22 September 2010). "Potato Glycoalkaloids: Chemistry, Analysis, Safety, and Plant Physiology". Critical Reviews in Plant Sciences. **16** (1): 55–132.
173. Beier R (1990). Reviews of Environmental Contamination and Toxicology. Springer New York. ISBN 978-1-4612-7983-9.
174. Jadhav SJ, Sharma RP, Salunkhe DK (26 September 2008). "Naturally Occurring Toxic Alkaloids in Foods". CRC Critical Reviews in Toxicology. **9** (1): 21–104.
175. Sc M, Th L (1984). "The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (Solanum tuberosum): a review". Food Technology in Australia.
176. Itkin M, Rogachev I, Alkan N, Rosenberg T, Malitsky S, Masini L, et al. Glycoalkaloid metabolism is required for steroidal alkaloid glycosylation and prevention of phytotoxicity in tomato. Plant Cell. 2011;23:4507–25.
177. Moehs CP, Allen PV, Friedman M, Belknap WR. Cloning and expression of solanidine UDP-glucose glucosyltransferase from potato. Plant J. 1997;11:227–36.