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Phytochemical and Hepatoprotective study of Solanum nigrum

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Abstract

Solanum nigrum is medicinal plants of solanaceae family. The flavonoids and steroids constituents posses more biological properties. Solamagine and solasodine present in alcoholic extract exhibits hepatoprotective properties. 3- 0- acetylbutulinic acid enhance potency of drugs. In CCl_4 induce hepatotoxic rats different liver enzyme showed significantly alteration in alcoholic extract treated groups. It shows preventive and curative responses in CCl_4 induced hepatotoxicity.

Key words: S. nigrum, SGOT, SGPT, Hepatotoxicity.

Introduction

India has an ancient birthright of traditional medicine. The material medica and medicinal plants of India provides a great deal of knowledge on the Ayurveda, folklore practices and various traditional aspects of therapeutic significance of natural products[1]. The plant extracted drugs even today remain important resources for treatment of various diseases especially in developing countries. Nature has provided a complete store-house of remedies to cure all aliments of mankind[2, 3]. This is where, nature provides us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown[4].

The *Sushruta Samhita* and the *Charaka Samhita* were influential works on traditional medicine during this era. *Solanum nigrum* is the most

variable species of the genus *Solanum*. These *Solanum* species display varying amounts of phenotypic variation, particularly in their vegetative features such as plant habit, leaf size and for, and stem winging. *Solanum nigrum* found in disturbed habitats, such as roadsides, often on arable land especially the edges of cultivated fields and plantations, in hedgerows, on railway cuttings, quaysides and rubbish tips, in areas around buildings and houses, under trees, on forest and grassland margins, as garden weeds, on shingle beaches, riverbanks and in gullies[2, 5]. Plants subglabrous to villous annuals up to 70-75 cm high, covered with simple multicellular hairs with glandular or eglandular heads. *S. nigrum* is a rich source of natural compounds with varying structural patterns and beneficial properties. To date, approximately 188 phytochemical compounds have been separated and identified from *S. nigrum*, containing steroids, alkaloids, organic acids, flavonoids, phenylpropanoids and theirglycosides, as well as other compounds[6, 7].

The effect of crude ethanolic extract of *S. nigrum* on blood sugar of albino rat after daily oral administration of dose at the level of 250 mg/kg b.wt. for five and seven days respectively[8]. It was noticed that the chronic administration for longer duration leads to significant decrease in blood sugar compared to control. Thus it can be concluded that *Solanum nigrum* has the anti-diabetic property[9].

S. nigrum is one of the richest sources of phenolic compounds like gallic acid, quercetin etc. which act as an active ingredient in controlling oxidation which results in the prevention of oxidative stress, acute liver toxicity and metabolic ailments such as diabetes, cardiovascular diseases and many types of cancer[10]. The role of S. nigrum is significant against acute liver toxicity due to presence of active ingredients. Out of all the parts, leaves and fleshy

portion of S. nigrum are used mostly for therapeutic functions. S. nigrum has the ability to act as an anti-tuberculosis, anti-viral, anti-oxidant and anti-inflammatory agent[11]. Antioxidant ability of S. nigrum is described by previous investigations, which prevents free radical oxygen species in hepatotoxicity. About 2 million deaths per year occuts due to liver toxicity worldwide and 60% of them have acute toxicity[12]. In an experimental design, phenylhydrazine has the ability to form reactive molecules like oxygen radicals and superoxide anions. These products causes the not only the lipid peroxidation but also the damage to membrane[13]. All the liver enzymes and bilirubin values are increased with the intake administration of single dose of phenylhydrazine, which in return cases the lipid peroxidation[14, 15]. The purpose of the study is to evaluate the potential of S. nigrum in carbon tetrachloride (CCl_4) induced acute liver toxicity by using different parameters like aspartate aminotransferase (AST) and alanine transaminase (ALT) etc.

Experimental

Collection of plant: The medicinal plants *Solanum nigrum* was identified and selected whole plant like stem, leaf, fruit and root were collected around agricultural field of Ayodhyay district U.P. in May 2022 and plant identified by the experts of botany department, Banaras Hindu University, Varanasi and also compare with herbarium specimen of *S. nigrum*. The total plants are washing thoroughly and dry in shade upto complete demonstration. Plant parts separated (stem, leaf and fruits, root) were clean and dried under shade. The dried plant materials were then ground well to fine powder (200 mace).

Preparation of extracts: Powdered plant materials were successively extracted with petroleum ether (60-80ºC), alcohol and acetone using soxhlet extractor. The extraction was continued for

62 hours. The petroleum ether, alcohol and acetone extracts were then filtered and distilled for concentration of extract and evaporate on water bath. Greenish brown and greenish black residues were obtained[16]. The yield of alcoholic extract was calculated. It was placed at 4ºC for phytochemical analysis and biological study.

Chromatographic Separation: The aqueous extract of *S. nigrum* was subjected to thin layer chromatography using silica gel G as stationary phase and petroleum ether: methanol (1:1) and petroleum ether: chloroform: methanol (5:2:1) asmobile phase. The chromatograms when developed provide seven and eight spots respectively[17] that showedzones for steroidal nucleus with Liebermann – Buchardvisualizing reagent.

Chemical and reagents: Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Novozym® 435, 10000 PLU/g) from *Candida Antarctica*, supported on a macroporous acrylic resin with a water content of 3 % (w/w) was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Chloroform, *n*-hexane was obtained from Fisher chemicals. Betulinic acid was purified from Malaysian *Callistemon speciosus* by previous method (Ahmad *et al*., 1999). Acetic anhydride was purchased from Acros Organics, Belgium. Ethyl acetate, celite® 545, Na_2SO_4, K_2CO_3 and HCl were purchased from Merck, Germany. All chemicals were of analytical reagent grade. 3-O-acetyl-betulinic acid could be prepared by the following procedure.

Identification of compounds: Active compounds are analysed on TLC study. Special group of compounds are isolated from column chromatography and purified by recrystallisation.

***CCl_4 induced hepatotoxicity*:** The Sprague-Dawley rats were divided into six groups, each

group had six animals. Group I (control) animals were administered a single daily dose of carboxymethyl cellulose (1 mL of 1%, w/v, p.o. body weight). Group II received carbon tetrachloride (1 mL/kg body weight, i.p. 1:1 v/v mixture of CCl_4 and liquid paraffin) alone while group III, IV and V received orally 100, 200 and 400 mg/kg body weight of extract of *Solanum nigrum* (1% w/v, CMC) respectively along with carbon tetrachloride as in group II. Group VI received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, p.o., along with carbon tetrachloride. The extract of *Solanum nigrum* was given daily while carbon tetrachloride was given for every 72h for 14 days. Animals were sacrificed 48 h after the last dose of the drug. The liver samples were dissected and blood was collected[18].

Result

The study examined the percentage yield of aqueous and ethanolic leaf crude extracts of *Solanum nigrum* as presented in Table-4.1.

Table-4.1: Percentage yield of *S. nigrum* leaves aqueous and ethanolic crude extracts

Extracts	Weight of powder residue (g)	Weight of extract (g)	Percentage yield (w/w)
Aqueous	100	9.07	8.92
Ethanol	100	8.16	8.16

T.L.C. study of alcoholic extract: Before reaching to most optimum solvent system a number of systems were employed. Chloroform crude extract was studied for thin layer chromatography

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(TLC) through different solvent systems. During the study TLC with solvent system Toluene: ethyl acetate: acetic acid (36:12:5) was most informative and showed five spots with R_f values of values 0.45, 0.46, 0.55, 0.82 (Table-4.3)[19, 20].

Table-4.2: Chromatographic results of alcoholic extract of *Solanum nigrum*

Conditions	Number of spots	R_f value
Short ultra violet (254nm)	10	0.01, 0.27, 0.33, 0.34, 0.40, 0.46, 0.49, 0.58, 0.71, 0.89
Long ultra violet (366 nm)	7	0.01, 0.27, 0.33, 0.41, 0.45, 0.71, 0.76
After spraying (Vaniline sulphuric acid)	8	0.07, 0.13, 0.15, 0.37, 0.48, 0.55, 0.68, 0.94

During the TLC different components were observed.

Selection of mobile phase for TLC alcoholic extract of S. nigrum

Extracts were analysed using thin layer chromatography to reveal most suitable solvent system suitable for separation of most of components present in extract. In randomly selected solvent system, Toluene: Ethyl Acetate: Formic acid (36: 12: 05), n-hexane: ethyl acetate: acetic acid (31:14:5) and n-hexane : ethyl acetate: formic acid (31:14:5)[21, 22] were found to be most effective in separation of components (Table-4.3). In which in first two solvent systems provided maximum spots (12). From previous publications it was observed that these solvent systems were used by in separation of components and they got good results using these solvent systems[23].

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Table-4.3: Showing TLC of different solvent systems

Solvent system	Ratio	Total spots observed	R_f value
Ethyl acetate : Acetic acid : Water	5:1:1	No spots	Negative
Chloroform : Methanol : Water	7:4:1	2	0.88, 0.91
n-hexane : ethyl acetate : formic acid	31:14:5	5	0.64, 0.67, 0.80, 0.85, 0.92
n-hexane : ethyl acetate : Acetic acid	31:14:5	7	0.53, 0.55, 0.58, 0.78, 0.81, 0.83, 0.90
Chloroform : Methanol : Glacial acetic acid	50:40:0.5	Taling	Negative
Toluene : Ethyl acetate : Acetic acid	36:12:5	12	0.65, 0.66, 0.72, 0.76, 0.82, 0.83, 0.84, 0.87, 0.89, 0.91, 0.97, 1

Spectral study of isolated compound

Isolated compound 1 appeared as white crystals. The structure of compound I was identified using H-1-NMR, C-13-NMR and 2-D data analysis. C-13-NMR revealed that compound 1 possesses an aglycone backbone related to a steroidal spirazolane-type alkaloid- four quaternary carbons at chemical shifts (□$_c$'s) 38.2 and 41.8 ppm were observed, including one linked to oxygen and nitrogen at □$_c$ 99.6 and one attached to a double bond at □$_c$ 142.1. Nine methane groups at □$_c$'c 31.8, 31.8, 42.9, 51.9, 57.9, 64.2, 79.5, 80.5 and 122.8 ten methylene groups at □$_c$'s 22.2, 30.9, 31.1, 32.9, 33.1, 33.4, 38.7, 39.7, 41.2 and 48.5 ppm and four methyl groups at □$_c$'s 15.6, 17.0, 19.9 and 19.9 ppm were identified[24, 25].

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The 1D NMR chemical shifts of the trisaccharide moiety indicated the structure O-[□-L-rhamnopyranosyl-(1→2)-O-[□-L-rhamnopyranosyl-(1→4)]-□ □ D-glucopyranoside. The proton NMR showed four distinctive aglycone methyl's at □$_c$ 0.83 (3H, s), 0.85 (3H, d, J [coupling constant] 6.4), 0.97(3H, d, J 7.2) and 1.05 (3H, s). Two multiples observed at □$_c$ 1.26 were attributed to 6-deoxyhexose methyl's, while a doublet at □$_c$ 5.38 with a J value of 4.6 was ascribed to an olefinic proton at position C-6. Four anomeric H-1-NMR resonances were identified at □$_c$ 4.34 (1H, m), 4.50 (1H, d, J 7.6), 4.84 (1H, s) and 5.21 (1H, s)[26, 27]. High resolution mass spectrometry (HRMS) data for compound 1 provided the molecular formula $C_{45}H_{73}NO_{15}$ with a molecular mass of m/z 868.5077 ($[M+H]^+$, 100%), identifying it as the steroidal alkaloid solamargine[28].

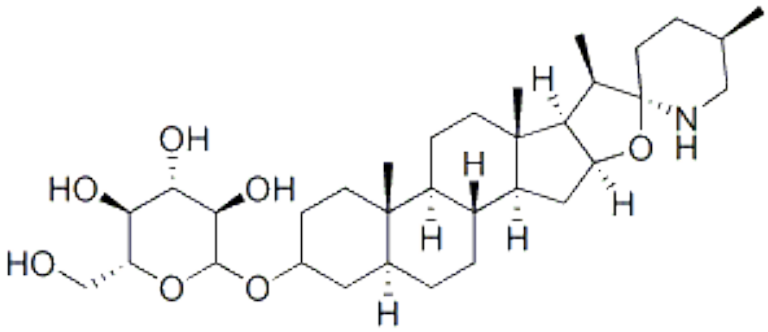

Figure:-1 Structure of Solamargine

Hepatoprotective Properties

Oral administration of CCl_4 significantly increase SGOT, SGPT and ALP. When animal were treated with *S. nigrum* extract for 7 days the toxicity of CCl_4 reduced due to which enzyme concentration reduced significantly[29, 30]. It was found that SGOT at 100 mg/kg b.w. treated group 164.1 in comparison to toxicant control 204.7 (1v/l)[31]. When extract dosages increase 100

mg/kg b.w. to 200 mg, 300 mg and 400 mg/kg b.w. SGOT concentration found 153.65, 143.5 and 136.4 (1v/l). Liver enzyme SGPT enhance by toxicant 30.20 to 109.57 on administration of toxicant PCM for 7 days. Prior administration of *S. nigrum* reduces 109.57 to 88.6, 81.5, 76.2, 72.7 (1v/l) in group 3, 4, 5 and 6. Alkaline phosphatase (ALP) also enhance on administration of toxicants from 142.2 to 430.5 (1v/l)[32, 33]. Prior administration of drug reduces formation of ALP in liver as shown in group 3, 4, 5 and 6. It was found that ALP in 100 mg/kg b.w. with toxicant was 344.4 (1v/l) reduces 291.40 in group 6 of 400 mg/kg b.w. treated Albino rats[34]. Hepatosites accumulates billirubin in their cell produced by toxicant administration. When toxicant CCl_4 administered billirubin enhance in blood 0.7 mg/dl to 1.05 mg/dl[35].

Table-4.4: Effect of S. nigrum on liver enzymes against CCl_4 induced toxicity

Group	SGOT (1v/l)	SGPT (1v/l)	ALP (1v/l)	Billirubin (mg/dl)	Protein (gm/dl)
Control	32.0±1.11	30.2±1.4	142.21±1.49	0.7±0.08	6.42±0.08
CCl_4 control	204.7±6.97	109.57±3.9	430.5±55.01	1.05±0.007	3.95±0.03
S.N. 100 mg + CCl_4	164.1±3.7	88.6±2.71	344.4±5.1	0.615±0.06	4.12±0.2
S.N. 200 mg + CCl_4	153.85±4.6	81.51±7.1	320.12±3.6	0.55±0.054	4.49±0.25
S.N. 300 mg + CCl_4	143.5±4.11	76.2±2.4	299.5±1.62	0.49±0.05	5.11±0.21
S.N. 400 mg + CCl_4	136.4±2.17	72.71±1.4	291.4±1.16	0.44±0.039	5.26±0.27

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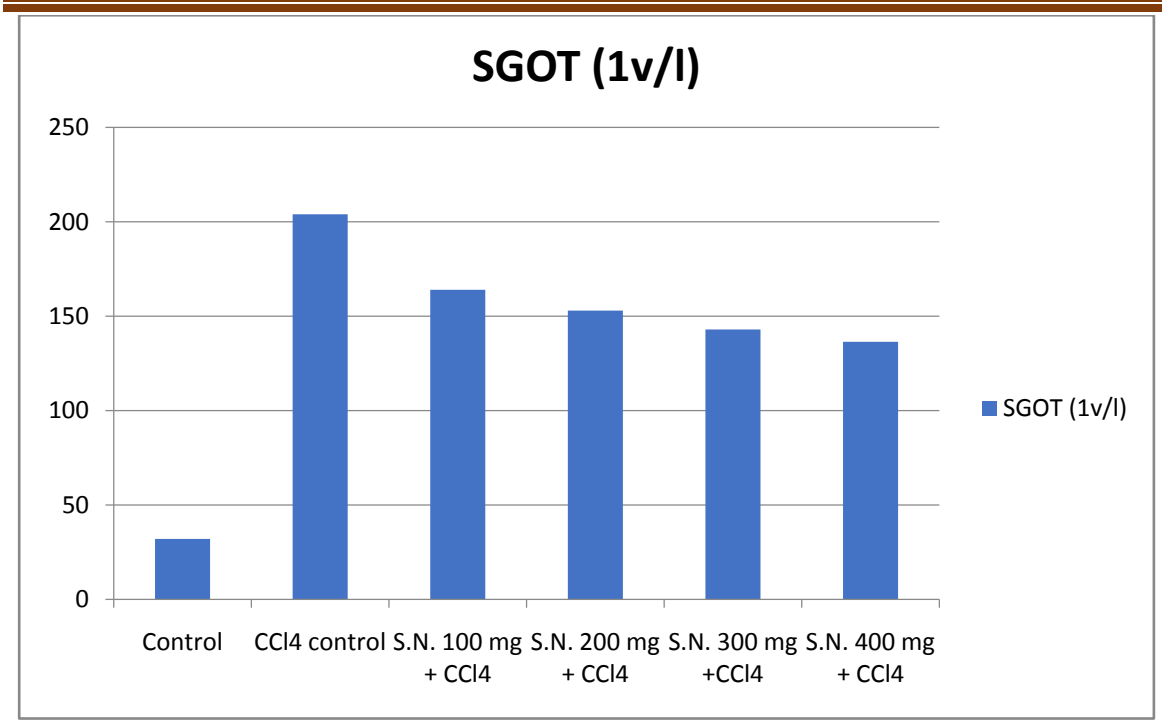

Figure:-2 Variation in SGOT value in blood of albino rats.

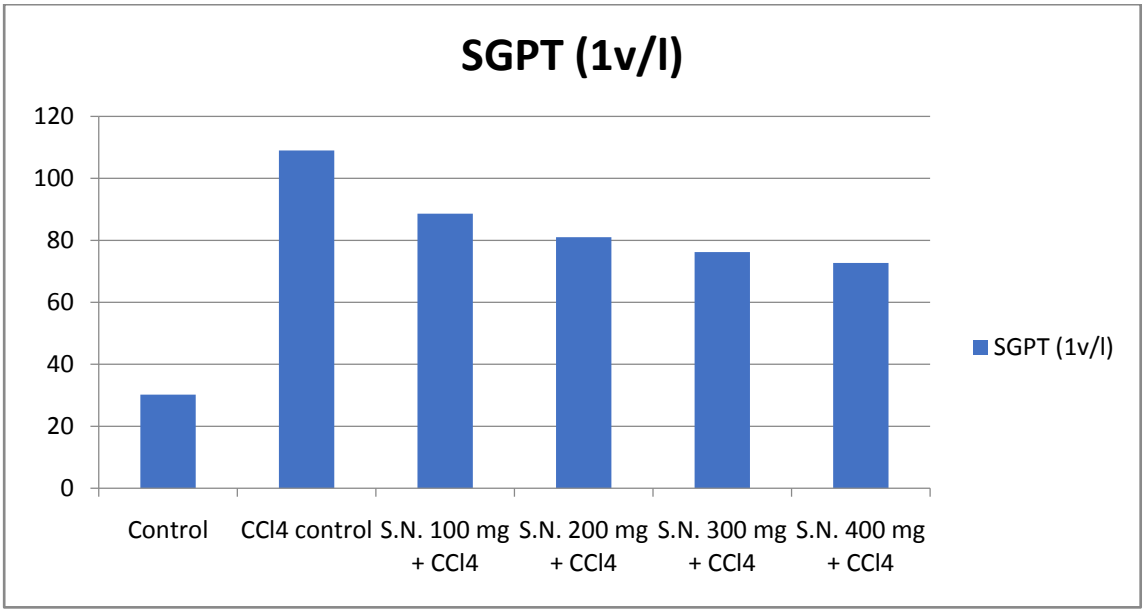

Figure:-3 Variation in SGPT value in blood of albino rats.

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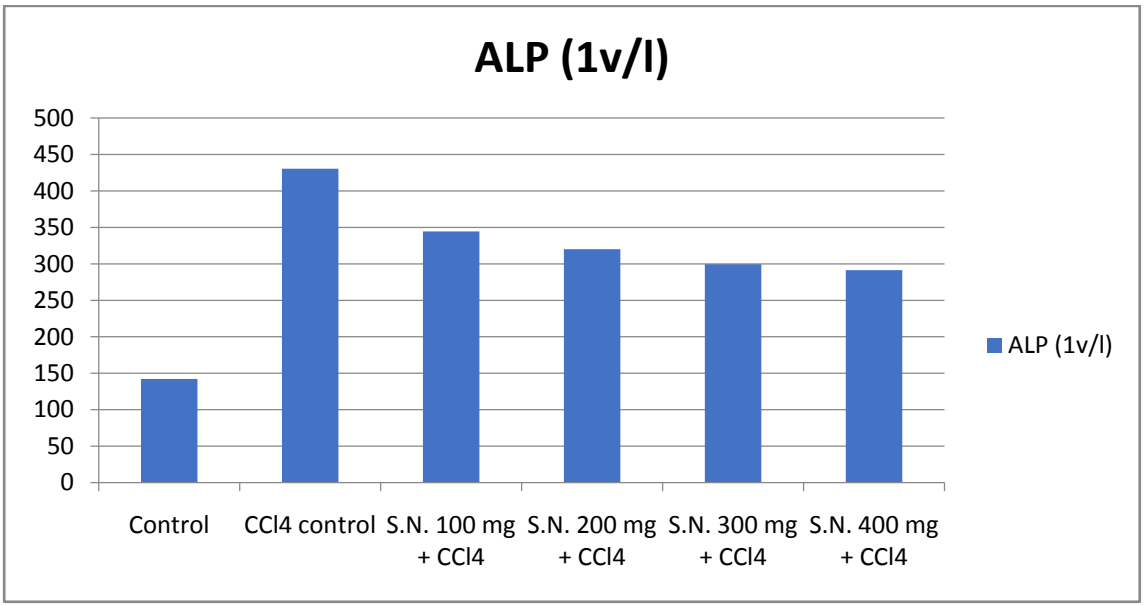

Figure:-4 Variation in ALP value in blood of albino rats.

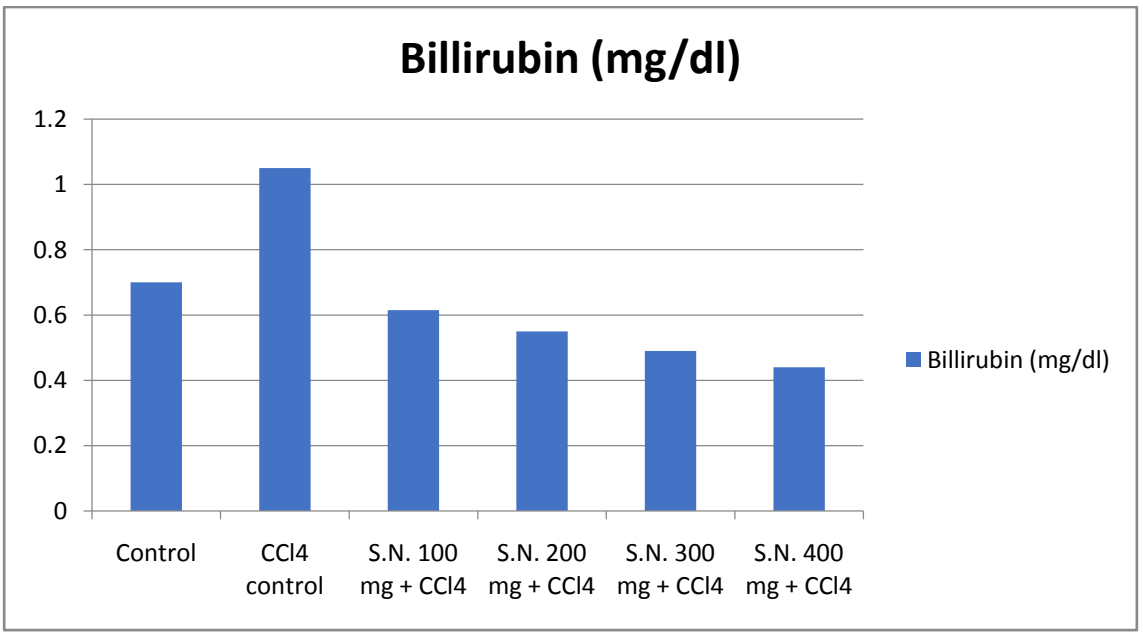

Figure:-5 Variation in Billirubin value in blood of albino rats.

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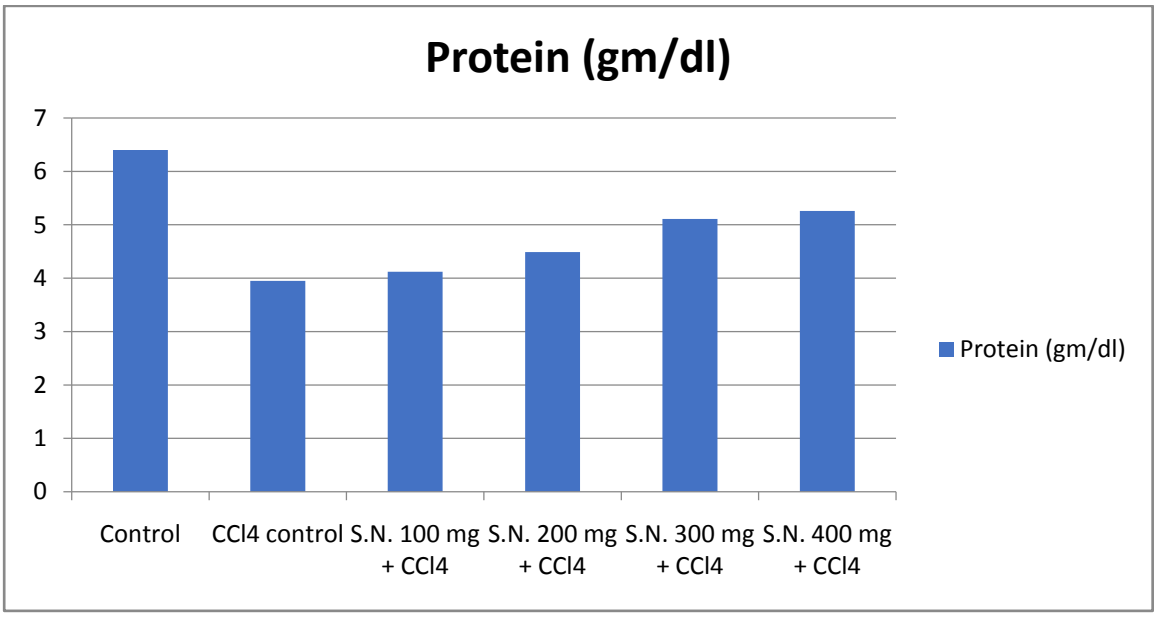

Figure:-6 Variation in Protein value in blood of albino rats.

When toxicant administered in group 3, 4, 5 and 6 billirubin concentration was found 0.615 mg/dl, 0.55 mg/dl, 0.49 mg/dl and 0.44 mg/dl. It indicate that *S. nigrum* retard mecrosis of hepatosites and reduced billirubin concentration in blood[36]. Protein is building unit of liver cell. When any toxicant produce inside the cell are administered orally it reduced protein concentration in blood serum. When CCl_4 administered in albino rats protein concentration was reduced 6.42 gm/dl to 3.95 gm/dl[37]. When CCl_4 administered along with different concentration of *S. nigrum* extract in group 3, 4, 5 and 6 the protein concentration again enhance in comparison to toxicant group as 4.12 gm/dl, 4.49 gm/dl, 5.11 gm/dl and 5.26 gm/dl. Active compounds present in *S. nigrum* minimize the effect of toxicant produce ROS which create

microsis hepatosites. Hepato protective effect of *S. nigrum* extract was further confirmed by histopathological studies of liver[38].

Conclusion: Solanum nigrum possess different phytoconstituent in which solamargine is one of them. Solamargine present in growthing parts of plants as considerable concentration. It has both preventive and curative role against CCl_4 induced liver toxicity. It is assume that active constitute interfere ROS synthesis in hepatocytes due to which necrosis in liver all minimize.

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