

EFFECT OF *SOLANUM NIGRUM* GLYCOALKALOID ON LIVER PROTEIN OF ALBINO RATS

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ABSTRACT

From medicinal plant *Solanum nigrum* (Solanaceae) phytochemically extracted glycoalkaloid from its CHCl_3 fraction by column chromatography. Spectral study and Co-TLC authenticate the structure of active principle Solasodine ($\text{C}_{27}\text{H}_{43}\text{NO}_2$). It has been claimed that *Solanum nigrum* particular of an excellent remedy for liver disorder. Present investigation is therefore to determine the effect of active compound Solasodine on protein content of liver and kidney after daily administration of dose at the level of 2 to 6 mg/kg b.wt. in albino rats for 4, 6 and 8 days respectively. It was noticed that the chronic administration for longer duration leads to significant increase in protein contents of liver and kidney. It has quicker response than alcoholic extract of whole plant. It is assumed that increase in protein content rapidly it also inhibits hepatocarcinoma cell growth by inducing G2/M phase arrest and apoptosis through synthesis of variety of protein.

Keywords: *Solanum nigrum*, liver, kidney, Solasodine, protein content.

INTRODUCTION

Solanum nigrum L., which belongs to the Solanaceae family, has been used traditionally to treat various ailments such as pain inflammation and fever¹. Locally known as 'Makoi'; this plant used in Indian system of medicine as analgesic, antiperiostic, antiphlogistic, diuretic, purgative and sedative treatment². The leaves, stem and roots are used as a poultice or wash to treat cancerous sores, boiled, leucoderma and wounds while extracts of the plant are claimed to possess anti-inflammatory, antispasmodic and vasodilator effects. Scientifically, most pharmacological studies reported that *S. nigrum* used for antitumor activity, antiulcerogenic, ulcer healing, hepatoprotective, cytoprotective, antioxidative, antimicrobial, molluscicidal, larvicidal, cercaricidal and centrally-mediated depressant activities³.

Medicinal property of plant derived from biochemical change in protein⁴. Antioxidant compound protects against oxidative damage, and include compounds to remove or repair damaged molecules. It can prevent/retard oxidation caused by free radicals and sufficient intake of antioxidant compounds is supposed to protect against disease. Our body produces many antioxidant enzymes such as superoxide,

dismutase, catalyses and glutathione peroxidase which neutralize of free radicals which produce disease. Plants which possess bulks antioxidant compound inhibit hepatocarcinoma cell growth by retarding free radical formation⁵.

Since *S. nigrum* is rich in polyphenolic compounds derived from naturally occurring substances⁶. Compounds reported *S. nigrum* are (i) steroidal glycoalkaloid-solasodine, 12 β , 27-dihydroxy solasodine, 23-o-acetyl-12 β -hydroxyl. Solasodine, N-methyl solasodine, solasonine (γ -solanigrine), α -solamargine, β -solamargine, solanavoil, solanine (α , β). Tomatidenol, solanocapsine, solasodi-3,5 ene, (ii) steroidal saponins-Diosgenin, Tigogenin, Desgalactotigonin, Nigrumnin I & II (iii) steroid-cholesterol, campesterol, stigmaterol and β -sisterol (iv) flavonoids-quercetin-3-glycosyl (1 \rightarrow 6) galactoside, quercetin-3-gentiobioside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-o-neohesperidoside, Isoquercitrin, Quercetin 3-o-(2^{gl}- α -rhamnosyl)- β -glucosyl (1 \rightarrow 6)- β -galactoside, quercetin. 3-o- α -rhamnosyl (1 \rightarrow 2)- β -galactoside (v) carotenoids- β -carotene (vi) vitamin s-vitamin c (vii) fatty acids-palmitic acid. Palmitoleic acid, stearic acid linoleic acid (viii) triterpenes-qualin (ix) oligofurostanosides-uttronin- β -D.

glucopyranosyl, utironin- β -D-xylopyranosyl, utlroside- β -D-glucosyl (x) Tricarboxylic acid-citric acid (xi) carbohydrates-fructose, D. glucose, L. Rhamnose⁷.

Steroidal glycoalkaloid containing 13 compounds possess variable properly as per growth of plants⁸. The unripe fruit of *S. nigrum* contain the highest concentration of toxin particularly solanine (α , β). Although there is a lot of disagreement over whether the leaves and fruits of *S. nigrum* are poisonous are not, but it is believed that the toxic effects vary considerably according to the part or cultivation of the plant being used or growth⁹.

The liver is largest gland and central metabolizing organ, so it is more responsible to metabolism dependent injury. Kidney is also most important organ of our body eliminate toxic substances from our body¹⁰. Our physiology and metabolism is governed by protein content present in form of enzyme or co-enzyme. Our present study delas the effect of active compound of *S. nigrum* i.e. glycoalkaloid on protein content of liver and kidney of albino rate.

MATERIAL AND METHODS

Plant material in ripening season *S. nigrum* plant was collected from rural area Jaunpur (U.P.) India and authenticated from Taxonomy Department of T.D.P.G. College, Jaunpur. It was shed dried and powdered in an electric grinder. The powdered plant material was extracted. The dried plant powder was dipped in n-hexane for 1 min to ensure the removal of surface fats and epicuticular was without disturbing the interior chemical makeup so that they may not interfere in alkaloid analysis.

Estimation of glycoalkaloid content

Total glycol-alkaloid contents of the defatted sample was determined by titrometric method¹¹. Sample was extracted with 100ml of methanol-chloroform (2:1) filtered and the extract was mixed with 100ml of 0.8% Na₂SO₄. The upper chloroform layer was separated, dried and the residue was dissolved in 15ml of 2NH₂SO₄. The solution was then heated for 2h and made basic with 10ml of 4N NaOH. The glycoalkaloids were extracted with benzene and after evaporating the benzene, the residue was taken up in 5 ml of methanol. Sample was filtered with a solution of 0.66% bromophenol blue and 10% phenol in absolute methanol, against a blank of methanol.

MATERIALS

α -chaconine, α -solanine, solasonine, demissidine, α -tomatine, tomatidine, solanidine were obtained from sigma (St. Louis, M.O., β -solanine, β 1-tomatine were isolated from a partial hydrolysis of mixture of the parent glycoalkaloid and characterized by HPLC and mass spectroscopy.

Animals

Adult male albino wistar rats weighing (150-170g) were purchased from Central Animal House BHU Varanasi. All animals were acclimatized for a week under standard husbandry condition. The animals were fed with commercial diet (Agro corporation Pvt. Ltd. Bangalore India) and water adlibitum was available to the animals throughout the experimental period which was replenished daily and maintained at 26-30°C with relative humidity at 60-70%.

Dose preparation

Oral administration of dose containing 2 mg/kg b.wt. of total glyco alkaloids was given to experimental animals.

Preparation of liver and kidney homogenate

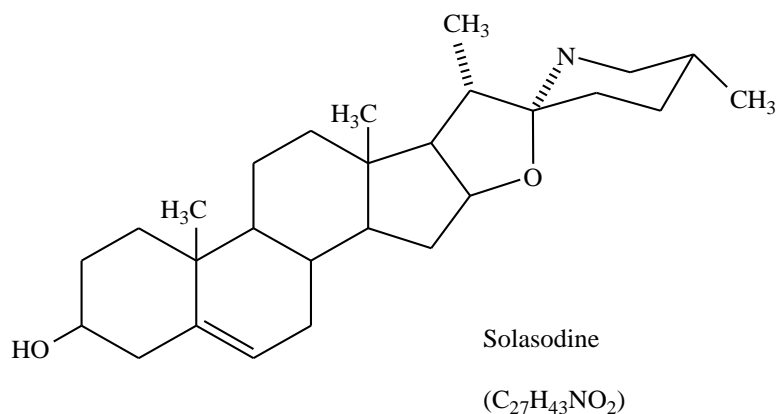
The rats were scarified under light anesthesia (ether vapour) at the end of 8 days of treatment. The liver and kidney were quickly removed, wash with cold water and weighed. After freed of fat they were homogenized in hypotonic solution (7% NaHCO₃ solution). This homogenate was used to determine the protein contents of various samples^{12,13}.

Experimental Protocol

Animals were divided in control and experimental group each containing 5 rats. Group 1 rats received normal standard diet and vehicle only. Group 2 experimental rats received 2 ml, 4 ml and 6 ml of dose (2mg/kg b.wt) for 4 days, 6 days and 8 days chronically. Results are reported as mean \pm S.E. The data were subjected to one way ANOVA followed by Tukey's multiple comparison test. PI 0.05 was considered statistically significant.

RESULT

TLC study reveal that steroidal glycoalkaloid containing 13 compounds. From column chromatography solasodine isolated which structure confired by Co TLC and mass spectroscopy.



Structure-1

Table 1: Effect of daily administration of glycoalkaloids on protein content of liver in adult rats (protein mg/100 mg liver tissue)

Drug	Duration after treatment (in days)		
	4	6	8
Control	17.6 ± 0.92	17.8 ± 0.94	18.0 ± 0.92
2 ml	18.6 ± 0.93	19.5 ± 0.78	20.2 ± 0.94
4 ml	21.0 ± 0.82	21.8 ± 0.65	22.1 ± 0.70
6 ml	23.1 ± 0.91	23.6 ± 0.67	23.9 ± 0.71

Table-1 shows the effect of solasodine on the protein contents in liver when 2 ml, 4 ml and 6 ml dose was orally administered daily for 4 days, 6 days and 8 days. In comparison to control group protein contents significantly increased in liver in experimental group at all doses even when administered for 4 days to 8 days. There was a successive increase in protein content of hepatocytes. Dose of 6 ml was relatively more potent.

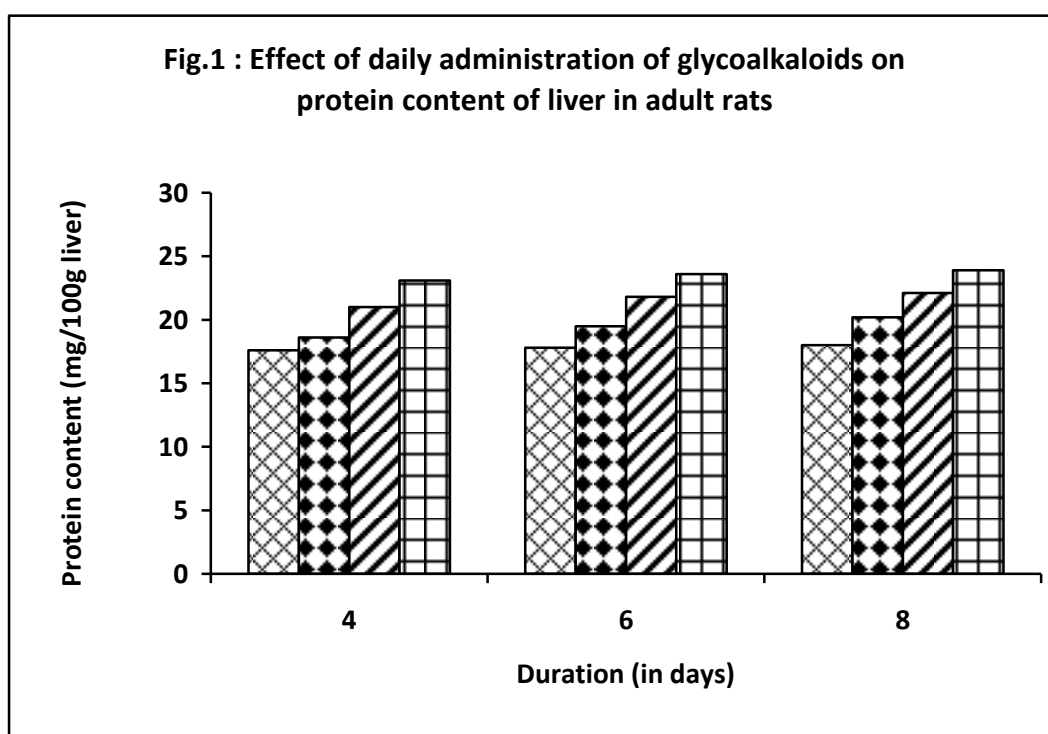
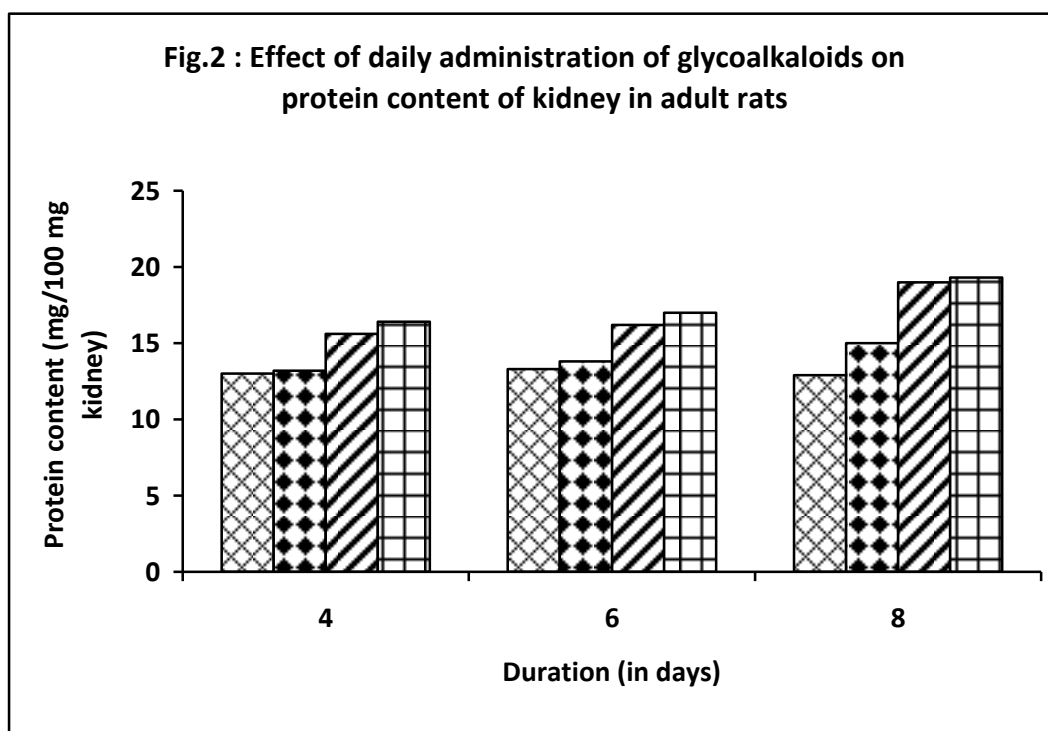


Table 2: Effect of daily administration of glycoalkaloids on protein content of kidney in adult rats (protein mg/100 mg kidney tissue)

Drug	Duration after treatment (in days)		
	4	6	8
Control	13.0 ± 0.64	13.3 ± 0.61	12.9 ± 0.62
2 ml	13.2 ± 0.63	13.8 ± 0.50	15.0 ± 0.58
4 ml	15.6 ± 0.60	16.2 ± 0.54	19.0 ± 0.39
6 ml	16.4 ± 0.62	17.0 ± 0.55	19.3 ± 0.41

Table-2 shows the effect of active compound solasodine extracted from *S. nigrum* on the protein contents in kidney. The administration of solasodine at 2 ml dose for 4 days not more effective but 4 ml and 6 ml dose for 6 days and 8 days the protein contents were gradually increased in kidney of albino rats.



DISCUSSION

Previous reported that ethanolic extract of *S. nigrum* slowly increase protein contents of liver and kidney of albino rats. In the present study active compound solasodine found to report the protein contents of liver and kidney rapidly even in small dose in albino rats. The whole plant *S. nigrum* shows preventive and curative role hepatotoxicity.

The solasodine treated groups did not shows any significant changes in protein content even at 4, 6 and 8 days treatment in chronic toxicity study from *S. nigrum* (spirostane, furostane, spirostane and pregnane) inhibit growth and spread of colon cancer pheochromocytoma¹⁴. Glycoalkaloids of *S. nigrum* also inhibits growth and spread of liver cancer by two distinct anticancer activities i.e.- apoptosis

(programmed cell death) and autophagy. Higher doses of glycoalkaloids induce apoptotic cell death while lower doses leads to autophagocytic death of cancer cells¹⁵.

Proteins are complex macro molecule with exquisite specific. The play key role in nearly all biological process¹⁶. The presence of protein in this plant sample could justify its use in the management of protein deficiency disease of the present finds suggest that glycoalkaloid in not toxic because no markable changes observed in protein content. It also boost hepatocytes by increasing protein content of glycoalkaloid containing drugs in normal therapeutic doses to be safe for the treatment of liver and kidney disorder^{17,18}.

Since *S. nigrum* L., is rich in polyphenolic compound derived from naturally occurring

substances; it has attracted increasing interest in its active compound role in preventing carcinogenesis. Aberrations in cell cycle progression and apoptosis dysregulation are the cause of oncogenic transformation. To evaluate the potential preventive effect of polyphenolic compound and glycoalkaloids on hepatocellular carcinoma development might be measured the Hep G₂ cells growth in experimental animals^{19,20}.

REFERENCES

1. Medina E, Aguiar G, Gomej M, Aranda J, Medina JD and Winter K. Taxonomic significance of the epicuticular wax composition in species of the genus *clusia* from Panama Bioch Syst Ecol. 2006;34(4):319-326.
2. Mohammad Abu Bin Nyeem, AKM Mamun Ur Rashid, Meher Nowrose and Md Abu Hossain. *Solanum nigrum* (Maku): A review of pharmacological activities and clinical effects. International Journal of Applied Research. 2017;3(1):12-17.
3. Rajathi D Modilal M, Anandan R, Sindhu R and Logeshwar MN. Screening of *Solanum nigrum* for its Phytochemical and Antimicrobial Activity Against Respiratory Tract Pathogens, International Journal of Pure and Applied Zoology. 2015;3(3):210-215.
4. Sethi S, Devnani P, Poonia M and Gupta S. Antimicrobial activity of medicinal plants on respiratory tract pathogens. IJBPR. 2013;4(21):48-152.
5. Khanam S and Sultana R. Isolation of β -sitosterol & stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae), Khanam and Sultana. UPSR. 2012;3(4):1057-1060.
6. Hsueh-Chun Wang, Pei-Jun Chung, Cheng-Hsun Wu, Kuang-Ping Lan, Mon-Yuan Yang and Chau-Jong Wang. *Solanum nigrum* L. polyphenolic extract inhibits hepatocarcinoma cell growth by inducing G2/M phase arrest and apoptosis, J. Sci. Food Agric. 2011;91:178-185.
7. Venkatesan D, Karrunakaran CM and Selva kumar S. Studies on phytochemical constituents, functional group indentificati antimicrobial activity of *Solanum nigrum* (Solanaceae), Ethnobotanical Leaflets. 2009;13:1485-1503.
8. Kap-Rang Lee, Nobuyuki Kozukue, Jaesook Han, Joon-Hong Park, Eun-young Chang, Eun-Jung Baek, Jong-Sun Chang and Mendel Friedman. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells, J. Agric. Food Chem. 2004;52:2832-2839.
9. Subash KR and Ramesh KS. Binoy Vargheese Charian, Francis Britto, N. Jagan Rao and S. Vijay Kumar. Study of hepatoprotective activity of *Solanum nigrum* and *Cichorium intybus*, International Journal of Pharmacology. 2011;7(4): 504-509.
10. Jayachitra A and Krithiga N. Study on antioxidant property in selected medicinal plant extracts, Int. J. Med. Arom. Plants. 2012;2(3):495-500.
11. Fitzpatrick TJ and Osman SF. A comprehensive methods for the determinations of total potato glycoalkaloids. Am Potato J. 1974;51:318-323.
12. Lowry WH, Roserbrough NJ, Farr ALand Randall RJ. Protein measurement with the folin reagent. Journal of Biological Chemistry. 1951;258:5696-5701.
13. Omale J, Okaor ON, Polycarp N and Irene II. Chemical composition and effects of aqueous extract of *Cissus multistriate* on some biochemical parameters in albino rats. Omt J Pharm Tech Res. 2009;1(3): 509-513.
14. Yesha Mohy-ud-din A, Zaheer-ud-din Khan, Mushtaq Ahmad and Muhammad Akram Kashmiri. Chemotaxonomic value of alkaloids in *S. nigrum* complex. Pak J Bot. 2010;42(1):653-660.
15. Ananthan Padmashree, Gopal Kumar Sharma, Anil Dutt Semwal and Chitrashekarachar Mahesh. Antioxygenic Activity of *Solanum nigrum* L. Leaves in Sunflower Oil Model System and Its Thermal Stability. Food and Nutrition Sciences. 2014;5:1022-1029.
16. Imran Ali, Waqar Ahmed, Muhammad Tariq, Rehana Asghar and Muhammad Altaf Hussain. Therapeutic potential of ethanolic extract of *Solanum nigrum* for lipofundin-induced hyperlipidemia in Rabbits, Pure and Applied Biology. 2016;5(1):85-90.
17. Rahul Mayee, Ambrish Thosar and Arun Kondapure. Evaluation of antiasthmatic activity of *calotropis gigantean* roots. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4(2):33-35.
18. Kanika Patel, Ravi B Singh and Dinesh K Patel. Medicinal significance, pharmacological activities and

- analytical aspects of solasodine : A concise report of current scientific literature. *Journal of Acute Disease*. 2013;92-98.
19. Bai Y, Lin CH, Luo WM, Wang LY, Sun C and Jia Q. Simultaneous quantification of four saponins, three alkaloids and three fatty acids in *S. nigrum* Linn. by HPLC-ELSD. *Journal of Medicinal Plants Research*. 6(20):3632-3639.
20. Shi-Yong Gao, Qiu-Juan Wang and Yu-Bin Ji. Effect of solanine on the membrane potential of mitochondria in HepG2 cells and $[Ca^{2+}]$ in the cells, *World J. Gastroenterol* . 2006;12(21):3359-3367.