THE CHEMISTRY BEHIND THE TOXICITY OF BLACK NIGHTSHADE, - *SOLANUM NIGRUM* AND THE REMEDY

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Summary

The cause of toxicity of *Solanum nigrum* extract (Sn) and its possible remedy are reported. The -N-NO derivative formed from –NH group of the glycoalkaloids of Sn, by interaction with systemic NO, would cause toxicity in animals ingesting the plant. In the present study, -N-NO derivative of glycoalkaloids was produced by using HNO_2 and the toxicity of the products were measured by swelling of mitochondria. The protective role of magnesium ion, when in conjugation with the glycoalkaloids, against N-nitrosation and resultant decrease in toxicity of the products were assessed. This finding indicated the preventive nature of magnesium conjugation in the formation of -N-NO bonding, which was replicated by similar sequence of reaction in L-proline. The results indicated that, Mg^{2+} ion complexation of the glycoalkaloids was responsible for the reduction of toxicity of Sn extract. Additionally, the occurrence and beneficent role of conjugation of other metal ions with the Sn-glycoalkaloids are reported.

Keywords: *Solanum nigrum*, glycoalkaloids, N-nitrosation, toxicity, magnesium, mitochondria, LD_{50}.

Introduction

Solanum nigrum (Sn), also known as Black nightshade, is generally regarded as a toxic plant [1]. However, in Europe, Sn is used as a home-remedy for convulsions and as a soporific, especially for children [2]. In India, it is used for a variety of therapeutic purposes in the indigenous systems of medicine, e.g. for the management and treatment of cardiac ailments [3], allergy [4], dropsy, arthritis and in restoring systemic homeostasis [5].

Black nightshade, a small erect annual herb that grows wildly in India, in shady habitats, often along with fodder crops, up to an altitude of 7000ft. Animal graze is often contaminated with this wildly growing toxic plant and, hence, it is a matter of concern for the health of livestock and of humans who consume the products (milk, meat) of such livestocks.

The toxicity of Sn is attributed to a number of agents and factors. The berries of this plant produce steroidal glycoalkaloids, viz. α-solanine and α-chaconine [6]; the leaves and stalks are replete with minerals [7], e.g. Ca, Mg, P, Fe, and nitrates [6]. The toxicity is attributed mainly to the glycoalkaloids and nitrates occurring in different parts of this plant. Nitrates are harmful to humans. When consumed and converted into nitrites, they interact with Fe^{2+} ions of the blood hemoglobin, resulting in decreased oxygen-capacity of the blood [8-10]. The often found high levels of nitrates in this plant, which reach a peak as the plant comes into flower, may also cause N-NO toxicity, by interacting with the –NH-group of the glycoalkaloids. This causal aspect was investigated and suitable remedial measures thereof are suggested.

Materials and Methods

Plant material- The plant material, *Solanum nigrum* was obtained from Messrs. Indian Herbs Ltd, Saharanpur (U.P.). A specimen of the sample has been preserved in our file for further reference.

Chemical and reagents- All the chemicals and reagents are of AR grade, standard α-solanine, α-chaconine, and Dowex 50WX8-100 were obtained from Sigma-Aldrich Chemicals, St. Lewis. Sodium nitrite, acetic acid, sucrose, mannitol, bovine serum albumin (BSA), ethylenediamine tetraaceticacid (EDTA) and magnesium acetate tetrahydrate were obtained from Merck (India). L-proline was obtained from Loba Chemie (India).

Extraction and isolation of bioactives of Sn- A general method of isolation of chemical constituents of Sn (Type-I to Type-III) compounds is given in Scheme-I.

In a typical experiment, dried and powdered whole plant material of Sn was hot extracted with petroleum ether followed by hot aq. methanol (40:60) (Soxhlet). Solvent was evaporated from the methanol extract, under reduced pressure, and the residue was partitioned between *n*-butanol and water. The *n*-butanol phase was dried and the residue was subjected to column chromatography over Silica gel (60-120 mesh) using $CHCl_3$-MeOH (9:1), followed by $CHCl_3$-MeOH-H_2O (20:10:1) as eluents.

Characterization of the steroidal saponins of Sn (Type-I compounds)- Enriched fractions of spirostanol saponins were obtained from the later $CHCl_3$-MeOH-H_2O eluates. The mixture of steroidal saponins, thus obtained, on mild acidic hydrolysis (0.5 N-MeOH-HCl, at ordinary temperature, overnight, followed by the usual processing), afforded genins (chloroform-soluble fraction of the hydrolyzed products) which were characterized by HPTLC analyses using reference markers of diosgenin, tigogenin and sarsasapogenin. The aqueous-soluble fractions were investigated for the presence of sugars by HPTLC and GC-MS analysis of the corresponding OTMS derivatives, using reference markers.

Characterization of Amino acids (Type-II compounds)- The mixture of amino acids (Scheme-I) was silylated and the products were analyzed by GC-MS using silyl derivatives of authentic amino acids as markers.

Characterization of glycoalkaloids (Type-III compounds)- Another part of the methanol extractives was suspended in 4% aq. acetic acid (100 mg/ 20 ml aq-methanol); it was kept overnight, at room temperature. The suspension was then extracted with *n*-butanol, to separate type-I and type-II compounds as the *n*-butanol extractive fraction. The aqueous acidic mother liquor was basified with Na_2CO_3 and the liberated bases were extracted with *n*-butanol.

Usual processing of the *n*-butanol extractives afforded a mixture of glycoalkaloids, viz., α-solanine and α-chaconine, which were separated by preparative TLC, using markers, on silica gel precoated plate and chloroform:methanol 50:50 as the developer. The identities of the glycoalkaloids were established by direct comparison with the respective markers and by correspondence of the physical and spectral properties [11].

Apparatus and Techniques:

HPTLC of type-I compounds- CAMAG winCATS HPTLC assembly with Silica gel $60F_{254}$/Merck plate was used with chloroform:acetic acid:methanol:water (64:32:12:8) as mobile phase followed by densitometric determination at 700 nm after derivatization with anisaldehyde spray reagent.

GC-MS of type- II compounds- GC-MS was carried out on a Varian GC-MS, Model: Saturn 2000, GC 3800; equipped with a VF-5 MS column (5% phenyl)- methyl polysiloxane (30 m x 0.25 mm i.d.). Carrier gas used was ultra pure helium with constant flow rate: 2 ml/min. The samples were derivatized by reaction with *N, O*-bis (Trimethylsilyl)-triflouro-acetamide at 70ºC for 30 min to form TMS derivatives. All the analytical data of GC-MS analysis were based on Varian MS workstation software.

HPTLC of Type-III compounds- Silica gel $60F_{254}$/Merck plate was used with butanol-acetone-acetic acid-water 35:35:10:20 as mobile phase followed by densitometric determination at 550 nm after derivatization with Carr-Price spray reagent.

Reagents. The following spray reagents were used –

Anisaldehyde reagent [12] (for type-I compounds)- 10 ml glacial acetic acid was added to 0.4 ml anisaldehyde, followed by addition of 85 ml methanol and 5 ml concentrated H_2SO_4. This solution was prepared 2 hr before use.

Carr-Price spray reagent [11] (for type-III compounds)- 20% antimony (III) chloride in acetic acid and dichloromethane (1:3).

Spectrophotometric estimation of metal ions- Jasco V-530 dual beam spectrophotometer was used for the estimation of iron content [13].

Titrimetric method for the estimation of metal ions- Calcium and Magnesium content were estimated using titrimetric method [14].

AAS for the estimation of metal ions [15] - GBC-AWANTA system; standard Certified Reference Material (CRM) were obtained from MERCK-India.

Preparation of N-NO conjugated glycoalkaloids- Metal ions depleted glycoalkaloids (obtained after passing through strong cation exchange resin) of Sn were treated with nitrous acid (HNO_2)(50mM). The solution was chased by N_2 gas, and the lyophilized product was analyzed by HPTLC and IR spectroscopy.

Preparation of N-NO conjugated L-proline- The N-NO derivative of L-proline was prepared in a similar way, as above, and the product was analyzed by HPTLC and IR spectroscopy.

Preparation of metal ion conjugated complex of glycoalkaloids- Mg^{2+} conjugated complex: Metal ions depleted glycoalkaloids of Sn, in water were separately treated with equimolar proportions of Mg-acetate. Each mixture was lyophilized and the product was analyzed by HPTLC and IR spectroscopy.
A portion of the Mg^{2+}-conjugated glycoalkaloids of Sn were treated with HNO_2 (50mM) as before. After chasing with N_2, the lyophilized product was analyzed by HPTLC and IR spectroscopy.

Preparation and characterization of Mg^{2+} ion conjugated complex of L-proline and HNO_2 treatment- Followed similar sequence of reactions as above.

Identification of N-NO bond resulting from glycoalkaloids-HNO_2 adduct identified by HPTLC and IR spectroscopy. The augmentation and /or inhibition of the formation of this bond by metal ion (Mg) were also demonstrated by the same. The toxicity of these compounds was assessed by mitochondrial swelling experiment [16].

Isolation of Mitochondria rich fraction- Mitochondria rich fraction was isolated from goat liver [17].

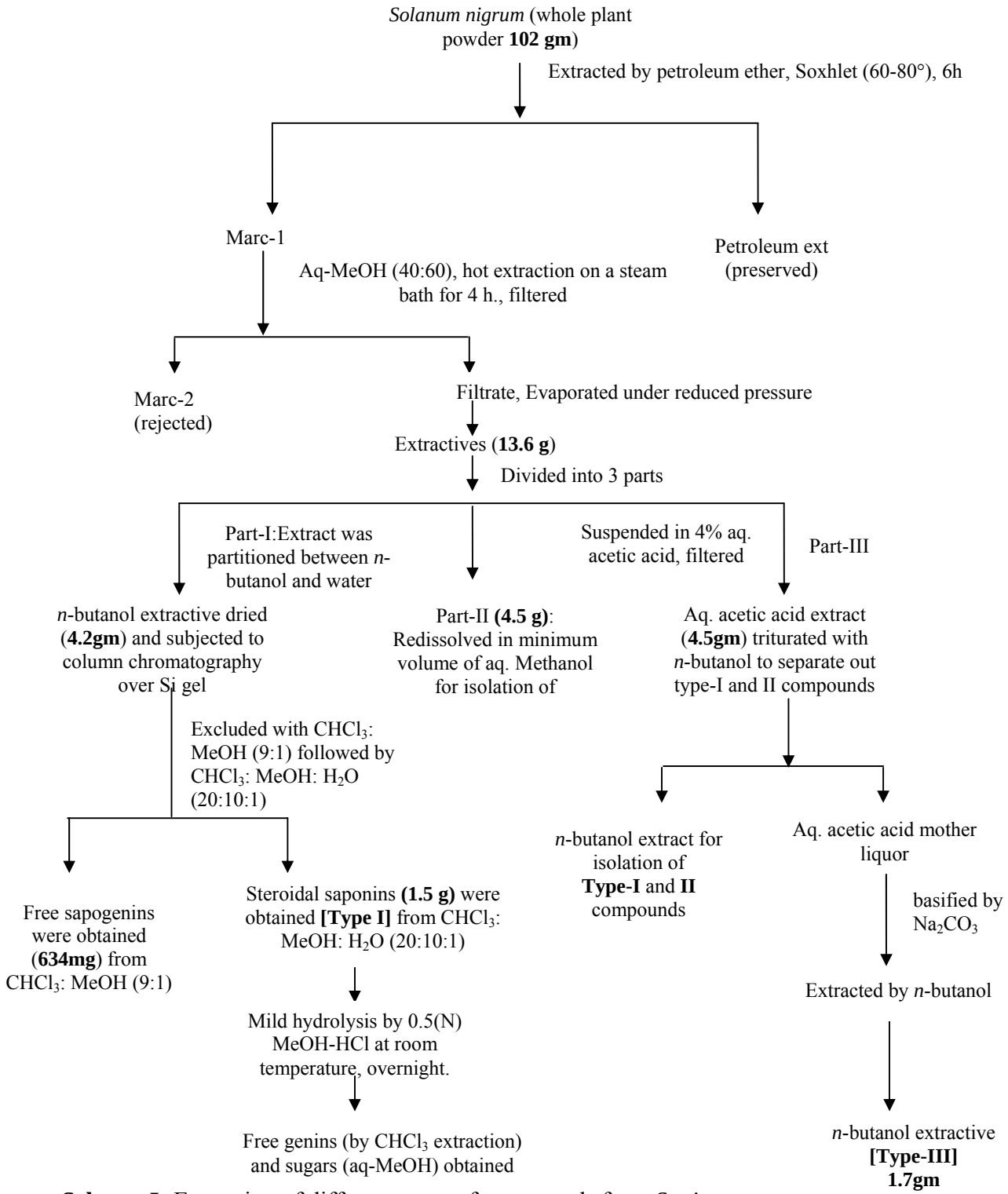
Toxicity Study- To evaluate the toxicities of Sn and the glycoalkaloids of Sn, with and without conjugated metal ions, two different modes of toxicity studies have been performed, viz., acute oral toxicity study (LD_{50} study) and sub acute oral toxicity study.

Animals- Swiss albino mice of either sex, 3-4 months old and weighing around 20-25 g, and Albino rats (Sprague Dawley strain) of either sex, 3-4 months old and weighing around 200 to 250 gm, procured from Central Research Institute (Ayurveda), Govt. of India, Salt lake City, Kolkata, were used. The animals fed with standard rodent diet and water *ad libitum* and were housed in an animal room with alternating light-dark cycle of 12 hr each. The animals were acclimatized for at least 5 days to the laboratory conditions before conducting experiments. Experiments were carried out between 0900 h and 1700h. The studies were conducted in accordance with Good Laboratory Practice (GLP) Regulations of WHO (WHO Document, 1998). The "Principles of laboratory animal care" (NIH Publication # 85-23, 1985) were also followed in the study. The 'Institutional Animal Ethics Committee' (IAEC) approved the experimental protocols.

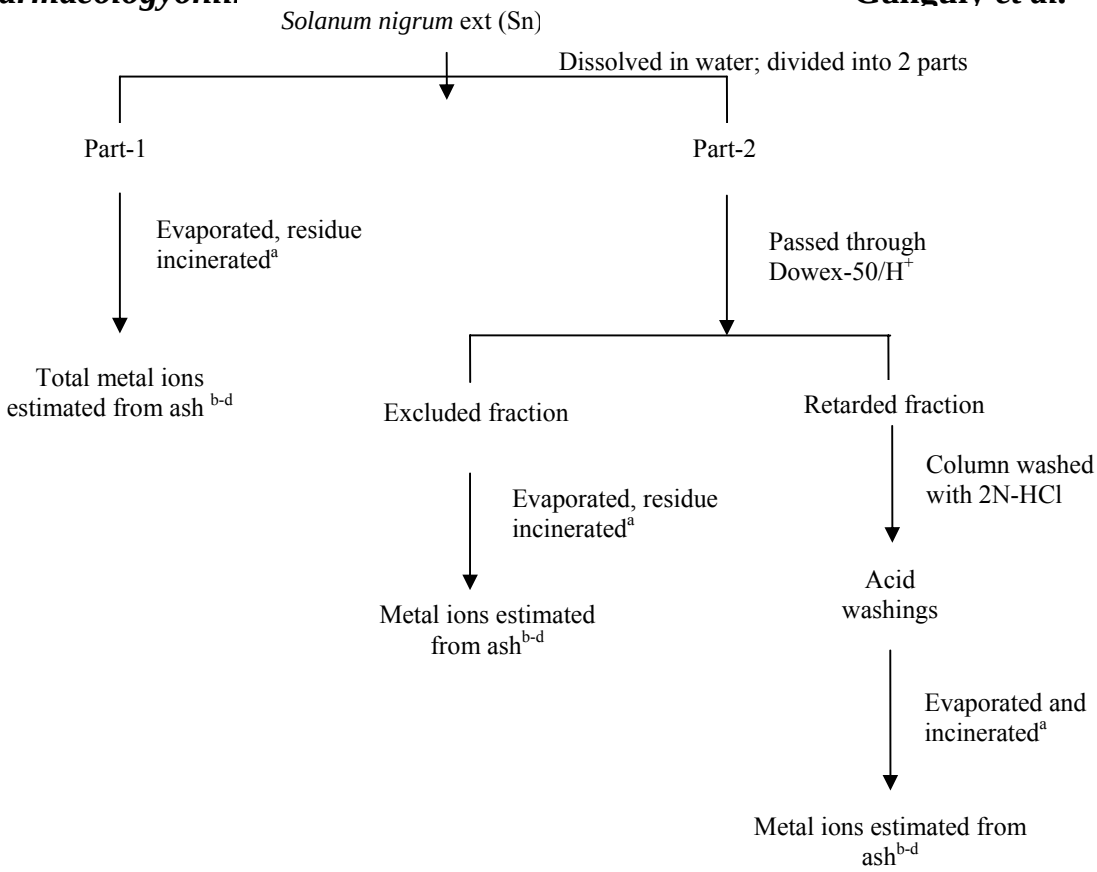
Acute Toxicity Study [18]- Swiss albino mice of both sex and approximately the same weights were divided into ten groups for each extract, each containing six animals for the purpose of determining the LD_{50} values of Sn and metal ions conjugated (Sn/glycoalkaloids) and metal ions depleted glycoalkaloids of Sn (Sn/glycoalkaloids/excluded). After 24 h of test compound administration, the number of dead animals in a group was recorded. The toxicological effect was assessed on the basis of mortality, which was expressed as an LD_{50} value.

Sub acute toxicity studies [19]- For sub acute oral toxicity study, albino rats weighing 200-250gm, were divided into 10 groups of six animals each.
Group-1: received only distilled water.
Group –2: received Sn 1g/kg body weight.
Group-3: received Sn 2g/kg body weight.
Group-4: received Sn 4g/kg body weight.
Group –5: received Sn/glycoalkaloids 100 mg/kg body weight.
Group-6: received Sn/glycoalkaloids 200 mg/kg body weight.
Group-7: received Sn/glycoalkaloids 400 mg/kg body weight.
Group –8: received Sn/glycoalkaloids/excluded 100 mg/kg body weight.
Group-9: received Sn/glycoalkaloids/excluded 200 mg/kg body weight.
Group-10: received Sn/glycoalkaloids/excluded 400 mg/kg body weight.
The rats were treated with the drugs for 21 days. On the 21st day, the blood samples were collected from hearts of animals by mild anesthesia by di-ethyl ether. The effect of different fractions of Sn on total RBC count, total WBC count, and Hb was evaluated [20]. The changes in the hepatic parameters (AST, ALT, ALP and total protein) were estimated [21].

Statistical analysis- Statistical analysis was carried out using Prism software ver.4.0 statistical software (Graphpad software Inc). All the results were expressed as Mean ± standard deviation of the mean (SD). Data were analyzed using one-way ANOVA followed by Dunnett's test. In the entire test, the criterion for statistical significance was $p<0.05$.



Scheme-I: Extraction of different types of compounds from *S. nigrum*.



Scheme-II. A general method for sample preparation and estimation of metal ions of Sn. A similar method was carried out for the estimation of metal ions of glycoalkaloids of Sn and for the preparation of metal ions depleted fraction of Sn/glycoalkaloids.

[a]500°C for 2.5 h.

[b] Fe ions measured by 1, 10-orthophenanthroline.

[c] Ca and Mg ions measured by complexometric (EDTA) titration.

[d] Cu, Zn, Pb, Cd, Hg by AAS.

Results

The isolation procedure of different compounds (type-I to type-III) present in Sn are given in Scheme-I. Phytochemical screening and characterization of different groups of compounds were accomplished by using comprehensive chromatographic analyses using markers. Steroidal saponins (type-I compounds), found in Sn are an important group of natural products having a number of potent beneficent properties [22]. Enriched fractions of spirostanol saponins (ca. 80-85%) were obtained from the solvent extracts by column chromatography. The mixture of steroidal saponins, thus obtained, on mild acidic hydrolysis afforded diosgenin, tigogenin and sarsasapogenin from the genin. The aqueous-soluble fraction showed the presence of different sugars. The identities of the genins and the monosaccharides were established by GC-MS analysis of the corresponding OTMS derivatives, using reference markers. The monosaccharides: steroidal sapogenins ratio was ≅5:1. The attachment of the monosaccharide moieties to the three spirostanol saponins were closely similar as suggested by their respective IR spectra and graded hydrolysis. The results are incorporated in Table-1.

Sn has been found to be a rich source of amino acids (type-II compounds), viz. arginine, aspartic acid, alanine, isoleucine, L-proline, serine and valine. These amino acids tend to protect recipients of Sn extracts, depleted of N-NO toxicity. The results are incorporated in Table-1.

Table-1: Amino acids and spirosta-steroidal saponins of Sn

Amino acids	**Spirosta-steroidal saponins**	
	sapogenins	**Sugars**
Arginine, aspartic acid, alanine, isoleucine, L-proline, serine and valine	Diosgenin, tigogenin, sarsapogenin	Myo-inositol, β-D-galactofuranose, L-altrose, β-DL-arabinoyranose, β-D-glucopyranose, gluconic acid lactone, D-altrose, D-xylose, arabinose, talose

The steroidal glycoalkaloids (type-III compounds), isolated from Sn, were characterized by HPTLC analyses using marker α- solanine and α-chaconine. The results are given in Table-2.

Table-2: Amounts of steroidal glycoalkaloids of Sn.

Parameters (in %)	Sn total extract (Aq: MeOH)
α-solanine	0.21 $\pm$ 0.01
α-chaconine	0.31 $\pm$ 0.02

Data represented as Mean $\pm$ SD; n=4.

Amounts of metal ions, in differently conjugated forms, in the total extractives and in different fractions thereof were measured. The strength of metal ions conjugation was obtained by release of metal ions from the conjugates by passing through a strong cation exchange resin, followed by metal ions analysis by spectrophotometry, titrimetry and AAS (Scheme II).

The abundance of metal ions of Sn and glycoalkaloids of Sn are incorporated in Tables 3-4.

Table-3: Amounts of metal ions in Sn obtained after through strong cation exchange resins.

Metal content in % of sample								
Samples	Fe	Ca	Mg	Cu	Zn	Pb	Cd	Hg
Sn	0.105±0.004	0.360±0.02	0.044±0.001	<0.0001	0.11±0.02	<0.0005	<0.0001	<0.0001
Sn/excluded	0.015±0.001	0.030±0.004	0.002±0.004	<0.0001	0.014±0.005	<0.0005	<0.0001	<0.0001
Sn/retarded	0.080±0.004	0.31±0.01	0.040±0.0003	<0.0001	0.094±0.006	<0.0005	<0.0001	<0.0001

Data represented as Mean ± SD; n=4.
Note that, chelated/complexed metal ions are largely arrested by cation exchange resin and the occurrence of negligible extent of heavy metal ions in the Sn extract.

Table-4: Amounts of metal ions in different fractions of steroidal glycoalkaloids (glyco) of Sn.

Metal content in % of sample								
Samples	Fe	Ca	Mg	Cu	Zn	Pb	Cd	Hg
Sn/glyco/total	0.095±0.004	0.367±0.02	0.047±0.001	<0.0001	0.11±0.02	<0.0005	<0.0001	<0.0001
Sn/glyco/ excluded	0.010±0.001	0.040±0.006	0.006±0.004	<0.0001	0.014±0.005	<0.0005	<0.0001	<0.0001
Sn/glyco/ retarded	0.072±0.005	0.32±0.01	0.038±0.0003	<0.0001	0.094±0.006	<0.0005	<0.0001	<0.0001

Data represented as Mean ± SD; n=4.
Note that, chelated/complexed metal ions are largely arrested by cation exchange resin and the occurrence of negligible extent of heavy metal ions in the natural extract.

Sn/glyco/excluded: Metal ion depleted glycoalkaloids of Sn

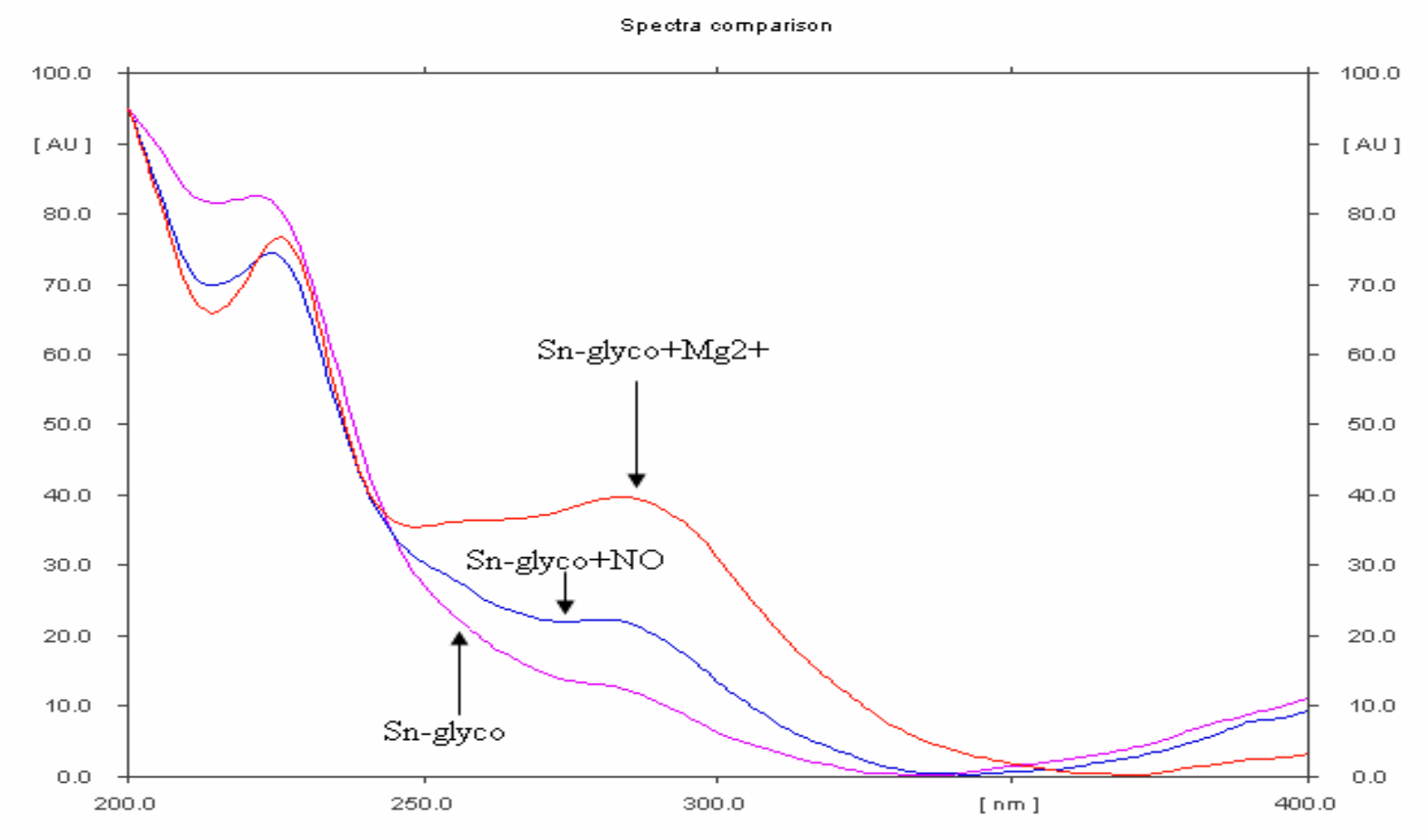


Fig 1: HPTLC reflectance spectra of Sn-glyco as such and after conjugation with NO and Mg^{2+}. (glyco stands for glycoalkaloids)

The formation of N-NO bond of the metal ions depleted glycoalkaloids of Sn (prepared by passing through a strong cation exchange resin) and of L-proline was established by HPTLC and IR spectroscopy. Conjugation of magnesium ion (Mg^{2+}) prior to nitrosation of glycoalkaloids and L-proline was also reflected in HPTLC and IR spectroscopic analyses.

HPTLC of N-NO and metal ion conjugated glycoalkaloids- Silica gel $60F_{254}$/Merck plate was used with chloroform-methanol 50:50 as mobile phase followed by densitometric determination at 254 nm.

The IR spectrum (in KBr) of the Sn/glycoalkaloids exhibited absorption bands at ν_{max} 3399, 2773, 1621, 1402, 1160, 1048, 996, 938, 845, 783 and 640 cm^{-1}. After N-nitrosation (with HNO_2) Sn/glycoalkaloids exhibited bands at ν_{max} 3441, 2551, 2074, 1622, 1382, 1262, 830, 541 cm^{-1} and a characteristic -N-NO weak band at 1445-1490 cm^{-1}. Magnesium conjugation of the glycoalkaloids modified the nature of the bands in the range: ν_{max} 3370 and 1184 cm^{-1} as broad signals. A characteristic -N-NO band at 1445-1490 cm^{-1} appeared in case L-proline after N-nitrosation with HNO_2.

The role of magnesium ion conjugation on N-NO toxicity was determined by extent of mitochondria swelling experiment. It has been found the formation of N-NO bond of the metal ions depleted glycoalkaloids of Sn elicited toxic effects by decreasing the extent of inhibition of mitochondria swelling. In contrast, magnesium ion conjugation of metal ions depleted glycoalkaloids and N-NO adduct of corresponding glycoalkaloids, prior to nitrosation, prevented the swelling of mitochondria in a better way. The results are expressed in Fig 2.

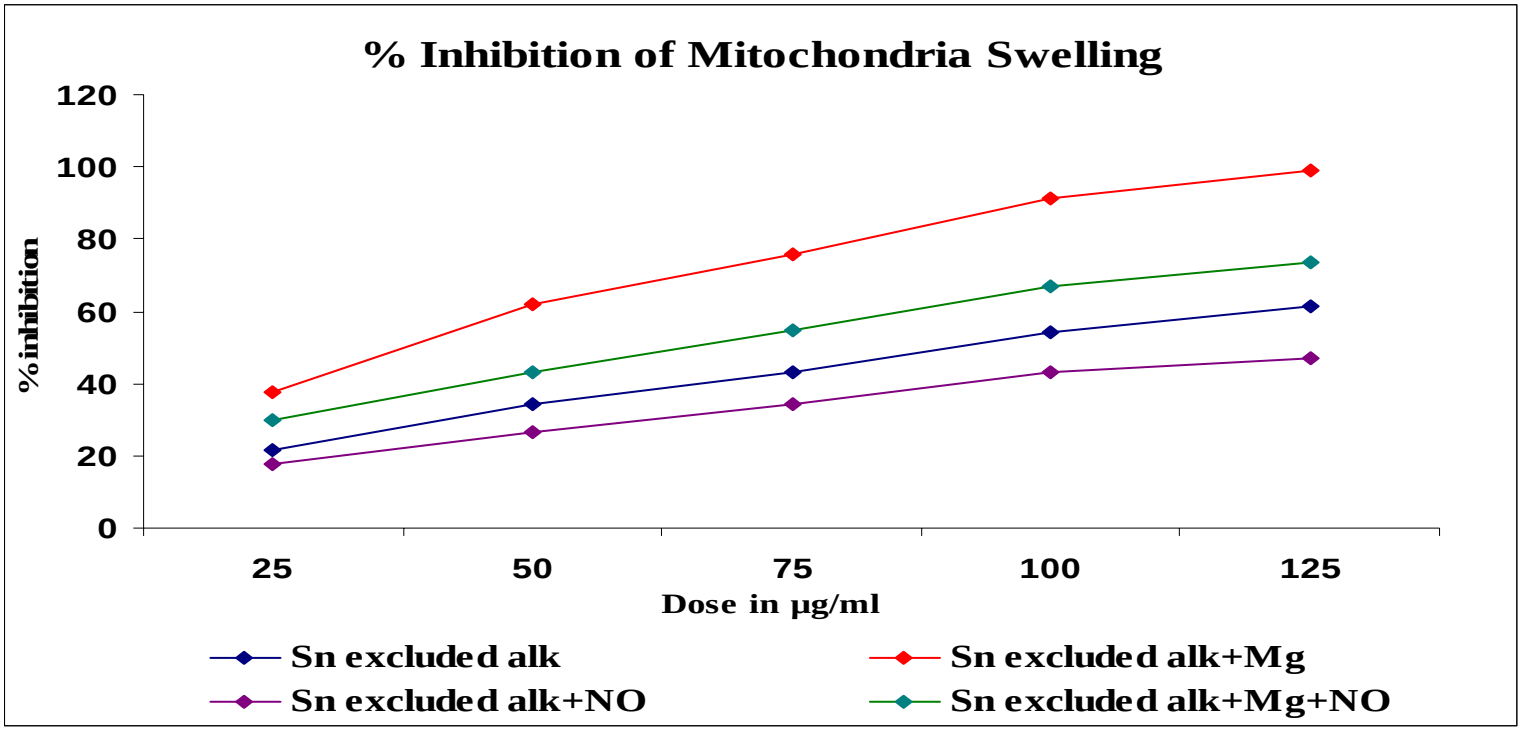


Fig 2: Extent of inhibition of Cu induced mitochondria swelling of glycoalkaloids of Sn and the role of NO and Mg conjugation.

The beneficial effect of glycoalkaloids of Sn, Mg^{2+} conjugated with was determined. The Sn total extract was found to be safe >5 g/kg body weight. This is presumably due to the fact that glycoalkaloids of Sn contain appreciable amount of conjugated metal ions (except Cu^{2+}) (Table 4). Metal-free excluded fractions were prepared by passing it through a strong cation exchange resin (Dowex $50/H^{+}$), This product (metal-free) was found to be more toxic than the total glycoalkaloids, as indicated by the LD_{50} values. The results are incorporated in Table 5.

Table- 5: Determination of LD_{50} value of different fractions Sn

Samples	LD_{50} in g/kg body weight
Sn	>5.0
Sn/glycoalkaloids	2.0
Sn/glycoalkaloids/excluded (metal ions depleted fraction)	1.6

Note: The toxicity in the metal ions depleted fraction of glycoalkaloids was increased considerably as indicated by lower LD_{50} value.

The increasing trend in toxicity of the metal ions depleted glycoalkaloids of Sn was further manifested in the sub acute toxicity study. The Sn total extract was found to be safe, as revealed by the hematological and hepatic parameters, upto a dose of 4 gm/kg, p.o. administered for 21 days as compared to normal control animals. Whereas glycoalkaloids fraction of Sn was found to be toxic at a dose of 200 and 400 mg/kg, p.o. treated for 21 days. The toxicity of the glycoalkaloids was further increased when the extract was made metal ions depleted by passing through a strong cation exchange resin. The results are incorporated in Table 6.

Table-6: Sub acute toxicity study of Sn total extracts and glycoalkaloids of Sn with and without conjugated metal ions

Groups	Hematological and hepatic parameters on 21st day						
	Total RBC count ($x10^6$/ μl)	**Total WBC count ($x10^3$/ μl)**	**Hb (g/dl)**	**AST (in U/L)**	**ALT (in U/L)**	**ALP (in U/L)**	**Total protein in mg/dl**
Normal control	7.30±0.39	5.51±0.38	14.35±0.49	64.06±2.72	56.25±2.24	19.31±1.40	7.62±0.83
Sn/-1gm/kg	7.36±0.47	5.68±0.38	14.50±0.51	62.04±1.71	54.62±2.92	19.93±2.01	7.75±0.61
Sn/-2gm/kg	7.29±0.27	5.72±0.39	14.71±0.95	63.29±2.13	54.32±2.68	19.71±1.52	8.41±1.02
Sn/-4gm/kg	7.52±0.69	5.59±0.36	14.68±0.39	63.55±1.99	51.51±2.08	19.01±1.35	7.91±0.93
Sn/glyco-100mg/kg	6.99±0.18	5.70±0.40	13.99±0.49	64.97±2.78	57.28±2.16	19.51±0.43	7.27±0.69
Sn/glyco-200mg/kg	6.83±0.33*	5.90±0.37	13.55±0.35*	68.35±2.22*	59.68±1.49*	19.88±0.39*	6.92±0.57
Sn/glyco-400mg/kg	6.67±0.26**	6.15±0.46*	12.93±0.36**	68.86±2.54*	62.57±2.04**	21.27±0.61**	6.16±0.49**
Sn/glyco/excluded-100mg/kg	6.87±0.21*	6.04±0.40	13.81±0.51	67.27±2.91	58.62±2.25	19.67±0.36*	7.15±0.79
Sn/glyco/excluded-200mg/kg	6.61±0.28**	6.19±0.34*	13.29±0.34**	68.35±2.37*	61.84±1.71**	20.28±0.56**	6.56±0.54*
Sn/glyco/excluded-400mg/kg	6.49±0.17**	6.35±0.38**	12.47±0.28**	69.63±2.28**	63.84±2.35**	22.22±0.88**	5.78±0.44**

Data represented as Mean ± SD; for 6 rats. *p<0.05; ** p <0.01; in comparison to Group 1 (normal control) rats treated with the vehicle.

Discussion

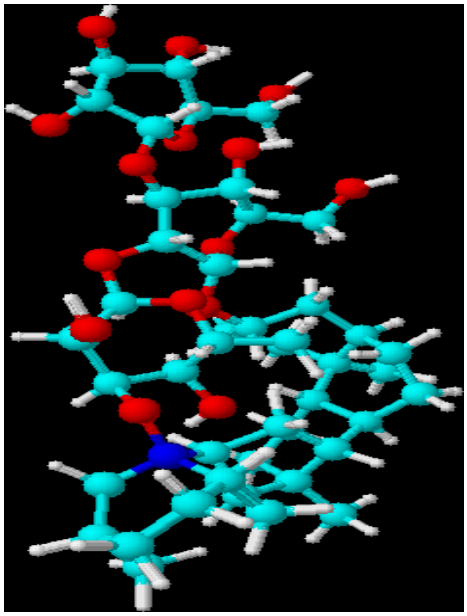
Traditional societies have always exploited edible wild plants to provide an adequate level of nutrition [23]. Agricultural development and cultivation in developing countries are primarily based on subsistence crops and edible wild plant species, and only secondarily on the cultivation or utilization of a wide diversity of food crops, whose total number of species is large [24]. However, dietary utilization of nondomesticated plants has received little attention and a dramatic narrowing of the food base in many traditional societies has occurred. Therefore, the domestication and cultivation of wild edible plants are essential to increase and diversify the food base which will ensure dietary balance and the intake of essential micronutrient.

Solanum nigrum (the black nightshade) is found worldwide and widely used as leafy herbs and vegetables, as a source fruit and various medicinal purposes [5]. Therefore, human consumption of their leaves and fruits as food is widespread, particularly in Africa and south-east Asia. Recently, the study on the steroidal glycoalkaloids of *Solanum nigrum* has created concern about its consumption. In this study, an attempt has been made to delineate the possible reasons for toxicity of Sn along with its remedial measures.

The detailed phytochemical studies showed the presence of, steroidal saponins, various essential amino acids along with essential trace elements which play an important role in the bioactivity and toxicity of Sn. The role of metal ion conjugation (especially magnesium) along with the glycoalkaloids of Sn in milieu was envisaged in view of the toxicity aspect.

The toxicity of Sn is primarily due to steroidal glycoalkaloids (viz., α-solanine and α-chaconine) and N-NO bond formed with the -NH group of glycoalkaloids and NO radical [6]. N-NO bonded compounds are known for its carcinogenicity and teratogenecity [25-26]. In the present study, the toxicity of synthetically prepared N-NO bonded compounds and the subsequent remedial measures were carried out in details. The formation of N-NO bond and metal ion conjugation has been substantiated by mimicking with L-proline, which also has -NH-group as steroidal glycoalkaloids of Sn. Detailed HPTLC and IR spectroscopic analyses proved the formation of N-NO bond and metal ion conjugation. Magnesium ion conjugated glycoalkaloids were found to be less toxic and beneficial in the inhibition of mitochondrial swelling than both metal ions depleted glycoalkaloids as well as synthetically prepared N-NO bonded glycoalkaloids. These findings clearly suggest the beneficial role of metal ion conjugation (especially Mg) in reducing toxicity of steroidal glycoalkaloids and corresponding N-NO adduct. These findings have been further substantiated by acute oral toxicity study in mice and sub-acute oral toxicity study in rats. In acute model, the LD_{50} value of metal ion depleted steroidal glycoalkaloids is almost 20% less than the metal ion conjugated corresponding glycoalkaloids which signifies the role of metal ions conjugation in toxicity lowering. Similar trends were obtained in case of sub-acute toxicity study when the toxicity of the glycoalkaloids of Sn was further increased when the extract was made metal ions depleted.

In metal ions depleted glycoalkaloids, an intermingling of the hydrophobic and hydrophilic components occurs. This intermingling results in severe cell-membrane perturbation and eventually manifests cellular toxicity (Fig 3). Magnesium ion (represented by green ball) complexation of the steroidal glycoalkaloids has produced a micellar (amphiphillic) structure (Fig 4a&b), thereby, facilitates smooth entry of magnesium via the cell membrane. This lipoidal complex compound acts as a strong neuroprotective agent [27]



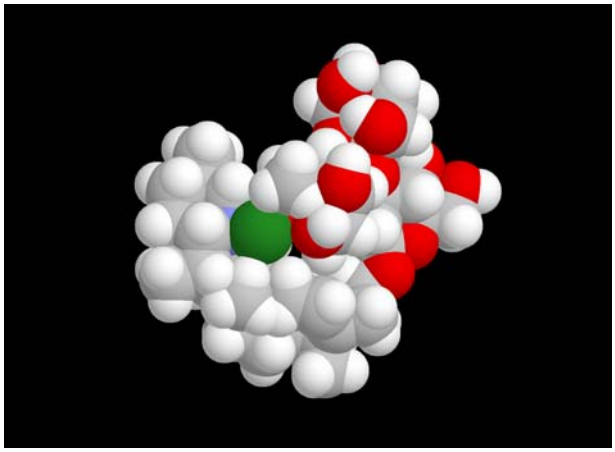


Fig 4a. (3D str. of Mg^{2+}-steroidal glycoalkaloids)

Fig 3 (3D str. of metal ions depleted steroidal glycoalkaloids)

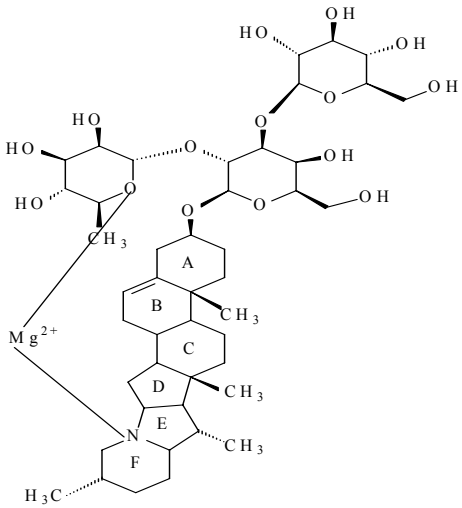


Fig 4b. **(**Mg^{2+}-steroidal glycoalkaloid conjugate)

Acknowledgement

PG and AKG thank M/S Natreon Inc. Kolkata, for providing instrumental facilities and for permitting to carry out independent research investigation under the supervision of the senior authors (UKM and SG).

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