Original Article

Evaluation of Comparative Immunomodulatory Potential of *Solanum xanthocarpum* Root and Fruits on Experimental Animal

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ABSTRACT

Aim: This study was aimed to justify the scientific basis in folklore use of *Solanum xanthocarpum* (solanaceae) as an Immunomodulatory agent in India. **Materials and Methods:** The ethanol and aqueous extracts of *S. xanthocarpum* fruit (SXE-1 and SXA-1), root (SXE-2 and SXA-2) and whole plant (SXE-3 and SXA-3) were evaluated for immunomodulatory activity by using cold water swim endurance stress test, delayed type hyper sensitivity reaction, carbon clearance test and CCl_4 induced oxidative stress model on experimental animals. The solasodaine content in *S. xanthocarpum* bioactive test samples were quantified by HPLC to establish phyto-pharmacological relationship. **Results:** The 200 mg/kg/day p.o. dose of test samples were showed significant changes in swimming survival time and at same doses test samples were significantly increased hypersensitivity, decrease in carbon clearance and reduced oxidative stress. Where, SXE-1 and SXA-1 exhibited maximum degree of immunomodulatory effect compared to SXE-2, SXA-2, SXE-3, SXA-3 and respective standard drugs used in this study. The hematological, biochemical and histopathological studies were supported respective results of the test samples. The solasodaine content in SXA-1 (1.146%), SXE-1 (0.894%), SXE-2 (0.643%) and SXA-2 (0.620%) were estimated by HPLC. **Conclusion:** These observations established the traditional claim and thus *S. xanthocarpum* could be a potent immunomodulatory agent for use in near future. The immunostimolatory effect may be due to presence of solasodaine in *S. xanthocarpum* extracts.

Key words: *S. xanthocarpum*, Solasodaine, HPLC, Immunomodulatory, CCl_4.

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INTRODUCTION

The body immune system is involved in the etiologic and pathophysiologic mechanisms of many human diseases. Therefore, Immunomodulation may be served as useful target for control of those human diseases.[1] The natural resistance of the body against infection can be enhanced by the use of herbal drugs.[2] Medicinal plants are rich sources of bioactive phytochemicals that enhance immunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells other complement factors.[1] With this background, it was planned to explore the immunomdulatory effect of locally available plant used in folklore health care system for related disorders. Based on consultation of literature survey, *Solanum xanthocarpum* Schrad and Wendl species was selected that are commonly used in the region for the autoimmune disease.

Solanum xanthocarpum Schrad and Wendl (Solanaceae) commonly known as Yellow Berried Nightshade (syn: kantakari), is a

prickly diffuse bright green perennial herb, woody at the base, 2 –3 m height found throughout India, mostly in dry places as a weed on road sides and waste lands.[3] The Mukundara tribals of Rajasthan used for treatment of hernia.[4] Local peoples of Taindol village, district Jhansi, Uttar Pradesh used for treatment of Asthma, Bronchitis, Cardio tonic, Chest pain, Cough, Diuretic, Fever, Gonorrhoea, Jaundice, Skin diseases, Toothache, Tuberculosis, Ulcers.[5] In Manipur, fruits are consumed as vegetable and local healers of Manipur are also used for treatment of various diseases.[6] In ancient Ayurveda, mentioned uses of the plant as pungent, bitter tonic, digestive, alternative astringent. The stem, flower, fruits have used as bitter tonic and carminative. Roots decoction used as ferifuge, diuretic and expectorant. Charaka and Sushruta used the extracts of whole plant and fruits oraly for treatment of bronchial asthma, tympanitis, misperistalsis, piles, rejuvenation and dysuria. The '*Kantkari Ghrita'* of *Charaka* is specific formulation for cough and asthma.[7] The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic and aphrodisiac activities.[8] The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions.[9] The hot aqueous extract of dried fruits used for treating cough, fever, and heart diseases.[10] The fruit paste is applied externally to the affected area for treating pimples and swellings.[11] Traditionally, the juice of the leaves is used for treatment of rheumatism, an autoimmune disorder,[12] and fruit juice is useful in sore throat and rheumatism.[13] the decoction of the of the fruit is used by traditional healers of Jharkhand and Orissa for the the treatment of diabetes. The different bioactive phytochemical including solasodine (steroidal alkaloid), tomatidenol (alkaloid), alpha-Solamargine (alkaloid), diosgenin (sapogenin), Apigenin (flavonoid), beta-sitosterol (steroid), linoleic acid (fatty acid), lupeol (triterpenoid) etc were reported to be present in *S. xanthocarpum* plant.[6]

The present investigation was aimed to justify the pharmacological basis of traditional use of *S. xanthocarpum* as immunomodulatory agent and to explore the bioactive component(s) which effect in physiological mechanism that caused immunomodulation.

MATERIALS AND METHODS

Plant material and extraction procedure

Fruit, root and whole plant including leaves, roots, roots and stems of *S. xanthocarpum* were collected from the surrounding fields of Mandsaur, Madhya Pradesh, India in the month of August and identified at Department of Botany, Govt. P. G. College, and Mandsaur, India. A voucher specimen (SX/002/2005/BRNCOP) has been deposited for future reference. Shade dried coarse powdered (10#) fruit, root and whole plant were defatted with petroleum ether, and extracted with 95% ethanol by soxhlation method (SXE-1, SXE-2 and SXE-3 respectively) and aqueous extracts obtained by occasional shaking of powdered fruit, root and whole plant with distilled water (SXA-1, SXA-2 and SXA-3 respectively).

Phytochemical screening

Preliminary phytochemical screening of extracts was carried out for the presence of phyto-constituents like alkaloids, flavonoids, steroids and saponins.[14]

Experimental Animals

Wistar albino rats (150-200 gm) of either sex obtained from Institutional Animal House of B.R. Nahata College of Pharmacy, Mandsaur for the study. Animals were maintained under normal environmental condition: (Room temperature - 27 ± 3°C, Relative humidity - 65 ± 10 %, 12 h light / dark cycle). The animals were fed with standard diet and water was given *ad libitum* under strict hygienic conditions. The permission of Institutional animal ethical committee was obtained before starting the experiments (approved proposal No. (34/M.Ph/06/IAEC/BRNCOP). All Experiments were performed in accordance with the current guidelines of CPCSEA, Government of India.

Acute Toxicity Study (ALD_{50})

The acute toxicity of the tested extracts was determined in Wistar albino rats, maintained under standard conditions. The animals (3 animals in a group) were fasted overnight prior to the experiment. Acute oral toxicity – acute toxic class method (OECD Guideline no. 423, Annexure – 2d) adopted by CPCSEA, Government of India was followed for toxicity studies. The mortality was observed after oral administration of 2000mg/kg b. w test samples SXE-1, SXA-1, SXE-2 and SXA-2.[15] Common side effects such as, mild diarrhoea and depression of treated groups of animals were recorded within the 72 h.

Cold water swim endurance stress test

Cold water swim stress test was performed according to Bhargava and Singh (1981) with some modifications.[16] The wistar albino rats were segregated into seven groups (n=6). Group I served as normal control, group II was stress control and received only purified water (10 ml/kg/day). Group III was treated with ashwagandha capsule, (Himalaya Drug Co. India) at dose of 50 mg/kg/day p.o. as standard drug. Group IV-VII was

administered with the tested extract SXE-1, SXA-1, SXE-2 and SXA-2 respectively at a dose of 200 mg/kg/day p.o. The animals of Group II-VII were allowed to swim till exhausted after 45 min of the treatment. The mean swimming time on 7th day for each group was calculated. On the 8th day, animals were individually placed in plastic container of capacity 400 ml and animals were immobilized in normal position, using adhesive tape. The container was placed in a refrigerator for 2 h. The blood was collected by heart puncture method for estimation of hematological studies like WBC count, differential leucocytes counts,[17] blood glucose, cholesterol and triglycerides (using commercially available kits from Span Diagnostics Pvt. Ltd, India).

Delayed type hypersensitivity

The delayed type hypersensitivity was induced according to the reported method of Agarwal *et al.* 1999 with some modifications using rabbit red blood cells (RRBC) as an antigen. The animals were divided into six groups of six animals each. Group I served as control (saline water, 10 ml/kg/day). Group II animals were administered levamisole 25 mg kg^{-1}orally as standard drug and group III -VI was administered with the test samples SXE-1, SXA-1, SXE-2 and SXA-2 respectively at a dose of 200 mg/kg/day p.o. for 5 days. On 5th day, 45 min latter of the treatment, the animals were challenged with $1x10^5$ (RRBC) subcutaneously in the hind paw. The right hind paw received saline only. Paw volume was measured with the help of plethysmograph on 1h, 24h and 48 h after the challenge. The difference in paw volume was taken as measure of delayed hypersensitivity (DHS).[18]

Carbon clearance test

In-vivo immunomodulation study, the change in macrophages (Phagocytic activity), was determined by using carbon clearance assay on albino rats according to the reported method of Ponkshe and Indap (2002) with some modifications. The animals were divided into six groups of six animals each. Group I (control group) received 10ml/kg body weight/p.o.), Group II were given Cyclophosphamide (50 mg/kg b.w. i.p for 3 days starting from day 4) as standard drug and Group III- VI received the z test samples SXE-1, SXA-1, SXE-2 and SXA-2 respectively at a dose of 200 mg/kg /day p.o. for 7 days. On 7th day of experiment, animals of the entire groups injected with 0.1 ml Indian ink dispersion (1 mg/ml) intravenously through the tail vein. The blood samples (50µl) were withdrawn from tail vein after 5, 10, 15 and 30 min after Indian ink injection (mean time is reported in Table 6). The individual aliquot was lysed with 2 ml of 1% acetic acid and absorbance was measured calorimetrically at 660 nm. Rate of carbon clearance (K) and phagocytic index (α) were calculated.[19]

CCl_4 induced oxidative stress

The animals were divided into five groups of six animals each. Group I served as normal control and received saline water (1 ml/kg, p.o.) daily for 5 days and received olive oil (1 ml/kg, S.C.) on days 2 and 3. Group II served as CCl_4 control and received distilled water (1 ml/kg, p.o.) daily for 5 days and received CCl_4: olive oil (1:1, 2 ml/kg, S.C.) on days 2 and 3. Group III was treated with the reference drug silymarin (50 mg/kg, p.o.) daily for 5 days and received CCl_4: olive oil (1:1, 2 ml/kg, S.C.) on days 2 and 3, 30 min after administration of reference drug. Groups IV and V were treated with SXE -1 and SXA -1 200 mg/kg p.o., respectively (as SXE-1 and SXA-1 exhibited maximum degree of immunostimulation in above stated bioassays), for 5 days and received CCl_4: olive oil (1:1, 2 ml/kg, S.C.) on days 2 and 3, 30 min after administration of extract. The animals were sacrificed on day 6th by cervical decapitation and blood was collected in centrifuge tubes. The serum was obtained by centrifugation. Biochemical parameters i.e. serum alkaline phosphate, SGOT, SGPT, bilirubin,[20] cholesterol and HDL (using kits from Span Diagnostics Ltd) were evaluated. In histopathological study the liver tissues were dissected out and fixed in 10% formalin. The paraffin sections were prepared and stained with haematoxylin and eosin and examined using light microscopy.[21]

HPLC fingerprinting method of solasodine in *S. xanthocarpum* extract

Experiments were performed on a HPLC system Shimadzu-$10AT_{VP}$, binary gradient equipped with detector Shimadzu UV –VIS SPD-10 A_{VP}, software Spinchrom, Chennai. The separations were performed on Merck's column [Lichrospher 100, C-18 (250×4.6 mm) and ODS RP-18 (250 x 4.6 mm, 5µ particle size)] using methanol: acetonitrile (70:30) mobile phase with flow rate 1.0 ml min−1.the wave length of detection was 254 nm.

Serial dilutions containing 2-20µg /ml reference solasodine in methanol were prepared from a stock solution of pure solasodine (10 mg/100 ml). Retention time of solasodine was observed to be 3.0667 min. The intercept and the slope of the standard plot were observed to be 53.14 and 121.1, respectively, with coefficient of correlation as 0.9935 (R^2).

RESULTS

Phytochemical screening

Preliminary phytochemical screening of all the tested extracts has shown presence of alkaloids, flavonoids, steroids and saponins (Table 1).

Acute Toxicity Study (ALD_{50})

In acute toxicity study of SXE-1, SXA-1, SXE-2 and SXA-2 does not showed mortality at the dose level of 2000mg/kg. Therefore, 2000 mg/kg dose was considered as ALD_{50} cut off the dose under GHS 5 (safe dose), as per Globally Harmonised Classification System (GHS) for Chemical Substances and Mixtures described in OECD guideline 423(Annexure 2d). Common side effects such as, mild diarrhea and depression in treated group of animals were not recorded within the 72 h of observation. So, in present study, 200 mg/kg body weight ($1/10^{th}$ of ALD_{50}) dose was selected for *in-vivo* immunomodulatory study.

Table 1: Preliminary phytochemical screening of *S. xanthocarpum* extracts.

Extracts	Alkaloids	Flavonoids	Steroids	Saponins
Ethanol extract of fruit (SXE-1)	Present	Present	Present	Present
Aqueous extract of fruit (SXA-1)	Present	Present	Present	Present
Ethanol extract of root (SXE-2)	Present	Present	Present	Present
Aqueous extract of root (SXA-2)	Present	Present	Present	Present
Ethanol extract of whole plant (SXE-3)	Present	Present	Present	Present
Aqueous extract of whole plant (SXA-3)	Present	Present	Present	Present

Cold water swim stress test

Animals administrated with test samples SXE-1 and SXA-1 have significantly ($p<0.001$) enhanced in swimming survival time as compare to stress control group. Whereas, the samples SXE-2 and SXA-2 were significantly ($p<0.01$) reduced swimming survival times (Table 2A). However, SXE-1, SXA-1, SXE-2 and SXA-2 Significantly reduced the elevated hematological parameters (Table 2B) and biochemical parameters (Table 2C). Results revealed fruit extracts have immunostimulant activity as compare to root extracts. Results of fruit extracts of *Solanum xanthocarpum* are better than the standard.

Delayed type hypersensitivity

SXE-1 and SXA-1 increased paw volume 61.25% and 63.15% respectively at 24 h when compared to paw volume at 1h, which was greater than control animals. SXE-2 and SXA-2 maximum percent (20.0% and

Table 2A: Effect of the tested extracts in cold stress swim test.

Groups	Parts	Swimming survival time (sec.)
Stress control	-	213.83 ± 17.00
Withania somnifera (Standard)	-	276.50±17.16**
SXE-1	Fruit ethanolic	267.50±32.57**
SXA-1	Fruit aqueous	365.83±32.84***
SXE-2	Root ethanolic	103.50 ± 7.58**
SXA-2	Root aqueous	108.83 ± 7.38**
SXE-3	Whole plant ethanolic	138.14 ± 3.01**
SXA-3	Whole plant aqueous	243.30 ± 3.57***

All values are expressed in mean ± SEM.
** $p<0.01$ and *** $p<0.001$ test group vs. stress group.

Table 2B: Effect of the tested extracts on hematological parameters.

Groups	WBC count (No. of cells /mm^3	Differantial leucocytes count			
		Polymorphs	Lymphocytes	Eosinophils	Monocytes
Control	4636.55±66.71	28.91±0.37	64.05±0.41	3.10 ±0.28	0.66±0.3
Stress control	12148.00±360.84	54.68±0.37	96.85±0.32	6.28±0.28	3.04±0.31
Withania somnifera	7141.65±94.03***	40.70±1.16***	74.17±1.96***	5.71±0.18**	2.13±0.25**
SXE-1	8419.40±44.54***	45.12±1.03***	80.63±0.83***	5.95 ±0.07**	2.60 ±0.25**
SXA-1	6441.80±90.08***	35.58 ±0.61***	66.92±2.27***	5.46±0.12**	1.93 ±0.14**
SXE-2	10879.41±327.22*	56.29±0.31***	96.12±0.56***	6.91±0.56***	3.48±0.56***
SXA-2	10287.56 ±297.21*	54.27±0.39***	94.56±0.43***	6.79±0.56***	3.34±0.46***
SXE-3	10012.42±334.33*	52.34±0.41***	92.62±0.51***	6.72±0.62***	3.30±0.41***
SXA-3	8912.62± 343.25**	46.12±0.43***	82.61 ±0.83***	5.97±0.07***	2.69±0.36***

All values are expressed in mean ± SEM,
* $p<0.05$,** $p<0.01$ and *** $p<0.001$ test group vs. stress group.

Table 2C: Effect of the tested extracts on biochemical parameters and organ weight in stress swim stress test.

Groups	Glucose mg/dl	Cholesterol mg/dl	Triglyceride mg/dl	Organ weight (Liver) gm / 100 gm body weight.
Control	84.791±1.399	44.316±1.058	70.88±0.7711	4.34±0.107
Stress control	115.72±7.12	97.16±4.21	126.101±1.88	7.02±0.123
Standard	89.5±6.2***	66.31±4.33***	105.31±1.19***	5.89±0.19***
SXE-1	49.46±3.15***	63.28±3.33***	112.91±1.43***	5.51±0.19***
SXA-1	67.38±1.91***	66.53±2.68***	95.59±0.79***	5.04±0.21***
SXE-2	67.38±5.18*	97.02±4.10*	126.34±4.21*	7.42±0.32*
SXA-2	69.23±5.91*	95.02±3.34*	123.31±3.78*	7.34±0.49*
SXE-3	40.65±4.16**	94.21±2.43**	120.29±2.91**	7.09±0.31**
SXA-3	42.46±4.15***	80.49±1.62***	114.23±1.28***	6.03±0.21***

All values are expressed in mean ± SEM,
*p< 0.05,** p< 0.01 and *** p< 0.001 test group vs. stress group.

Table 3: Effect of the tested extracts on delayed hypersensitivity.

Groups	Paw edema (ml)		
	1 h	24 h	48 h
Control	0.08±0.006	0.17±0.08 (52.94)	0.14±0.007 (42.85)
Standard	0.081±0.010	0.24±0.01	0.16±0.043**
SXE-1	0.062±0.009***	0.16±0.008*** (61.25)	0.12±0.07*** (48.33)
SXA-1	0.07±0.010***	0.19±0.01*** (63.15)	0.17±0.4*** (58.82)
SXE-2	0.064±0.011***	0.08±0.011** (20.0)	0.07±0.023*** (8.57)
SXA-2	0.056±0.016**	0.086±0.012*** (34.88)	0.072±0.034** (22.22)
SXE-3	0.056±0.014***	0.12±0.01*** (53.33)	0.10±0.032*** (44.0)
SXA-3	0.046±0.01***	0.11±0.008*** (58.18)	0.098±0.04*** (53.06)

Values are expressed in mean ± SEM, values in parentheses have indicated percentage increase in respect to 1 h. ** p< 0.01 and *** p< 0.001 test groups vs. control group.

34.88% respectively) increase in paw volume was found at 24 h as compare to at 1 h, which was less than control animals (Table 3). This shows the maximum degree of immune system mediated hypersensitivity with fruit extracts compared to root extracts and standard drugs.

Carbon clearance test

SXE-1 (0.877±0.01), SXA- 1 (0.748±0.02), SXE-2 (0.638±0.01) and SXA-2 (0.796±0.02) significantly decreased carbon clearance, when compared to Control mean time 0.574±0.01 (Table 4).

CCl_4 induced oxidative stress

CCl_4 treatment significantly increases in levels of SGOT, SGPT, bilirubin, cholesterol, but decrease in HDL when

Table 4: Effect of the tested extracts on carbon clearance assay.

Groups	Carbon clearance
Control	0.574±0.01
SXE-1	0.277±0.01***
SXA-1	0.148±0.02***
SXE-2	0.638±0.01***
SXA-2	0.796±0.02**
SXE-3	0.418±0.003***
SXA-3	0.336±0.008**

All the values are expressed in mean mean ± SEM.
** p< 0.01 and *** p< 0.001, test group vs. control group.

compared to control animals. These levels were restored by SXE-1 and SXA-1. Result of SXE-1 was better than standard (Table 5).

Histopathological studies

Results supports by histopathological study. Normal control group showed normal hepatic globular structure, central vein and portal tract and kuffer cells. Whereas in carbon tetrachloride treated group hepatic globular architecture is normal but hepatic cells have fatty degeneration like ballooning of hepatocytes, fatty cyst and infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids are congested. The Group treated with ethanol extract of fruit has normal hepatic globular architecture and very few areas shown with lymphocytic infiltration. Majority of hepatocytes were normal (Figure 1A, 1B, 1C and 1D). In Standard drug treated group; the hepatic globular architecture is almost normal. Few fatty cells and some cells have shown hyaline and cytoplasm.

Estimation of solasodine in S. xanthocarpum

HPLC analysis has shown 0.894%, 1.146%, 0.643% and 0.620% of solasodine present in the SXE-1, SXA-1,

Table 5: Effect of sxe-1 and sxa-1 on ccl_4 induced oxidative stress.

Group	SGPT (IU/L)	SGOT (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	Alkaline phosphate (IU/L)
Control	46.81±1.65	95.66±0.66	0.896±0.054	0.174±0.010	103.55±0.438	46.76±0.274	121.826±1.909
CCl_4 treated	284.58±21.05	523.22±15.95	4.259±0.095	1.587±0.08	164.84±1.815	26.81±0.36	250.591±1.778
SXE-1	62.58±1.69***	131.0±0.95***	1.256±0.112***	0.40±0.028***	117.24±0.695***	44.31±0.361***	80.635±0.92***
SXA-1	208.11±6.54***	361.49±4.89***	3.051±0.068***	0.841±0.028***	154.06±2.71 ***	31.23±0.416***	196.456±1.554***
Standard	91.03±1.30***	130.76±1.51***	1.535±0.068***	0.544±0.023***	120.63±0.69***	40.77±0.53***	89.804±0.987***

All the values are expressed in mean mean ± SEM,
*p< 0.05,** p< 0.01 and *** p< 0.001 test group vs. CCl_4 treated group

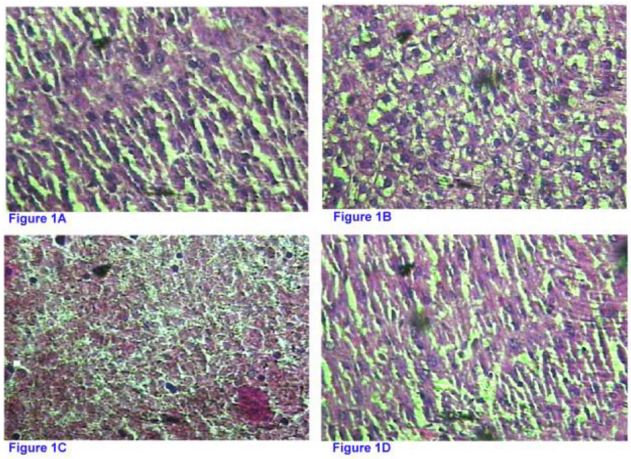


Figure 1A: Normal Control (-Ve Control); Hepatic Globular Structure, Central Vein, Portal Tract and Kuffer Cells Are Normal.
Figure 1B: Carbon tetrachloride treated group (+ve control); hepatic globular architecture is normal, hepatic cells have fatty degeneration like ballooning of hepatocytes, fatty cyst, and infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids are congested.
Figure 1C: sxe-1 treated group; the hepatic globular architecture is normal. Very few areas are with lymphocytic infiltration. Majority of hepatocytes are normal.
Figure 1D: SXA-1 treated group; the hepatic globular architecture is normal with some fatty changes. A few areas are with lymphocytic infiltration. Majority of hepatocytes are normal.

Table 6: Content of solasodine in *S. xanthocarpum* Extract.

Sample	Solasodine content [content ± SD; n=3]		
	Content % (w/w)	S.D.	% R.S.D
S. xanthocarpum aqueous fruit extract (SXA-1)	1.146	0.011	0.045
S. xanthocarpum ethanol fruit extract (SXE-1)	0.894	0.045	0.022
S. xanthocarpum ethanol root extract (SXE-2)	0.643	1.12	1.043
S. xanthocarpum ethanol root extract (SXA-2)	0.620	0.821	0.175

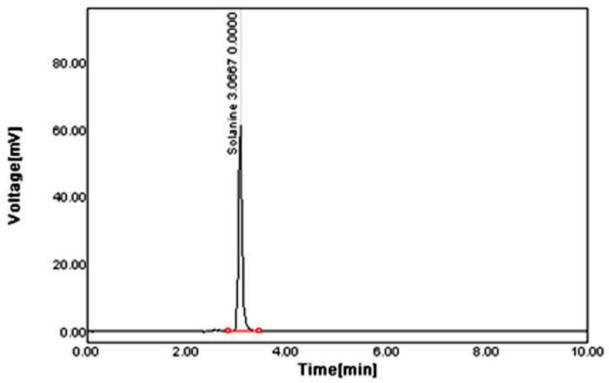


Figure 2: RP-HPLC chromatograph of Solasodine.

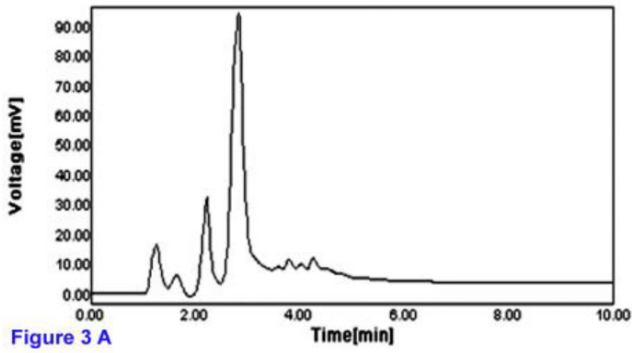
SXE-2 and SXA-2 respectively (Table 6; Figure 2, 3A and 3B).

DISCUSSION

To detect the presence of various phytoconstituents in formulations as well as in raw materials phytochemical investigations were performed. The tests were performed on alcohol and water, extract. It has conforms the presence of alkaloids, flavonoids, steroids and saponins.

200 mg/kg body weight ($1/10^{th}$ of ALD_{50}) dose was selected for *in-vivo* immunomodulatory study by *Acute Toxicity Study (ALD50).*

Biochemical parameters and hematological parameters are the index of immune system, imbalance of those parameters may disturb immune system. Our present study investigated that stress alters normal biochemical and hematological parameters, which may disturb immune system.[22] The adoptive reaction enables the body to heighten its power of resistance towards stresses and to adopt external conditions. Pretreatment with *Withania somnifera,* SXE-1 and SXA-1 enhanced swimming survival time, when compared with stress control group in cold water swim stress. Present study revealed that stress causes alteration of normal biochemical parameters as well as change in vital organ weight and blood



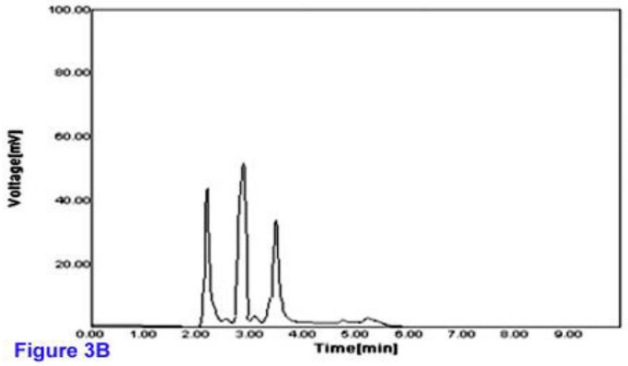


Figure 3A: RP HPLC chromatograms of solasodine in *S. xanthocarpum* ethanol fruit extract (SXE-1).
Figure 3B: RP HPLC chromatograms of solasodine in *S. xanthocarpum* aqueous fruit extract (SXA-1).

cell count. The biochemical parameters like glucose, cholesterol, triglycerides were increased in stress control group but these parameters recovered significantly in SXE-1 and SXA-1 treated groups. Increased blood cell count in stress control group was also significantly decreased in the entire treated group except SXE-2 and SXA-2 treated.

Whereas, aqueous and ethanol extract of *Solanum xanthocarpum* fruits reported for protection of experimental animal against Cyclophosphamide induced immunosuppression.[23-24]

Modification of immune functions by pharmacological agents is emerging as a major area of therapies in those cases where undesired immunosuppression is the result of therapy. Such efforts were previously being carried out by using gluco corticoid in combination with cytotoxic drugs like cyclophosphomide. In contrast, desired immunostimulation (i.e. hyper-reactivity) is a common side effect with drugs like quinine, salicylate etc. In delayed type hypersensitivity model i.e. type T lymphocytes and activated macrophage mediated reaction, sensitize animals, when challenges with same allergens, result in a significant increase in paw edema when compared with control. Delayed type hypersensitivity also demonstrated that SXE-1 and SXA-1 as immunostimulant. Enhanced phagocytic activity of macrophages is evaluated by the rate of elimination of exogenously administered antigen such as carbon particles. In addition, *in-vivo* assay macrophages probably secrete a number of cytokines which in turn stimulate other immunocytes. This may give defense ability to counter the infectious stress to the host.[19] Hepatic injury induced by CCl_4 is due to its conversion into highly reactive radical trichloromethyl radical, which further react with oxygen to give trichloromethylperoxy radical. Cytochrome P ^{450} 2EI is the enzyme responsible for this conversion which is a main culprit for hepatotoxicity.[25] Trichloromethyl peroxy radical forms covalent bond with sulahydryl group of various membrane molecules like glutathione, protein thiol, lipid or unsaturated lipid. This covalent binding of free radical with the cellular macromolecule is considered the initial step in chain of events which leads to lipid peroxidation.[25] This result in the release of marker enzymes like SGPT, SGOT, ALP etc. in addition billiary excretion of bilirubin level are raised and HDL level are decreased. Since GSH involve in the scavenging of highly reactive free radicals, liver damage is not seen until the significant depletion of GSH level. Therefore decreased level of GSH levels are observed in liver tissue.[26]

The two extracts SXA-1 and SXE-1 were further subjected for screening of hepatoprotective activity to find any possible correlation between immunostimulant and hepato-protective properties. Toxic liver injury produced by drugs and chemicals may virtually mimic any form of naturally occurring liver disease. Drugs like carbon tetrachloride or one of its metabolites is either directly toxic to the liver or it lowers the host immune defense mechanism.[27- 28]

The observations in present study established the traditional claim and thus *S. xanthocarpum* could be a potent immunomodulatory agent for use in future. In our present study we conclude that aqueous and ethanol extract of *Solanum xanthocarpum* fruit have significant the immunostimulant potential. Whereas, aqueous and ethanol extract of root does not have immunostimulant potential.

In addition, aqueous and ethanol extract of *Solanum xanthocarpum* fruit are having significant hepatoprotective activity which may be attributed to the immunostimulant property. . Further the HPLC analysis of *S. xanthocarpum* revealed the presence of 1.146% and 0.894% solasodine in aqueous and ethanol extracts of *S. xanthocarpum* fruit respectively. But, 0.643% and 0.620% estimated in ethanol and aqueous extracts of *S. xanthocarpum* root respectively

CONCLUSION

The observations in present study established the traditional claim and thus *S. xanthocarpum* could be a potent immunomodulatory agent for use in future. In our present study we conclude that aqueous and ethanol extract of *Solanum xanthocarpum* fruit have significant the immunostimulant potential. Whereas, aqueous and ethanol extract of root does not have immunostimulant potential. HPLC analysis of *S. xanthocarpum* revealed the presence of higher level of solasodine in aqueous and ethanol extracts of *S. xanthocarpum* fruit compared to ethanol and aqueous extracts of *S. xanthocarpum* root. Since solasodine is a lead constituent of the plant, hence solasodine may be responsible for the above immunostimulant property. However, further bioactivity guided phytochemical analysis in future could be reveal phytopharmacological relation of immunostimulant property and solasodine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SXE-1: Ethanol extracts of *S. xanthocarpum* fruit; **SXA-1:** Aqueous extracts of *S. xanthocarpum* fruit; **SXE-2:** Ethanol extracts of *S. xanthocarpum* root; **SXA-2:** Aqueous extracts of *S. xanthocarpum* root; **SXE-3:** Ethanol extracts of *S. xanthocarpum* whole plant; **SXA-3:** Aqueous extracts of *S. xanthocarpum* whole plant; **SGOT:** Serum glutamic-oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvic transaminase; **CCl_4:** Carbon tetrachloride; **HPLC:** High-performance liquid chromatography.

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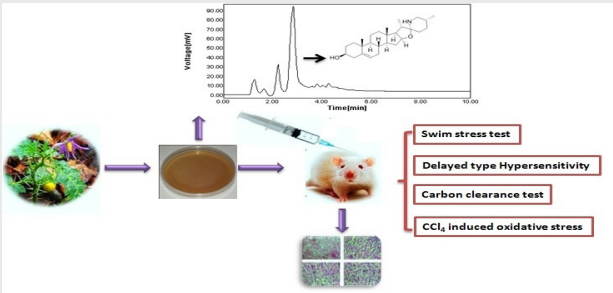
PICTORIAL ABSTRACT



SUMMARY

- *Solanum xanthocarpum* used in the Indian traditional system of the medicine as an immunomodulatory agent.
- The fruit extracts (aqueous and ethanol) of *S. xanthocarpum* have exhibited higher degree of immunomodulatory effect compared to root and whole plant extract.
- The bioactive principal, Solasodaine contents more in fruit extracts compared to root and whole plant extract. Therefore, Solasodaine may responsible for immunomodulatory effects of the plants.

About Authors

Dr. Ravindra Kumar Pandey (RKP), M.Pharm, Ph.D.Professor, Columbia Institute of Pharmacy (CIP), Raipur, was a main researcher for this work. Ravindra executed all experiments required for this work in Pharmacology lab, B. R. Nahata College of Pharmacy (BRNCP), Mandsaur-458001, Madhya Pradesh, India as per the instructions of supervisors and compiled the work as a manuscript in Columbia Institute of Pharmacy (CIP), Raipur-492001, Chhattisgarh, India. He also had done literature survey.

Dr. Avijeet Jain, M. Pharm, Ph.D. Professor & Principal at Dr. Satayendra Kumar Memorial College of Pharmacy. He has participated in designed od overall pharmacognostical and phytochemical screening required for the thesis work and supervised the same. He also helps for compilation of research data in BRNCP.

Dr. Shiv Shankar Shukla, M.Pharm, Ph.D.Professor, Columbia Institute of Pharmacy (CIP), Raipur has participated in compilation research data, prepared tables, analyzed data and helps in writting the Manuscript in CIP.

Dr. Vipin Bihari Gupta, M.Pharm, Ph.D. Ex. Professor & Director, B. R. Nahata College of Pharmacy (BRNCP), Mandsaur. Vipin has supervised overall research activities and review the manuscript in BRNCP.

Dr. Anurekha Jain (AnJ), M.Pharma, Ph.D., Ex. Professor, B. R. Nahata College of Pharmacy (BRNCP), Mandsaur. Anurekha has designed phytochemical analysis required for this work and supervised respective work in BRNCP

Dr. Lokesh Deb, M.Pharma, Ph.D. Scientist – C (Pharmacology),IBSD- Sikkim Centre (Department of Biotechnology, Govt. of India). Lokesh has designed and mentored overall project including pharmacological experiments in BRNCP. He also helps for writing the manuscript, finalized figures and tables with help of the team members and communicated the Manuscript from IBSD-SC.

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