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Research Article

STUDIES ON THE PHYSICO-PHYTOCHEMICAL PROPERTIES AND HEPATOPROTECTIVE EFFECT OF *SOLANUM TORVUM* SWARTZ IN CCl_4 INDUCED EXPERIMENTAL TOXICITY IN ALBINO RATS

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ABSTRACT

Solanum torvum Swartz (Family: Solanacea) is a popularly used medicine in Tamilnadu for treating various illnesses in the native system of Medicine. The hydroalcoholic extract of fruits of this plant at dose level 200 mg/ kg b w showed promising hepatoprotective activity as evidenced by biochemical parameters like SGPT (Serum glutamate pyruvate transminase), SGOT (Serum glutamate oxaloacetate transminase), Cholesterol, Bilirubin, Total protein and LDH after CCl_4 administration to rats. Further histopathological examination of the liver was also studied. Nevertheless the overall hepatoprotection exhibited by the extract is found to be low in comparison to standard drug silymarin at a dose of 25 mg / kg. Preliminary phytochemical analysis of the plant in question was attempted. The results are highlighted and discussed.

Keywords: *Solanum torvum*, Physico-Phyto chemical parameters, Hepatoprotective activity, CCl_4 induced toxicity in rat model, Ethnomedicine.

INTRODUCTION

Solanum Torvum Swartz (Family: Solanaceae) is known as Devil's fig in English, Kaatuchunta in Malayalam and Sundaikkai in Tamil. It is prickly, tomentose, erect shrub, 1.5-3m high, leaves having no prickles, white bell-shaped flowers and lobed fruits seated on the calyx[1]. It is a common plant found throughout the Indian subcontinent and West Indies, Bermuda, Indonesia, Malaya, China, Philippines and tropical America[2, 3]. The fresh fruits are made into a paste and given orally to reduce fever by the Nilgiri Irulas. The Nilgiri Paniyas consume freshly collected fruits to relieve chest conjestion and cough[4]

The fruits are useful for treating liver and spleen enlargement, cough and haematopoietic, antimicrobial and analgesic[5-7]. Many valuable phytoconstituents of therapeutic importance such as steroidal alkaloids, chlorogenone, neochlorogenone, isoflavanoid sulfate, steroidal glycosides, 2, 2 o-spirostannol.(Torvonin-A), Solasonine, sterolin (Sitesterol-D-glucoside), Protein, fat and minerals have been earlier isolated classes of constituents reported[8-12]. People of Tamil nadu use the fruit of *S.torvum* as vegetables in the daily diet.

Liver is one of the important vital organs which regulate many crucial metabolic functions in the humanbody[13]. The toxins absorbed from the intestinal track first go to the liver causing variety of liver diseases[14]. Hepatitis is nothing but an inflammation of liver caused by certain viruses, and other agents. Cirrhotic changes the structure of the liver leads to dysfunction and retention of toxins in the blood. Therefore damage to the liver inflicted hepatotoxic agents[15]. Drug also induce liver toxicity which is a common cause of liver injury[16, 17]. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders[18]. A number of plants have been shown to posses hepatoprotective property by improving antioxidant status[19]. Natural remedies from medicinal plants are believed to be effective and safe alternative treatment for hepatotoxicity. The diverse medicinal properties of *solanum torvum* swartz inspired us to investigate its hepatoprotective activity.

In the light of skimpy data on hepatoprotective potential of above plant used in the traditional system of medicine, the present work was undertaken to throw more light on the pharmacological activities of *Solanum torvum*. This work supplements additional information on the topic.

MATERIALS AND METHODS

Collection of Plant Material

Solanum torvum fruits were collected during the months March and April from the fields of Thanjavur (TN). The collected specimens were authenticated by Botanist Dr.S.Rajan by comparing them with the herbium specimen of survey of Medicinal plants and collection unit (CCRH), Ooty. The voucher specimens were deposited in the herbarium of Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Tamil Nadu, India for future reference. The work was carried out in the Department of Pharmacology, Periyar college of pharmaceutical sciences, Tiruchirappalli, Tamil Nadu, India. The clearance of Animal Ethical Committee has been obtained from the college. The samples were washed with distilled water and dried under shade, mechanically pounded to get coarse powder and passed through member 40 sieve mesh. The sample powders were processed in such a way that they are useful for carrying out both powder studies and phyto chemical analysis.

Preparation of plant extracts

The coarse powder 100 grams of the given *S.torvum* was extracted using 400ml of hydroalcohol (20: 80) by continous hot percolation with help of soxhlet apparatus until the extraction procedure is complete. The successive extractions were done separately for each solvents namely water and ether. The powder solvent ratio employed for the present study was 1: 4. On completion, the extracts were filtered and the solvents were removed by distillation and dried under reduced pressure and controlled temperature 50-60°C and the sample was refrigerated until use. The hydroalcoholic extract was subjected to various analysis such as organoleptic characters[20], fluorescence studies[21], physico-chemical parameters[22], Preliminary photochemical screening[23] and quantitative analysis of various phytoconstituents[24]. The quantification of various metals present in *S.torvum* were analysed using Atomic absorption spectrophotometer. The hepato protective activity of the hydroalcoholic extract was evaluated as detailed below.

Evaluation of Toxicity

The LD_{50} study was carried out by Miller and Trainter method and result was reported.

Animal Studies

Albino rats of either sex weighing 150 to 200g belonging to Wistar strain were used in this study. The animals were procured from a registered animal dealer of the college (Sri Venkateshwara Enterprises, Bangalore). The rat pellet food was also supplied by the same firm (Hindustan Lever Company). The animals were accilimatized to the laboratory condition by subjecting them to dark and light cycles for 12hours period before commencement of work. All the animals were given food and water ad libitum.

Evaluation of hepatoprotective activity

- Group I served as control 1ml/kg b.w (Normal Saline)
- Group II Served as a hepatotoxic control (CCl_4 Induced) 1ml /kg b.w
- Group III received hydroalcoholic extract of *Solanum torvum* dry fruit powder 200mg/kg b.w
- Group IV Served as a reference standard drug Silymarin 25mg/kg b.w

Hepatotoxicity was induced by intrapretonial injection of CCl_4 (By S.D. Fine Chemicals Pvt Ltd.,) at a dose of 1ml/kg b.w.were given for Group II, Group III and Group IV whereas, Group I received similar volume of vehicle (Normal Saline) 1ml / kg b.w. The hydroalcoholic extract of *solanum torvum* was administered for Group III orally by using catheter after CCl_4 induction. For Group IV reference standard drug silymarin was given orally after CCl_4 induction. Treatment continued to fifteen consecutive days, at the end of fifteenth day serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) where measured by the method of Reitman and Frankel[25, 26]. Cholesterol, Bilirubin, Total protein, LDH were determined using standard proceedures[27, 28]. The results were tabulated and analysed and discussed.

Histopathological Studies

Hepatotoxic animals were sacrificed at the end of fifteenth day after treatment and their hepatic tissues were collected and washed with saline and then were fixed in 10% buffered formalin and embedded in paraffin wax for light microscopic examination of HPE sections. They were shown in the figure 1.

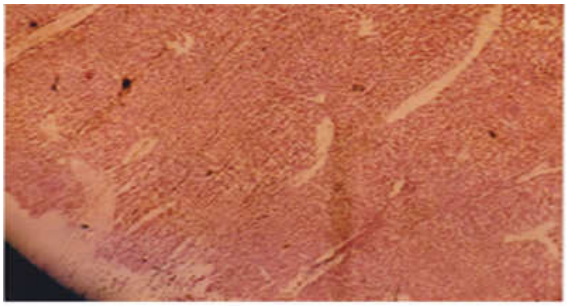

Fig.1a: Histopathological section of Normal liver

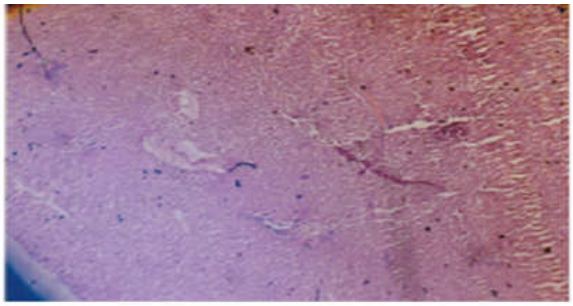

Fig.1b: Hepatic tissue after CCl_4 induction of hepatotoxicity

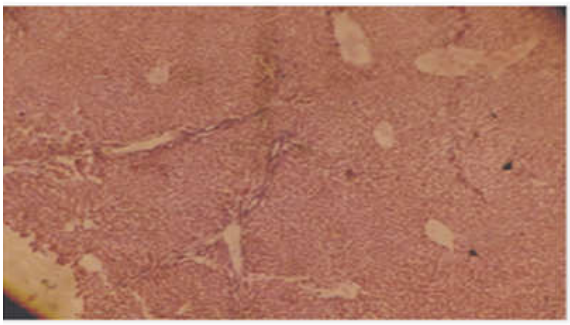

Fig.1c: Hepatic tissues after drug treatment with the study extract

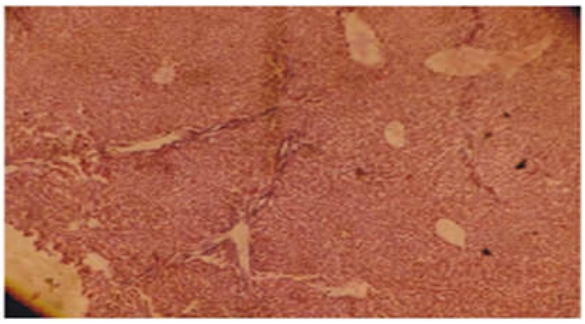

Fig.1d : Hepatic tissues after treatment with reference standard silymarin

Statistical analysis

The results were analysed as mean of ±SE., n=6. Data were tabulated analysed and discussed using student 't' test.

RESULTS AND DISCUSSION

Tables 1-5 narrate the results of physico-phytochemical studies on the dry fruit of *solanum torvum*. The dry fruit appears orange red colour and posses bitter taste with no discernible odour with coarse texture. These powders when viewed under UVlight at 365nm appear green in colour, under normal light also they appear green in colour. After treating with various biochemical reagents they displayed narrow ranging colour variations only. The hydroalcoholic extractive values are higher than water and ether soluble extractive values. Both total ash and sulphated ash values are recorded higher, when compared to moisture content values. Preliminary phytochemical screening of *S.torvum* indicates the presence of alkaloids, carbohydrates, reducing sugars, flavanoids, gums and mucilage and proteins. The tannin is absent. The alkaloid, flavonoids, and saponin contents of S.torvum are 1.25mg, 18mg, and 0.31mg respectively per 100 mg of the sample.

Table 1: Organoleptic characters of *Solanum torvum* Swartz

Organoleptic characters	*Solanum torvum*.Swartz
Colour	Raw fruits appear green in colour. Dried fruits are orange red in colour.
Taste	Bitter
Odour	Not Discernible
Texture	Coarse

Table 2: Fluorescence Studies of *Solanum torvum* Swartz

Characters	Day light	UV Light at 365nm
Sample	Green	Green
Sample+1N Sodium hydroxide	Greenish Yellow	Greenish Visible
Sample+1N Hydrochloric acid	Green	Invisible
Sample + 50% Sulphuric acid	Green	Pale green

Table 3: physicochemical characteristics of the hydroalcholic extract of *Solanum torvum*.Swartz

Character	*Solanum torvum*.Swartz (g)
Total ash	0.30
Water soluble ash	0.09
Acid insoluble ash	0.11
Sulphated ash	0.50
Moisture content	0.12
Alcohol soluble extractive	0.32
Water soluble extractive	0.26
Ether soluble extractive	0.11

*All values represent per 1g of the sample

Table 4: Qualitative phytochemical analysis of the hydroalcoholic extract of *Solanum torvum* Swartz

Phyto constituents	*Solanum torvum*.Swartz
Alkaloid	(+)
Carbohydrate	(+)
Reducing sugar	(+)
Tannins	(-)
Flavonoids	(+)
Gums and Mucilage	(-)
Saponins	(+)
Protein	(+)

(+) indicates presence; (-) indicates absence

Table 5: Quantitative analysis of various phytoconsituents present in the hydroalcholic extract of *Solanum torvum*

Phyto constituents	*Solanum torvum*.Swartz (mg)
Alkaloid	1.25
Carbohydrate	3.50
Reducing sugar	11.60
Tannins	-
Flavonoids	18.00
Saponins	0.31
Protein	8.00

*All values represent per 100 mg of the sample

The quantification values of various elements such as Iron, Copper, Zinc, Magnesium, Chromium and Manganese are shown in (Table 6). The magnesium and Iron are present in higher (286.50 and 206.25 ppm) whereas chromium is present in low amount (9.31 ppm). Metals and minerals present in biological system play significant role in the metabolism[29]. Magnesium plays very crucial role in the function of Mg-dependent enzymes whose deficiency leads to tissue damage. Metals and trace elements have a decisive role to play in the patho-physiology of human disease[30].

Table 6: Quantification of various elements in the fruits of *Solanum torvum* Swartz in ppm

Elements	Fe	Cu	Zn	Mn	Cr	Mg
Amount in ppm	206.25	171.47	121.20	79.30	9.31	286.50

Table 7: LD_{50} value of *S.torvum* hydroalcoholic extract

Test sample	LD_{50} Value (mg / kg.b.w)
Solanum torvum swartz	2000

Table 7 shows the LD_{50} value of *S.torvum* hydroalcoholic fruit extract which was found to be 2000mg/kg bw 1/10 th of this dose was chosen as initial dose in this work.

Table 8 summarizes the results of hepatoprotective activity of *solanum torvum* hydroalcoholic fruit extract. The serum enzyme levels SGPT and SGOT, Cholesterol, Bilirubin, Total protein, and LDH levels were distinctly elevated in the group of those rats administered with the CCl_4. It is clear from the results that hydroalcoholic extract of fruit powder showed antihepatotoxic effects as evidenced by the reduction of the CCl_4 elevated serum enzyme levels and other biochemical parameters($p<0.001$). The CCl_4 is metabolized by the mixed function oxidase system in the endoplasmic reticulam of the liver. Cleavage of carbon chloride bond results in the formation of trichloromethyl radical (CCl_3·), which is highly unstable and immediately react with cellular membrane components. They form covalent bonds with unsaturated fatty acids or abstract a hydrogen atom from the unsaturated fatty acids of membrane lipids results in the production of chloroform and lipid radicals. They reacts with molecular oxygen, that initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum. The peroxidation process results in the release of soluble products that affect cell membrane[31]. It is generally believed that microsomal oxidation of endoplasmis reticulam by the chloroform will result in the formation of phosgene. It is a secondary metabolite which is known to cause cell death.

These preliminary findings indicate that after CCl_4 administration the liver function is impaired which is supported by elevation in the serum bilirubin levels which is indicative of liver toxicity. It is to be noted here that although both SGOT and SGPT levels get elevated during liver damage. GOT is a liver specific enzyme[32] whose elevation suggests liver damage. Lactate dehydrogenate (LDH) activity may be elevated in all cases of liver diseases but not as high as to the elevation of SGOT and SGPT levels. But in the present studies LDH elevation is more than SGPT and SGOT which deserves further examination of this trend through future studies.

The S*olanum torvum* fruit extract treatment afforded a significant protection against CCl_4 induced toxicity and the elevated levels of various enzymes were brought to normal levels significantly ($P<0.001$) as compared to that of control. However, the overall hepatoprotective effect was found to be less than standard reference drug silymarin.

Table 8: Effects of *Solanum torvum* raw fruit extract on various on biochemical parameters in CCl_4 induced hepatotoxicity

Treatment	SGPT (µ /L)	SGOT (µ /L)	Cholesterol (mg/DL)	Bilirubin (mg/DL)	Total Protein (g/DL)	LDH (µ /L)
Control (Group1)	97.3± 1.18	35.08± 0.2	15.92 ± 0.72	14.0± 0.16	4.2± O.22	892.2± 12.3
CCl_4 (1ml/kg) (Group2)	186.7± 1.82	196.9± 1.94	98.3± 7.9	26.0± 0.14	14.2± 1.1	1820.2± 10.76
Solanum torvum.Swartz sample 200mg/kg (Group3)	62.08 ±4.80	153.0*± 5.2	84.0* ±4.6	12.6± 0.20	5.5± 0.12	1264.8*± 12.06
Silymarin 25mg/kg(Group 4)	47.3*±4.3	49.4*± 3.6	34.8*± 2.9	6.5± 0.14	7.2± 0.16	696.45*± 10.03

All values are expressed as mean ±S.E, n=6, df-10 t test $p<0.001$ assignificant compared to that of control.

CONCLUSION

Liver injury can be caused by toxic chemicals, drugs and virus infiltration either from ingestion or infection caused by toxins which one absorbed from the intestinal tract by the liver resulting in variety of liver ailments. Thus, liver disease remain one of the series public health problems [33, 34, 35]. The administration of CCl_4 elevates the levels of serum marker enzymes SGPT, SGOT, and other biochemical parameters like Bilirubin, Protein LDH[36] and thus their toxic effects confirmed by histopathological studies of hepatic cells. The *S.torvum* hydroalcoholic fruit extract effectively prevented CCl_4 induced hepatotoxicity. The *Solanum torvum* fruit extract treatment clearly indicate regeneration changes in the liver tissue to normalcy after CCl_4 damage as reflected in the HPE of liver tissue. From these results, it is concluded that *S.torvum* extract has hepatoprotective effect. However further studies are necessary for the identification of bioactive constituents responsible for hepatoprotection present in the extracts of the study plant.

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