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Research Paper

Exploration of Hepatoprotective Activity of Aqueous Extract of Solanum Nigrum

Pharmacology

KEYWORDS : Carbon tetrachloride; Free radical scavenger; Hepatotoxicity

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ABSTRACT

Aim

To explore the hepatoprotective potential of Solanum nigrum.

Materials and Methods

Albino Wistar rats weighing 150-200g of either sex were divided into six groups of six animals each. Group I was given normal saline, group II carbon tetrachloride(CCl4), group III Liv.52 syrup for twenty days followed by CCl4, group IV, V & VI received aqueous extract of S. nigrum (1ml/100g twice daily) orally for 10, 20 & 30 days respectively followed by CCl4 administration. Blood was collected from anaesthetized animals. Alanine transaminase (ALT), Alkaline phosphatase (ALP) & Total bilirubin were estimated.

Results

ALT, ALP & Total bilirubin levels were significantly increased in CCl4 treated group while S. nigrum displayed significant reduction in rise in these parameters in group IV, V and VI.

Conclusion

It can be concluded from the present study that S. nigrum extracts are potent hepatoprotective agent.

INTRODUCTION

The liver is a vital organ which regulates homeostasis in the body.[1] This organ is expected not only to perform physiological functions but it also protect against the hazards of harmful drugs and chemicals.[2] Most of the liver diseases are well associated with oxidative stress which is caused by free radicals. Free radicals are generated inside the human body as a result of the exposure to exogenous chemicals as well as endogenous metabolic processes.[3,4] Thus, it is very important to maintain a healthy liver.

Solanum nigrum has been shown to be hepatoprotective by Ayurvedic physicians. Some studies also indicate the hepatoprotective activity of *S. nigrum.*[5]

Therefore, the present study is envisaged to strengthen the hepatoprotective activity of an aqueous extract of *S. nigrum.*

MATERIALS AND METHODS

Preparation of plant extract

After collection of the aerial parts of the plant *S. nigrum* in required quantity, it was carefully washed and dried in shade. Dried stem and leaves of the plant were powered and prepared powder was kept in airtight glass jar and stored at 4°C until used.

100 g of the prepared powder weighing was macerated and soaked in 500 ml of distilled water for 24 h. It was then filtered and the filtrate was concentrated to a dark green residue by heating at 40°C. 100 mg of this concentrated extract dissolved in 1ml of distilled water and the resulting solution was administered in rats. [5]

Animals

Albino rats (Wistar strain) of either sex weighing 150-200g were obtained and after one week of acclimatization, the animals were considered suitable for study.

Study was reviewed and approved by the Institutional Animal and Ethics Committee of S. N. Medical College, Agra, India, and was in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity study

The animals were divided into five groups (n = 6). The aqueous extract of *S. nigrum* was administrated orally in increasing dose up to 800 mg/kg. The rats were observed continuously for 2 h for behavioural, neurological, and autonomic profiles and after 24 and 72 h for any lethality. [6, 7]

STUDY DESIGN

This experimental study was undertaken in the Department of Pharmacology, S. N. Medical College, Agra from June' 2014 to September' 2014.

The animals were divided into six groups of six animals each.

Group-I: This group was given normal saline 1ml/100g twice daily orally in addition to the standard rat pellet diet and tap water for a duration of 20 days.

Group-II: This group was given 1 ml/kg of a 50% v/v solution of carbon tetrachloride in olive oil intraperitoneally once only.

Group-III: This group was given Liv.52 syrup (1 ml/100g twice daily) orally for twenty days followed by CCl_4 intraperitoneally. CCl_4 dose was given concomitantly with the last (20^{th} day) dose of Liv.52.

Group-IV: This group received the *S. nigrum* extract in the dose of 1ml/100g twice daily orally for a total period of 10 days. CCl_4 dose was given concomitantly with the last (10^{th} day) dose of *S. nigrum.*

Group-V: Animals in this group received the *S. nigrum* extract (1ml/100g per orally twice daily) for a total period of 20 days. CCl_4 dose was given concomitantly with the last (20^{th} day) dose of *S. nigrum.*

Groups-VI: This group received the *S. nigrum* extract (1ml/100g per orally twice daily) for a total period of 30 days. CCl_4 dose was given concomitantly with the last (30^{th} day) dose of *S. nigrum.*

Animals of all the groups were fasted for 24 hours (during this duration water remained freely available) after which they were sacrificed under Ketamine (75 mg/kg i.p.) and Diazepam (10

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mg/kg i.p.) anaesthesia. [8] Blood was collected from the anaesthetized animals from retro-orbital plexus.

Biological study parameters
The collected blood, after a standing time of half an hour, was centrifuged in Remi R-8 centrifuge at 2500 rpm for 10 min. The serum so obtained was used to estimate the biochemical parameters viz. Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Total bilirubin using standard diagnostic kits. (Span Diagnostics Ltd., India)

Statistical Analysis
Results were expressed as Mean ± Standard deviation (SD). Statistical differences between the groups were tested by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparisons.

RESULTS
Acute toxicity studies revealed the nontoxic nature of the aqueous extract of *S. nigrum* until the end of the study period.

EFFECT ON ALANINE AMINOTRANSFERASE (ALT) (Table 1)
ALT level in normal saline treated group was significantly increased ($p<0.001$) with administration of CCl_4.

Pretreatment with known hepatoprotective preparation Liv.52 significantly ($p<0.001$) limited the rise in ALT levels after CCl_4 administration.

Although aqueous extract of *Solanum nigrum* in the doses of 2 ml/100g for 10 days and 20 days showed a significant limitation ($p<0.001$) of ALT rise when compared to CCl_4 treated group, its effect was not comparable to that of Liv.52 treated group. However, in dose of 2 ml/100g for 30 days, the *Solanum nigrum* extract limited the ALT rise after CCl_4 administration which was highly significant ($p<0.001$).

EFFECT ON SERUM ALKALINE PHOSPHATASE (ALP) (Table 2)
A highly significant ($p<0.001$) rise in serum ALP levels was seen in CCl_4 treated group as compared to the normal saline treated group.

The rise in serum ALP was significantly low ($p<0.001$) in Liv.52 treated group after CCl_4 administration as compared to only CCl_4 treated group.

The effect of *Solanum nigrum* treatment on serum ALP levels was time related. As with ALT, the doses of 2ml/100g for 10 days of *Solanum nigrum* produced significantly less ($p<0.001$) increments in serum ALP as compared to the CCl_4 treated group. However, in dose of 2ml/100g for 30 days, *Solanum nigrum* showed significantly better ($p<0.001$) prevention in rise of ALP than Liv.52 treated group.

EFFECT ON TOTAL SERUM BILIRUBIN (Table 3)
The administration of CCl_4 significantly increased ($p<0.001$) the serum bilirubin as compared to normal saline treated group. The rise in serum bilirubin was significantly low ($p<0.001$) in Liv.52 treated group after CCl_4 administration as compared to only CCl_4 treated group.

The total bilirubin levels achieved with *Solanum nigrum* in the doses of 2 ml/100g for 10, 20, and 30 days were significantly low($p<0.001$).

DISCUSSION
Liver damage induced by CCl_4 is very commonly used model for the screening of hepatoprotective drugs.[9]

Serum alanine transaminase, and alkaline phophatase were found to be significantly elevated after CCl_4 administration (Table 1, 2) though the rise in bilirubin level was not to the same extent as ALP and ALT. This phenomeneon can be explained by the fact that bilirubin reaches peak serum level in the second hour after CCl_4 administration and then declines afterwards. [10]

Our study reveals hepatoprotective effect of *S. nigrum.* The pharmacological constituents separated out from *Solanum nigrum* are reported to be steroidal glycoalkaloids, steroidal genin (titogenin), saponins and vitamins.[11]

The hepatoprotective effect of *S. nigrum* may be related to glutathione-mediated detoxification which is found in the cell cytosol of the living system.[12] It is a potent antioxidant and cofactor for enzymatic reactions. [13]

CONCLUSION
It can be concluded from the present study, that *Solanum nigrum* aqueous extract is potent hepatoprotective agent.

TABLES
Table 1: Effect of Liv.52 (1 ml/100g twice daily, po), ***Solanum nigrum*** **(SN) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Alanine Transaminase. (n=6).**

TREATMENT	ALANINE TRANSAMINASE (IU/l) (Mean ± **SD**)
Normal Saline	25.87±5.30
Carbon Tetrachloride(1 ml/kg, ip)	423.08±22.02^
Liv.52 + CCl_4 on 20th day	105.75±7.06**
SN x 10 days + CCl_4 on 10th day	298.74±19.42**
SN x 20 days + CCl_4 on 20th day	186.64±18.82**
SN x 30 days + CCl_4 on 30th day	104.54±13.90**$^{\neq}$

^$p<0.001$ as compared to normal saline treated group. $^{\neq}p>0.05$ as compared to Liv.52 treated group.

**$p<0.001$ as compared to CCl_4 treated group. $^{*}p<0.05$ as compared to CCl_4 treated group.

Table 2: Effect of Liv.52 (1 ml/100g twice daily, po), ***Solanum nigrum*** **(SN) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Alkaline Phosphatase. (n=6).**

TREATMENT	ALAKALINE PHOSPHATASE (IU/l) (Mean ± **SD**)
Normal Saline	23.73±**11.26**
Carbon Tetrachloride(1 ml/kg, ip)	237.84±**35.83**^
Liv.52 + CCl_4 on 20th day	67.69±**11.63****
SN x 10 days + CCl_4 on 10th day	147.60±16.30**
SN x 20 days + CCl_4 on 20th day	119.46±11.31**
SN x 30 days + CCl_4 on 30th day	72.10±14.31**$^{\neq}$

^$p<0.001$ as compared to normal saline treated group. $^{\neq}p>0.05$ as compared to Liv.52 treated group.

**$p<0.001$ as compared to CCl_4 treated group.

Table 3: Effect of Liv.52 (1 ml/100g twice daily, po), *Solanum nigrum* **(SN) (1 ml/100 gm twice daily, po) for the duration of 10, 20 and 30 days, on carbon tetrachloride (CCl_4) induced changes in Serum Total Bilirubin. (n=6).**

TREATMENT	TOTAL BILIRUBIN (mg/dl) (Mean ± **SD**)
Normal Saline	0.25±**0.11**
Carbon Tetrachloride(1 ml/ kg, ip)	1.88±**0.21**^
Liv.52 + CCl_4 on 20th day	0.60±**0.07****
SN x 10 days + CCl_4 on 10th day	1.43±0.23*
SN x 20 days + CCl_4 on 20th day	1.25±0.11**
SN x 30 days + CCl_4 on 30th day	0.80±0.10**$^{\pi}$

^p<0.001 as compared to normal saline treated group. *p<0.05 as compared to CCl_4 treated group.

$^{\pi}$p>0.05 as compared to LIV.52 treated group. **p<0.001 as compared to CCl_4 treated group.

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