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Original Article

Antidiabetic Potential and HPTLC Fingerprinting of *Schleichera oleosa* (Lour) Oken

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ABSTRACT

Introduction: *Schleichera oleosa* (Lour) Oken., also known as Lac tree, is a traditional plant used to treat different ailments since ancient time. The folkloric use of this plant as hypoglycemics is still un-revealed scientifically. So, the present study includes the *in-vivo* evaluation of hypoglycemic activity with HPTLC fingerprinting, qualitative and quantitative phytochemical screening of the extracts of the plant. **Methods:** Ethanolic extract of the plant (SOE) was subjected for phytochemical screening and HPTLC finger printing (using CAMAG LINOMAT 5 instrument). For the evaluation of hypoglycemic activity Streptozotocin (STZ) induced diabetic rats were treated with glibenclamide (0.5mg/kg body weight) and ethanolic extract (100 mg/kg and 200 mg/kg body weight) of the plant. Acute and chronic studies were performed for the evaluation of blood glucose levels. **Results:** The presence of alkaloids, tannins, phenolics and flavonoids were confirmed in the preliminary phytochemical screening. Quantitative screening of total tannins (6.15±4.20), total flavonoids (25.13±3.21) and total phenolic compounds (48.09±2.58) were reported. HPTLC fingerprinting analysis of SOE affirmed the presence of quercetin. **Conclusion:** The positive outcome was observed in the results of hypoglycemic activity, as all the treatments significantly decreased blood glucose level. During the study, SOE and glibenclamide maintained the body weight of the rats while diabetic control reduced the body weight by 11.33%. In support of its traditional use *Schleichera oleosa* (Lour) Oken. was proved to be a potent antidiabetic herbal plant.

Key words: *Schleichera oleosa* (Lour) Oken, Hypoglycemic, HPTLC, Quercetin.

INTRODUCTION

Hyperglycemia is a result of high blood glucose in plasma and diabetes is the authentication mark of it.[1] Diabetes mellitus is a group of metabolic disorder mainly characterized by high blood glucose level in the blood (hyperglycemia), kidney (glycosuria), hyperlipidemia and ketonaemia. Type II diabetes also called as maturity onset diabetes mellitus, is caused due to abnormal function of glucoreceptors of β-cells of the pancreatic islet of Langerhans resulting in insufficient production of insulin.[2] The global scenario of diabetes is very alarming, as the counts of diabetic affected people will be more than 640 million by 2040. In India, the management of diabetes is very challenging mainly because of lacking public awareness and expensive cost of medications.[3]

Diabetic-kidney disease, liver diseases and coronary artery diseases are the common and severe complications associated with diabetes.[4] The oral hypoglycemic drugs have potential adverse effects like low blood sugar, obesity, gastrointestinal disturbances, dizziness etc.[5] The choice of medicinal plants as an alternative approach for the management of diabetes is helpful to get rid of the diabetic complications.[6]

Schleichera oleosa (Lour) Oken., belongs to Sapindaceae family, is a medicinal plant having the plethora of ailment treating properties. The plant possesses several biological effects like antiulcer, anticancer, antimicrobial activities. Traditionally the plant is used as an antidiabetic.[7]

Though the *in-vitro* study of the antidiabetic activity of *Schleichera oleosa* (Lour) Oken. has been reported recently by evaluating alpha-amylase inhibition,[8] scientific *in-vivo* evaluation has not been done yet.

The present research deals with the *in-vivo* evaluation of hypoglycemic activity on streptozotocin induced diabetic rats and the High Performance Thin Layer Chromatography (HPTLC)-fingerprinting analysis of *Schleichera oleosa* (Lour) Oken.

MATERIALS AND METHODS

Collection and authentication of the plant

The plant *Schleichera oleosa* (Lour) Oken., was collected from Maharajganj, Uttar Pradesh, India and the leaves of the plant were authenticated by Indian Council of Agricultural Research (ICAR)-Kamla Nehru Krishi Vigyan Kendra, Sultanpur, Uttar Pradesh, India (Specimen No. 02/2017).

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Crude extracts preparation

The shade-dried cleaned leaves were powdered in grinder mixer, passed through sieve no. 40. The crude ethanolic (ethanol 95.5% v/v) extract of the powder was prepared by hot percolation process using Soxhlet apparatus at 70°-80°C for 32 h followed by distillation and drying. The crude extracts (SOE) were kept at desiccators for further experiments.

Qualitative and quantitative Phytochemical screening

The qualitative identification of phytoconstituents of the ethanolic extract for alkaloids, tannins, flavonoids and phenolic compounds were performed by following standard methods. For alkaloids Mayer's, Dragendroff's, Hager's and Wagner's tests were performed. Chloride test and Vaniline tests were performed to identify tannins. Flavonoids were identified by the treatment of extract with aqueous sodium hydroxide, concentrated sulphuric acid and Shinoda's test, whereas, ferric chloride, lead acetate and bromine water were used to assure the presence of phenolics.[9]

The quantitative estimations of tannins (Folin–Ciocalteau assay),[10] flavonoids (Aluminium chloride colorimetric assay)[11] phenolic compounds (Folin–Ciocalteau reagent-15% sodium carbonate assay)[12] were examined by the standard methods with minor modifications in triplicates and the results were represented as mg of Tannic acid, Quercetin and Gallic acid equivalents per gm dry weight respectively.

HPTLC fingerprinting

The study of HPTLC fingerprinting was done according to the standard procedures.[13-14] The analysis was done by CAMAG LINOMAT 5 instrument (Camag; Muttenz, Switzerland), operated by WINCATS software. The ethanolic extract of *Schleichera oleosa* (Lour) Oken. was dissolved with HPTLC grade ethanol (100 mg in 1ml) followed by centrifugation. Quercetin was taken as standard. Sample solution and standard solution (each of 2µl) were sprayed on Silica gel $60F_{254}$ TLC plate as bands of 5mm length 10 mm from the bottom edge, 10 mm from the side edge and 6 mm apart using 100µl Hamilton syringe. Saturation of the chamber was done with Toluene: Ethyl acetate: Formic acid (13:11:2), as the mobile phase for 25 min at room temperature. The chromatogram was developed by following ascending technique in CAMAG twin trough developer from the point of sample application to approximately 80 mm solvent front in given mobile phase followed by the drying of the plates in hot air to remove the solvent. The plates were kept in CAMAG REPRO-STAR 3 (photo-documentation chamber). The images were taken at the different wavelength of 254 nm and 366 nm. After derivatization, the plates were fixed in CAMAG TLC SCANNER 3 for densitometric scanning, done at 500nm. All the data like peak height, peak area, R_f values, peak densitogram were denoted.

Hypoglycemic activity

Experimental animals

For the experimental design healthy albino Wistar rats (160±20 g of body weight) were selected and the animals were kept in the animal house at the standard condition with commercial pellet diet and water. The animals were kept for 1 week before commencement of experiments and all the experiments were carried out by the approval of Institutional Animal Ethics Committee (IEAC) of Suresh Gyan Vihar University (Approval No: SGVU/PH/IAEC/2017/01).

Diabetes induction

Healthy selected rats were fasted overnight before induction of diabetes through Streptozotocin (STZ). Intraperitoneal injection of STZ at 50mg/kg body weight in 0.1M sodium citrate buffer was administered. Induction was confirmed after 48 h of administration. >200mg/DL value of fasting glucose level was confirmed as diabetic animals and screened for the further experiments. Glibenclamide (GLI) was used as standard drug. The dose of the plant extract was selected by following the acute toxicity study.[15]

Experimental design

The following five diabetic groups (n=5) were designed for the activity:

Group I – Non-diabetic control (NC)

Group II – Diabetic control (DC)

Group III – Diabetic rats administered with GLI (0.5 mg/kg body weight) (DC+GLI)

Group IV – Diabetic rats administered with SOE (DC+SOE) in the dose of 100mg/kg

Group V – Diabetic rats administered with SOE (DC+SOE) in the dose of 200 mg/kg

Acute study

After the administration of GLI and SOE, the blood glucose level was noted at 0,2,4,6 and 24 h.

Chronic study

After daily dosing of GLI and SOE plant extracts, the body glucose of the rats was monitored for 28 days at the interval of 0, 7, 14, 21 and 28 days.

Blood glucose estimation

The blood samples were collected from all the rats through tail-vein and blood glucose levels were measured (in mg/dL) instantly by using glucometer (Accu-chek, Roche Diagnostic, Mannheim, Germany).[16]

Body weight estimation

Body weight of the rats was recorded before (at 0 days) and after (at 28 days) of the study. The percentage change of the body weight was recorded and tabulated.

Statistical analysis

The results were expressed in ±SEM (n=5) and the comparison between the groups were determined followed by Tukey's multiple comparisons test, * $p<0.01$, ** $p<0.001$ were considered as significant. All the graphs were drawn in GraphPad Prism 7.4 software.

RESULTS

Qualitative and quantitative phytochemical screening

The ethanolic extract showed the presence of alkaloids, tannins, flavonoids and phenolic compounds, tabulated in Table 1. Quantitative estimations of total tannin, total flavonoids and total phenols were reported in Table 2 as 6.15±4.20 mg TAEg, 25.13±3.21 mg QEg, 48.09±2.58 mg GAEg dry weight respectively.

HPTLC fingerprinting for Quercetin

HPTLC fingerprinting of SOE was performed with the suitable solvent system and visualized under UV 254nm and 366 nm. The chromatogram and densitogram were given in Figure 1 and 2. The densitogram evidenced 12 bands of R_f values of 0.10, 0.23, 0.46, 0.49, 0.57, 0.62, 0.69, 0.74, 0.77, 0.84, 0.93 and 0.99, in which fourth one confirmed as quercetin while compared to standard (Figure 3). The R_f values, peak areas, height has been tabulated in detail in Table 3.

Hypoglycemic activity

Acute and chronic studies were performed to assess the hyperglycemic effect of SOE in two different doses which exhibited significant activity. To predict the acute effect, the blood glucose levels at the different time interval (0,2,4,6 and 24 h) were measured and reported in Figure 4. The reduction of blood glucose levels of glibenclamide on diabetic rats were

Table 1: **Phytochemical screening of SOE.**

Phytochemicals	Ethanolic extract of leaves
Alkaloids	+ - + +
Tannins	+ +
Phenolic compounds	+ + +
Flavonoids	+ + +

(+ indicates 'Presence' and – indicates 'Absence' of phytochemicals)

Table 2: **Quantitative phytochemical estimations of SOE.**

Estimations	SOE
Total Tannins (mg TAEg DW)	6.15±4.20
Total Flavonoids (mg QEg DW)	25.13±3.21
Total Phenols (mg GAEg DW)	48.09±2.58

(All Values represent Mean± SEM; All the samples were analyzed in triplicate)

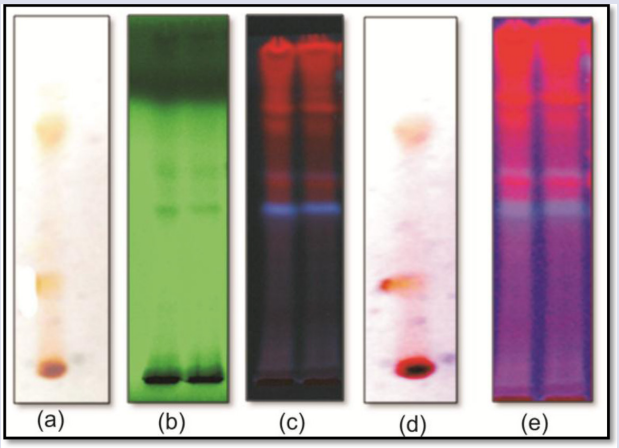

Figure 1: HPTLC chromatogram profile of SOE.
Before derivatization (a) Visible light, (b) UV 254 nm, (c) UV 366 nm;
After derivatization (d) Visible light, (e) UV 366 nm.

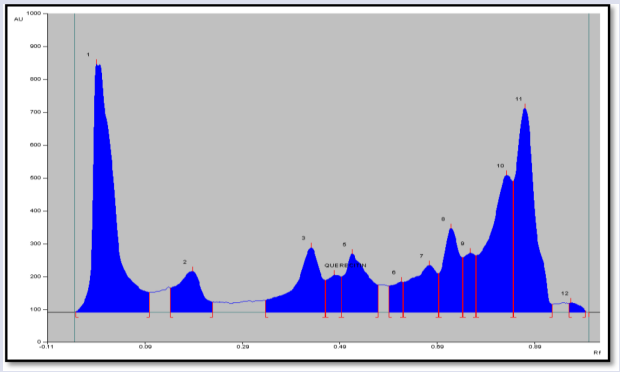

Figure 2: HPTLC densitogram for SOE at 254 nm.

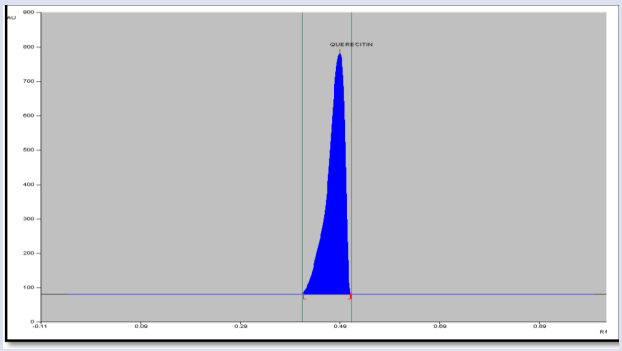

Figure 3: HPTLC densitogram for Quercetin standard.

highest (21.5%) than the higher dose of SOE (19.16%) and the lower dose of SOE (15.59%) in 24 h.

For the long-term effect of the extract on hyperglycemia, the chronic study was conducted and the blood glucose levels were reported after the study of 28 days at 7 days interval (Figure 5). Among the two extracts, high dose of SOE (200mg/kg) lowered the blood glucose more significantly (from 287±5.10 to 140±5.70 mg/dl) than low dose of SOE (100mg/kg) (from 295±4.70 to 180±3.54 mg/dl); however, the effect of glibenclamide was greatest among all (from 293±4.20 to 129±5.10 mg/dl).

The preventing effect of body weight loss of SOE was conducted at first day and 28th day after the study (Table 4). Glibenclamide, SOE (200mg/kg) and SOE (100mg/kg) showed 7.5, 10.37 and 11.39 % increase in body weight, whereas, diabetic control decreases the body weight by 11.33% (Figure 6).

DISCUSSION

Binding of insulin at alpha-subunit of insulin receptor activates tyrosine kinase activity at beta-subunit of the receptor followed by cascade of phosphorylation and dephosphorylation reactions which causes the activation of secondary messengers, MAP (Mitogen-Activated Protein)

Table 3: **HPTLC profile of SOE with Rf, height and area.**

Track	Peak	Rf	Max Height (AU)	Area (AU)	Assigned substances
SOE	1	0.10	755.8	32595.7	Unknown 1
SOE	2	0.23	124.6	6441.9	Unknown 2
SOE	3	0.46	196.8	10512.8	Unknown 3
SOE	4	0.49	113.8	3115.2	Flavonoid 1(Quercetin)
SOE	5	0.57	177.6	8585.6	Unknown 4
SOE	6	0.62	93.1	2248.6	Unknown 5
SOE	7	0.69	143.2	7463.1	Unknown 6
SOE	8	0.74	255.4	8553.6	Unknown 7
SOE	9	0.77	180.4	4025.3	Unknown 8
SOE	10	0.84	416.6	19885.3	Unknown 9
SOE	11	0.93	620.7	24028.5	Unknown 10
SOE	12	0.99	28.7	660.7	Unknown 11
STD	13	0.51	700.4	24011.3	Quercetin Standard

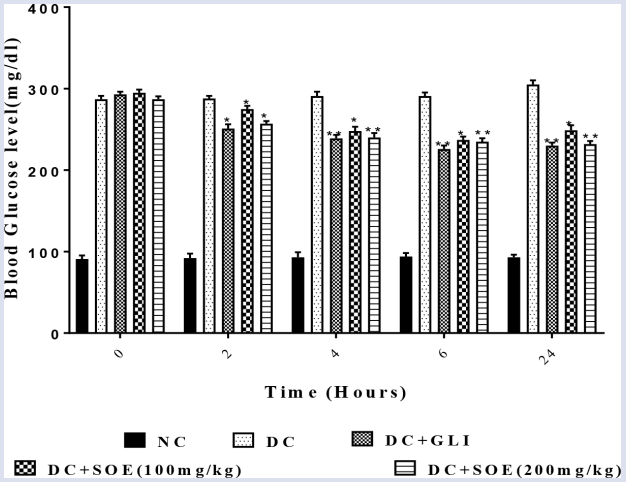

Figure 4: Acute hypoglycemic effect of SOE.
All values were expressed as ± Standard error of the mean (n=5), followed by Tukey's multiple comparisons test, * *p<0.01*, ** *p<0.001* were considered as significant.

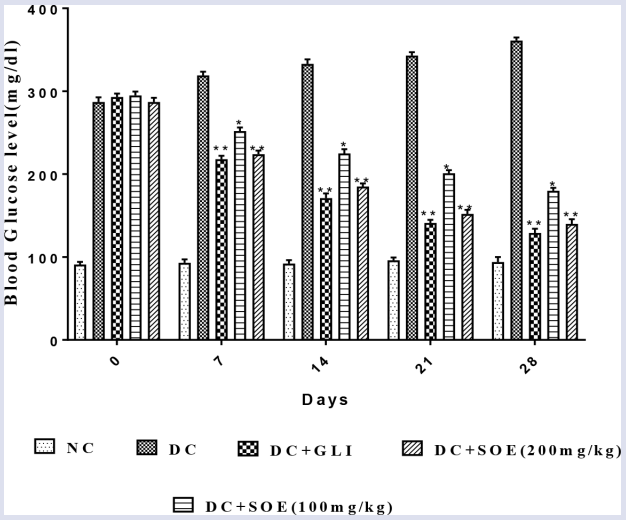

Figure 5: Chronic hypoglycemic effect of SOE.
All values were expressed as ± Standard error of the mean (n=5), followed by Tukey's multiple comparisons test, * *p<0.01*, ** *p<0.001* were considered as significant.

kinase and Glucose Transporters (GLUT) to maintain the normal blood glucose. The insufficient production of insulin fails to regulate normal blood glucose level and causes diabetes. The phytochemical screening of SOE affirmed the presence of tannins, flavonoids and phenolic compounds. Tannins increase the glucose uptake by activating GLUT-4, MAP kinase and secondary messengers like PI3K (Phosphoinositide 3-Kinase).[17] Stimulation of insulin production, preventing gluconeogenesis, enhancing insulin sensitivity and glucose uptakes are the few possible mechanisms of flavonoids in the regulations of diabetes.[18] α-amylase and α-glucosidase enzymes of the digestive tract can delay the absorption of

Table 4: Effect of SOE on body weight of STZ induced diabetic rats.

Groups	Body weight before the study (g)	Body weight after the study (g)
Group I	132.4±2.4	134.6±2.3
Group II	157.1±1.7	139.3±5.8
Group III	142.5±2.7	153.2±4.1
Group IV	146.6±3.21	163.3±2.7
Group V	156.2±4.2	172.4±3.4

All values were expressed as ± Standard error of the mean (n=5)

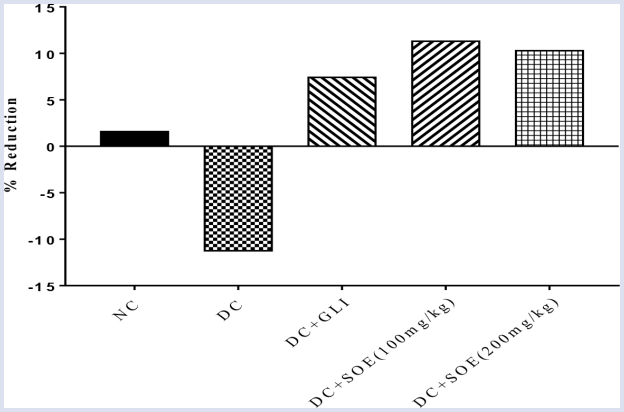

Figure 6: Effect on body weight (in percentage reduction) of SOE.

glucose, so, their inhibition by phenolic compounds helps to control the post-prandial hyperglycemia.[19]

HPTLC fingerprinting is an accurate and precise instrumental technique to identify the phytoconstituents and adulterants present in medicinal plants.[20-21] HPTLC serves as an important highly efficacious technique to establish the appropriate quality control parameters for the herbal extracts.[22] The present investigation of HPTLC fingerprinting of SOE affirmed the presence of quercetin along with twelve other compounds. Quercetin which is a part of the subclass of flavonoids groups of polyphenols, is an important aspect of research because of its versatile effect mainly on degenerative diseases by inhibiting lipid peroxidation.[23] The earlier studies reported that quercetin has significant hypoglycemic effect in STZ induced rats perhaps by increasing the insulin secretions.[24-26]

Despite all the effective researches in synthetic chemistry for oral hypoglycemic drugs, the reliability on medicinal plants for the management of hyperglycemia is a long practice almost all over the world due to its affordability.[27] The use of medicinal herbs is the important aspect of an alternative approach to manage diabetes.[6] The presence of several active phytoconstituents in medicinal herbs serve a protective and therapeutic effect on diabetes mellitus by the reduction in insulin resistance, preventing glycogenolysis and gluconeogenesis.[28] The present work evaluated the hypoglycemic activity of SOE in STZ induced diabetic rats and the results were significant (* *p<0.01*, ** *p<0.001* were considered as significant) as compared to diabetic control. STZ, obtained from a gram-positive bacterium, is uptake by pancreatic β-cells (via GLUT-2) to destroy them by several proposed mechanisms.[29] In case of acute study, the reduction of blood glucose of glibenclamide treated diabetic rats in first 6 h were found higher than SOE-high dose and SOE-low dose. The similar result was reported for *Rhizophora mucornata* in which high

dose (200mg/kg) of hydroalcoholic extract of the plant was found more potent than the low dose in first 6 h of the study.[16] For the daily treatment of SOE and glibenclamide up to 28days, it was found that the higher dose of SOE (51.21%) was more significant than SOE low dose (38.98%) in blood glucose lowering while compared to glibenclamide (55.97%). The similar study of the hypoglycemic effect of *Kyllinga triceps* plant extracts at 200mg/kg on STZ induced rats was reported as a significant (47.2% blood glucose reduction) than low dose.[30] The result of improvement in body weight of the rats for the daily treatment of SOE was important aspect in maintaining the body weight without altering the normal health, as obesity is the important issue for the management of type-2 diabetes.[31]

CONCLUSION

In recent scientific research the alternative approach for the management of diabetes is an important exposure. The present study proved *Schleichera oleosa* (Lour) Oken. as a hypoglycemic agent. The presence of quercetin will help to isolate and characterize the active constituents responsible for the hypoglycemic activity and will be the future scope.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ABBREVIATIONS

SOE: Ethanolic extract of *Schleichera oleosa* (Lour) Oken.; **STZ:** Streptozotocin; **GLI:** Glibenclamide; **NC:** Non-diabetic control; **DC:** Diabetic control.

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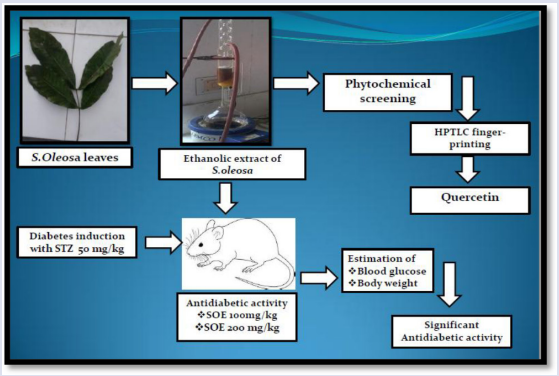
GRAPHICAL ABSTRACT



SUMMARY

- The qualitative and quantitative phytochemical screening for the ethanolic extract of *Schleichera oleosa* (Lour) Oken was performed. Presence of alkaloids, tannins, phenolic compounds and flavonoids were reported.
- HPTLC fingerprinting was performed and presence of quercetin was assured.
- Hypoglycemic activity was performed with two different doses.
- Significant outcome was noted.

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Shambaditya Goswami: He is a research scholar of Suresh Gyan Vihar University, Jaipur. His area of interest is phytochemistry and synthetic chemistry.

Dr. Ravindra Pal Singh: He is a renowned researcher in Pharmaceutical Science field. He is having 14 years of vast academic experience. His areas of interests are floating drug delivery system and phytochemistry.