Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 2, 2013 ISSN - 0974-2441

Research Article

ISOLATION OF SOY LECITHIN FROM SOY SLUDGE, ITS STANDARDIZATION AND BEHAVIOURAL STUDY

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Received: 22 January 2013, Revised and Accepted: 06 February 2013

ABSTRACT

The purpose of this study was to isolate soy lecithin from soy sludge by bleaching it with hydrogen peroxide give treatment with calcium chloride and finally dried. Standardization of soy lecithin was done by TLC analysis and other official tests like acid value, hexane-insoluble matter, acetone-insoluble matter etc. The behavioural study was done on albino rats, it included forced swim test and elevated plus-maze test. Soy lecithin was successfully isolated economically from soy sludge and all parameters for which it was evaluated were within specified limits Chromatographic and FTIR-spectrophotometric analysis of soy lecithin suggested the presence of triglycerides, free fatty acids. Results obtained from the behavioural study performed on albino rats indicate that soy lecithin has potent anti-depressant activity, which mainly have been contributed by its chief constituent, phosphatidylcholine

KEYWORDS: Soy lecithin, Isolation, Standardization, Behavioural Study.

INTORDUCTION

Lecithin is mixture of phosphatides, which varies in colour from light tan to dark reddish brown. Soybeans are by far the most important source of commercial lecithin and lecithin is the most important by-product of the soy oil processing industry [1].

All varieties of soy lecithin can be classified into three broad classes: unrefined, refined and chemically modified. Structurally, the phosphatides in soy lecithin consist of glycerides, in which one fatty acid radical has been replaced with phosphoric acid. Lecithin is composed mostly of fatty acids, 50-57% linoleic acid and 5% linolenic acid.

The most concentrated natural and refined sources of lecithin are soybeans (1.48-3.08%), peanuts (1.11%), calf liver (0.85%), etc. Lecithin is used for emulsification, solubilization, suspension, lubrication, complexation, as a wetting agent, anti-spatter agent, viscosity modifier, etc.

OBJECTIVE

Literature survey reveals that no work has been done so far to evaluate the effect of soy lecithin on behavior. Our objective is to isolate soy lecithin from soy sludge, perform its standardization and evaluate its behavioural activity on rats.

EXPERIMENTAL METHODS

a) Isolation: Soy sludge was bleached with Hydrogen peroxide. Fluidizing additive, calcium chloride was then added. Finally the product was dried. The oil in unrefined lecithin was removed by extraction with acetone.

b) Standardization: The standardization of soy lecithin was done by studying the physio-chemical characteristics like state, colour, taste, etc. TLC analysis was also performed. Other official tests like acid value, hexane-insoluble matter, acetone-insoluble matter, etc. were carried out.

FTIR spectrometric analysis of soy lecithin was performed using KBr pellet technique. HPTLC analysis of soy lecithin was performed using chloroform: methanol: conc. ammonia: distilled water (70: 25: 2.5: 2.5) as eluent and 10% Copper Sulfate ($CuSO_4$) solution in Phosphorous acid (H_3PO_4) (6.85%) as staining agent.

c) Behavioural study: The behavioural study was done on albino rats. It included forced swim test and elevated plus-maze test.

Forced swim test; - Animals are subjected to two trials during which they are forced to swim in an acrylic glass cylinder filled with water, and from which they cannot escape. The first trial lasts 15 minutes. Then, after 24-hours, a second trial is performed that lasts 5 minutes. The time that the test animal spends without moving in the second trial is measured. This immobility time is decreased by antidepressants

Elevated plus –maze test: - The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40–70 cm from the floor. The model is based on rodents' aversion of open spaces. This aversion leads to the behavior termed thigmotaxis, which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space. In EPM this translates into a restriction of movement to the enclosed arms. [2][3][4][5]

Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms), and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). Total number of arm entries and number of closed-arm entries are usually employed as measures of general activity [6].

RESULTS AND DISCUSSION

a) Physio-chemical characteristics:

Table: 1 shows the results of various physio-chemical tests :

S. no.	Test	Result
1.	State	Granular solid
2.	Colour	Creamish yellow
3.	Odour	Slight, characteristic
4.	Taste	Salty
5.	pH	6.1
6.	Melting point	152-155°C
7.	Solubility	Soluble in methanol, chloroform, ether, petroleum ether and benzene

b) Thin Layer Chromatography:

Table 2: shows the Thin Layer Chromatogram of Soy lecithin when dissolved in benzene.

S. No.	Solvent system	Proportion (%)	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	R_f-value
1.	$CHCl_3$:CH_3OH:NH_3(conc.)	80:15:5	2.2	5.3	0.41
2.	$CHCl_3$:CH_3OH:NH_3(conc.)	70:25:5	2.1	4.9	0.43
3.	$CHCl_3$:CH_3OH:NH_3(conc.): Dist. water	70:25:2.5:2.5	2.4	5.2	0.46

c) Official Tests

Table 3 shows various official tests.

S.N.	Characteristic test	Observation	Std. value	Reference
1.	Acid value	29.4525	36	USP
2.	Hexane-insoluble matter	0.2%	0.3%	USP
3.	Acetone-insoluble matter	97.8%	62	USP
4.	Peroxide value	3.8	10	IP 1996
5.	Benzene-insoluble matter	0.5%	0.3%	IP 1996
6.	% Loss on drying	1.95%	2%	IS: 1996
7.	Phosphorus content	0.2368 g/g of $Mg_2P_2O_7$	-	IS: 1996
8.	Lead content	38.70 ppm	10mg/kg	IS: 1996
9.	Arsenic content	0.23 ppm	3mg/kg	IS: 1996

d) FTIR spectrometric analysis of Soy lecithin:

FTIR spectrometric analysis of soy lecithin showed peak at 3710 cm^{-1} and 3400cm^{-1}, which indicated the presence of N-H stretching and O-H stretching of amino group and hydroxyl group respectively. Another band at 2960-2860 cm^{-1} indicated the presence of C-H group of alkane. Band at 1745 cm^{-1} indicated the ester linkage. Another band at 1640cm^{-1} was found due to aliphatic alkane. Band at 1245 cm^{-1} indicated presence of P=O (phosphomoyl) group.

e) HPTLC analysis of soy lecithin:

Table :4 shows the comparative study of the R_f-values of soy lecithin sample and standard is as follows:

	R_f-values									
Standard	0.01	0.17	0.25	0.43	0.49	0.54	0.59	-	-	0.93
Sample	0.01	0.17	0.26	0.48	0.51	0.55	0.61	0.65	0.88	0.91

f) Behavioural study on rats

i) Table: 5 Forced swim test :

Treatment	Dose(g/kg)	Immobility time (sec)
Control	-	92.80 ± 1.715
Soy lecithin	1.25	15.80 ± 1.582

ii) Elevated plus maze test (EPM) :

Treatment	% of entries in open arm	% of entries in closed arm	Time spent in open arm (sec)
Control	16.79 ± 0.948	83.31 ± 1.112	8.2 ± 1.772
Soy lecithin	48.13 ± 1.942	51.87 ± 1.943	21.8 ± 1.985

CONCLUSION

Soy lecithin was successfully isolated economically from soy sludge and all parameters for which it was evaluated were within specified limits, except for benzene-insoluble content.

Chromatographic and FTIR-spectrophotometric analysis of soy lecithin suggested the presence of triglycerides, free fatty acids, phosphatidylethanolamine, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine.

Results obtained from the behavioural study performed on albino rats indicate that soy lecithin has potent anti-depressant activity, which mainly have been contributed by its chief constituent, phosphatidylcholine, which contributes to the cholinergic neurotransmission.

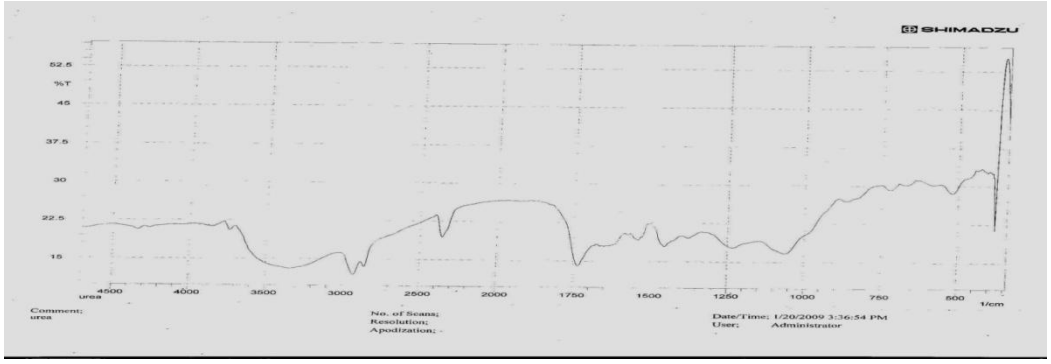

Fig 1: FTIR Spectra of Stndard Soya –Lecitin

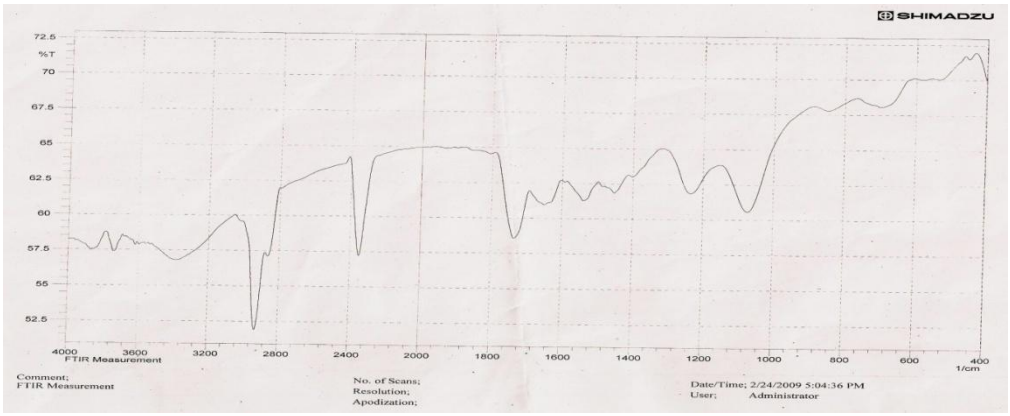

Fig 2: FTIR Spectra of Soya –Lecitin Sample

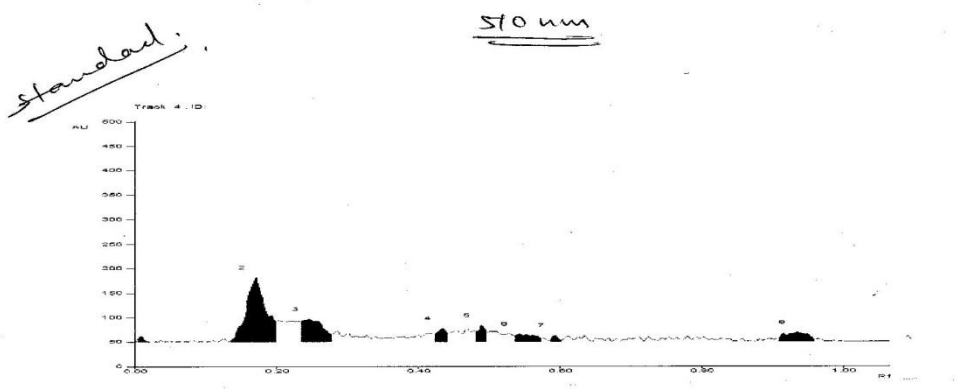

Fig 3:- Comparative study of the R_f-values of standard soy lecithin.

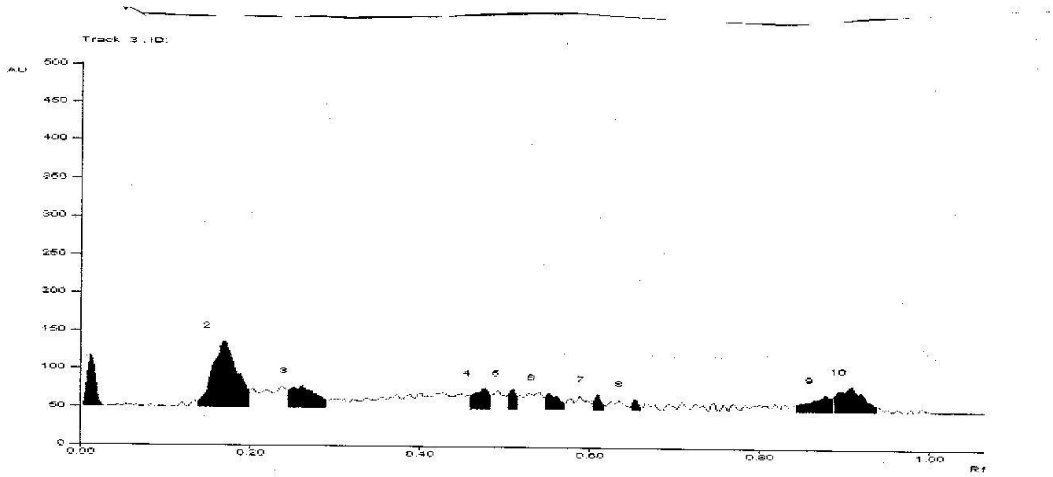

Fig 4:- Comparative study of the R_f-values of soy lecithin sample.

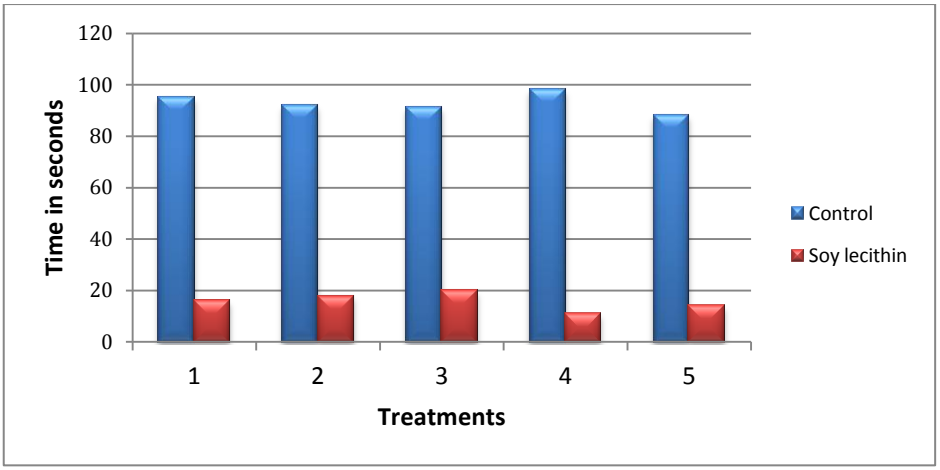

Fig 5:Forced swim test on rats

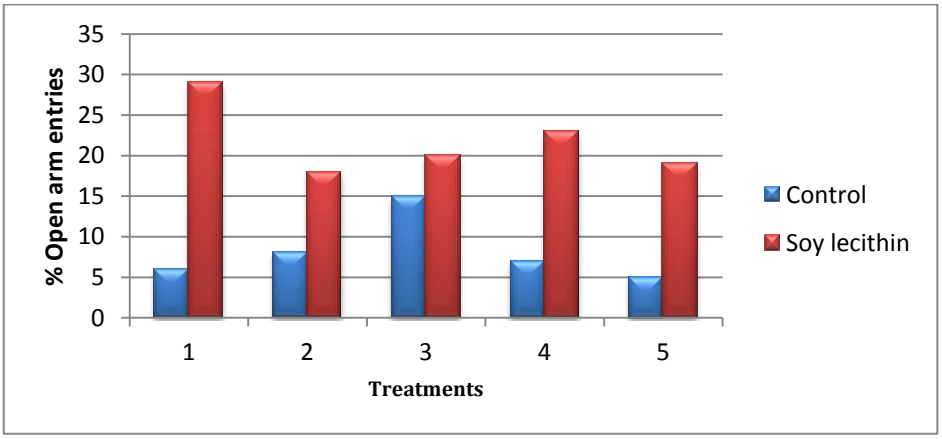

Fig 6:- Elevated plus maze test on rats

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