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Isolation of Cystine from Human Hair, Characterization, Pharmacological Screening on Frog Heart Muscle and Hepatoprotective Activity in Mice

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Hairs are elastic keratinised threads which develop from the epidermis and extend downwards into the subcutaneous tissue. Keratin is made from 16 amino acids. The most abundant of these amino acids is cystine, which was isolated by a new method and compared with the reference standard obtained from reported method. The crude extract was physicochemically characterized and pH adjusted to 7.4 for observation of its effect on frog heart muscle and for hepatoprotective activity in mice. Extract showed positive inotropic action and the ability to protect hepatotoxicity.

Key Words: Human hair, Cystine, Hepatoprotective, Heart muscle.

INTRODUCTION

Hairs are one of the specialized structures of the skin[1]. Indeed, these are elastic keratinised threads which develop from the epidermis and extend downwards into the subcutaneous tissue. The hairs are not only present in scalp, these are present all over the skin except for palms, soles and urogenital apertures. They vary in length from 1 to 1500 mm and in thickness 0.05 mm. Hairs, itself not only improve the beauty but play a vital physiological role in human body[2].

Hair is made up of a strong structural protein responsible for the hardness of the hair, which originates in the hair follicle. In the papilla, as the cells mature, they fill up with a fibrous protein called keratin. These cells loss their nucleus and die as they travel up the hair follicle.

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The keratin found in hair is called 'hard' keratin and made up of 16 amino acids. This type of keratin does not dissolve in water and is quite resilient. Approximately 91 % of the hair is protein made up of long chains of amino acids. The amino acids are joined to each other by chemical bonds called peptide bonds or end bonds. The long chain of amino acids is called a polypeptide chain and is linked by peptide bonds. The polypeptide chains are intertwined around each other in a helix shape. The most abundant of these amino acids is cystine which gives hair much of its strength[3].

In this compilation, a new method for isolation of cystine is reported and the character of cystine was compared with a reference standard isolated from hair only by following the standard method[4]. Further, the liquid extract of hair was freed from melanin pigment and physico-chemically characterized and thus pH was adjusted to 7.4, which was applied to frog heart muscle in different concentration and screened for hepatoprotective activity in mice, to observe the effect with the aim to utilize the extract as protein substitute in deficiency.

EXPERIMENTAL

The melting point reported here was determined in open capillaries method and are uncorrected. The IR spectra of the compounds were recorded in BXII-FTIR Perkin Elmer. The pH values were observed on pH meter, Toshniwal Instrument Mfg. Pvt. Ltd. Ajmer, CAT no-CL 54.

New method for isolation of cystine: 50 g of dried human hair was placed in a round bottom flask and to that 300 mL of 5 % (w/v) potassium hydroxide solution was added. The mixture was warmed at 20 ºC for 2-3 min and then left for 24 h at room temperature for complete hydrolysis. The hydrolysate was treated with about 3.5 g of activated charcoal and then filtered. To the filtrate 1 N HCl was added drop wise with shaking until a white precipitate was found to be produced. The solid precipitate was filtered and washed with warm water followed by ethanol and ether. The crude product of cystine was collected and air-dried.

The cystine was also isolated by following the standard method[4] and the yield was 5 % (melting point: 260-262 ºC).

Physico-chemical characterization of crude extract: After dissolution of hair in the solvent system, the melanin pigment was removed from the extract by treating with activated charcoal. The pH of the extract was adjusted to 7.4 by 1 N HCl. Further the colour, density, IR spectral analysis and chemical tests[5-7] for amino acid identification and specifically for cystine were carried out. The TLC[8,9] of the crude extract was also performed by taking solvent system *n*-butanol:glacial acetic acid:water:::4:1:1 as mobile phase and 0.2 % (w/v) ninhydrin in acetone as detection agent. The results obtained are displayed in Table-1.

TABLE-1
OBSERVATION FROM TLC STUDY

Spot no.	Solvent travels (cm)	Spot travels (cm)	R_f value	Colour in ninhydrin	Amino acid (probable)
1	12.7	0.7	0.05	Grey-violet	Histidine
2	12.7	0.8	0.06	Violet	Arginine
3	12.7	1.3	0.10	Violet	Cysteine
4	12.7	1.8	0.14	Violet-yellow	Proline
5	12.7	2.2	0.17	Blue-violet	Aspartic acid
6	12.7	2.6	0.20	Violet	Threonine
7	12.7	3.0	0.24	Violet	Glutamic acid

Mobile phase: *n*-butanol:glacial acetic acid:water = 4:1:1
Stationary phase: Silica gel-G

Effect on frog heart muscle: During experiment, ringer solution was used as physiological salt solution and water for injection as vehicle (control) to dilute the test drug solution (crude extract). Kymodrum was arranged for kymograph of frog heart muscle. Different dilution of the stock drug solutions were applied individually and the responses were recorded. The test drug solutions were prepared in different concentrations- 0.01, 0.1, 0.2 and 0.25 mg/mL.

Hepatoprotective activity in mice[10]: Adult albino Swiss male mice weighing between 18-22 g were used for the study. The animals were divided into four groups. Each group contains six animals. Normal saline solution is used as vehicle. (i) Group I served as control which received only vehicle 0.5 mL/kg body wt. at 12 h interval four times through intraperitoneal injection. (ii) Group II received only carbon tetrachloride in 0.8 mL/kg body wt. once and next three times treated with vehicle by intraperitoneal injection. (iii) Group III received silymarin (standard drug) dissolved in normal saline in 100 mg/kg body wt. four times through intraperitoneal injection. This group also received carbon tetrachloride in 0.8 mL/kg body wt. through intraperitoneal injection after 0.5 h of first dose of silymarin administered. (iv) Group IV received the test drug (crude extract) solution in 200 mg/kg body wt. as like as silymarin.

All the animals received four doses of administration,where anaesthesized after 12 h of the last dose by diethyl ether. Blood sample were collected by penetrating the retro-orbital plexus. The serum was separated after coagulating at 37 ºC for 0.5 h and centrifuged at 250 rpm for 10 min.

SGOT, SGPT, alkaline phosphatase, total bilirubin and direct bilirubin content were determined for every animal. The results obtained are depicted in Table-2.

TABLE-2
SERUM ENZYMES AND BILIRUBIN LEVELS OF MALE MICE

Treatment	Mice no. (wt. in g)	SGOT (unit/mL)		SGPT (unit/mL)		Alkaline phosphate (unit/mL)		Total bilirubin (mg/dl)		Direct (conjugated) bilirubin (mg/dl)	
		Individual	Average ± SEM	Individual	Average ± SEM	Individual	Average ± SEM	Individual	Average ± SEM	Individual	Average ± SEM
Control	1(18)	69	70.8 ± 0.316	49	51.2 ± 0.34	291	290.5 ± 0.273	0.58	0.61 ± 0.0115	0.29	0.30 ± 0.003039
	2(18)	73		48		292		0.57		0.33	
	3(20)	69		53		288		0.63		0.31	
	4(22)	70		54		289		0.62		0.30	
	5(19)	72		52		290		0.61		0.28	
	6(19)	72		51		293		0.63		0.27	
Toxicant (CCl_4)	1(20)	176	173.2 ± 0.34	129	129 ± 0.4137	769	765.2 ± 0.409	1.89	1.91 ± 0.0115	0.88	0.90 ± 0.003039
	2(21)	170		126		768		1.88		0.92	
	3(19)	171		130		765		1.93		0.91	
	4(18)	173		131		764		1.95		0.89	
	5(18)	175		133		763		1.91		0.89	
	6(20)	174		125		762		1.92		0.93	
Standard (Silymarin)	1(21)	80	80.8 ± 0.34	67	69.5 ± 0.2739	438	435.2 ± 0.237	1.19	1.17 ± 0.0115	0.64	0.63 ± 0.002049
	2(22)	79		69		434		1.18		0.62	
	3(22)	83		71		433		1.20		0.65	
	4(20)	84		72		437		1.16		0.63	
	5(19)	78		70		435		1.14		0.64	
	6(18)	81		68		434		1.15		0.61	
Extract (Hair) of pH 7.4	1(19)	114	115.3 ± 0.33	123	125.7 ± 0.4867	337	334 ± 0.293	1.28	1.31 ± 0.0036	0.77	0.78 ± 0.00395
	2(20)	113		124		336		1.32		0.76	
	3(20)	117		128		333		1.31		0.79	
	4(22)	116		130		332		1.33		0.80	
	5(21)	118		122		332		1.34		0.75	
	6(18)	114		127		334		1.27		0.82	

RESULTS AND DISCUSSION

Human hairs dissolved in warm 5 % (w/v) KOH solution was freed nicely from melanin pigment after the treatment by activated charcoal. The cystine was isolated and confirmed by its melting point 259-262 ºC, which was compared to that of reference standard. The IR spectra in KBr pellet obtained was showing stretching at 2924 cm^{-1} for $^{+}NH_3$, 3499 for COOH: -OH free, 2499 for COOH: -OH bound (may be due to salt formation), 1653 for C=O of COOH, 1541 for $-COO^{-}$ (salt form), 1410 for -COO- sym. vibration, 1478 for $-CH_2-$ and 1361 and 1250 for C-S.

The crude extract (freed from melanin) was adjusted to the pH 7.4 and its colour was grayish pink, density - 2.0437 g/mL. From the qualitative chemical tests, it has been confirmed that the extract was containing amino acids and cystine specifically. Further, the TLC observations (Table-1) showed the presence of histidine, arginine, cysteine (two molecule of cysteine if joined with one disulfide bond, gives cystine[11]), proline, aspartic acid threonine and glutamic acid.

On treatment with different concentrations of extract in the isolated frog's heart muscle, it has been found that extract exhibited positive inotropic effect. The normal kymograph was recorded as 0.5 and 1.8 cm was recorded for 0.01 mg/mL concentration in highest and 2.2, 2.4 and 2.7 cm were recorded for 0.1, 0.2 and 0.25 mg/mL, respectively. It has been found that extract was able to inhibit the hepatotoxicity created by CCl_4 but not at par to the standard silymarin.

From the above observation it could be expected that the crude extract of human hair may have the use as protein supplement in deficiency, for which further more biological studies are essential. The reported procedure for isolation of cystine is more easy than the standard referred procedure, though the yield percentage was found less in case of present designed procedure.

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REFERENCES

1. C.C. Chatterjee, Human Physiology, Medical Allied Agency, Calcutta, Vol. 2, p. 79 (1988).
2. S. Subrahmanyam and K.M. Kutty, Text Book of Human Physiology, S. Chand and Company Ltd., New Delhi, India, pp. 464-466 (2001).
3. www.google.com:mhtml:file

4. B.S. Furniss, A.J. Hannaford, V. Rogers, P.W.G Smith and A.R. Tatchell, in: A.I. Vogel's Text book of Practical Organic Chemistry, The English Language Book Society and Longman, Great Britain, p. 560 (1978).
5. P.C. Dandya and P.K. Sharma, Biochemistry and Clinical Pathology, Vallabh Prakashan, Delhi, India, p. 31 (1999).
6. N. Murugesh, Biochemistry and Clinical Pathology, Sathya Publishers, Madurai, p. 2.03, 2.23 (2001).
7. V.K. Malhotra, Practical Biochemistry for Students, Jaypee Brothers, New Delhi, India, edn. 4, pp. 22-25 (2003).
8. C.K. Kokate, Practical Pharmacognosy, Vallabh Prakashan, Delhi, India, p. 128 (2001).
9. J.B. Harborne, Phytochemical Methods, Chapman and Hall Ltd., London, pp. 179-181 (1984).
10. S.K. Panda, S.R. Swain, A. Samanta and P.K. Sahu, *Indian J. Nat. Prod.*, **21**, 1 (2005).
11. A.L. Lehninger, D.L. Nelson and M.M. Cox, Principles of Biochemistry, CBS Publishers & Distributors, Delhi, India, p. 116 (1993).

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