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Phytochemical Studies and Hepatoprotective activity of *Melia azedarach* Linn, against CCl_4 induced Hepatotoxicity in rats

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ABSTRACT

The aim of the study is to investigate the phytochemicals and hepatoprotective activity of *Melia azedarach* L leaves extracts against carbon tetrachloride (CCl_4) induced hepatotoxicity. The phytochemical screening was carried on the leaves extracts of *Melia azedarach* revealed the presence of some active ingredients such as Alkaloids, Tannins, Saponins, Phenols, glycosides, steroids, terpenoids and flavonoids. Leaves of *Melia azedarach* was successively extracted with ethanol against carbon tetrachloride (CCl_4) induced hepatotoxicity using Standard drug Silymarin (25 mg/kg). There was a significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP),serum bilirubin and decrease the total proteins content.) in CCl4 treated rats, which were restored towards normalization in *Melia azedarach* (500 mg/kg) treated animals. Thus the present study ascertains that the leaf extract of *Melia azedarach* possesses significant hepatoprotective activity.

Key words: *Melia azedarach*, phytochemical screening, hepatoprotective activity, CCl_4, ethanol and Silymarin.

INTRODUCTION

Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction. [(1)] The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. [(2)] Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders. [(3)]The disorders associated with the liver are also numerous and varied.[(4)] CCl4 is toxic to the liver and its toxicity is dose dependent and based on time of exposure.[(5-6)] In the liver, CCl4 is metabolized in to the highly reactive trichloromethyl radical. This free radical causes autooxidation of the fatty acids present in the cytoplasmic membrane phospholipids resulting in functional and morphological changes in the cell membrane.[(7)] The metabolism of CCl3 free radical released from CCl4,initiates peroxidation and cleavage of fatty acids in membranes. Thus, trichloromethylperoxyl free radical elicits lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death.[(8)]

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Melia azedarach linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. Melia azedarach linn (synonym: Melia dubia Cav, Indian lilac, Persian lilac) belonging to the family Meliaceae is a tree found in India. It is popular as Indian lilac. Different phytochemicals present in leaf, root and stem, are meliacarpins, limonoids, sendanins, trichilins and azedarachins. [(9)] The plant is traditionally used for the treatment of leprosy, inflammations, Analgesics and cardiac disorders. Its fruits extracts possess ovicidal [(10)]and larvicidal activity.[(11)] The leafs extracts also possess antiviral[(12)] and antifertility activity.[(13)] Hence the present study was aimed at investigating the phytochemicals and hepatoprotective activity of *Melia azedarach* L leaves extracts against carbon tetrachloride (CCl4) induced hepatotoxicity.

MATERIALS and METHOD

Plant materials

The basic plant material of *Melia azedarach Linn* used for the investigation was obtained from Mount Opera Garden, Near Ramoji Film City, and Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul- loom College of Pharmacy, Hyderabad.

Preparation of ethanolic extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of ***Melia azedarach*** were used for extraction. The shade dry coarsely leaves of ***Melia azedarach*** were used for extraction with ethanol. ***Melia azedarach*** leaf powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol extracts. [14, 15 & 16]

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of NIZAM INSTITUTE OF PHARMACY, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animals experiment was carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registeration number. (1330/AC/10/CPCSEA)

Acute toxicity study

Melia azedarach in the dose range of 110 mg-630 mg/kg were administered orally to different group of mice comprising of ten mice in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of Litchfield and Wilcoxon. [17]

Experimental design for hepatoprotective activity

Animals are divided into 4 groups, each comprising 6 rats.

Group I : Control group
Group II : CCl_4 treated group
Group III : CCl_4+*Melia azedarach* leaf extract (500mg/kg, p.o)
Group IV : CCl_4+Silymarin (25mg/kg, p.o)

Hepatoprotective study was carried out as described by Brijesh et al. (2008) [18]. Albino rats of either sex (150-200 gm) were selected and divided into four groups of six animals each. The first group was fed with 1 ml/kg p.o of saline solution (S.S.) for 4 days. The second group was fed with 1 ml/kg p.o. of S.S. for 4 days along with 2 ml/kg of CCl_4 by S.C. on the second and third days. The third group was fed with *azedarach* leaf extract (500mg/kg, p.o) for 4 days along with 2 ml/kg of CCl_4 by S.C. on the second and third days. Fourth and fifth groups were fed with Silymarin (25mg/kg, p.o) for 4 days along with 2 ml/kg of CCl_4 by S.C. on the second and third days. On the fifth day, all the animals were sacrificed by mild ether anesthesia.

Blood biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT [19], ALP [20] and bilirubin [21] by standard method. Serum total protein was measured according to the method of Lowry et al, 1951. [22]

Histopathology

Histopathology of liver was carried out by a modified Luna (Luna LG., 1999) [23]. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h.Then the livers were paraffin embedded and 5 ì thickness microtone sections were made. [24] The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

Statistical analysis

The data obtained were analyzed by One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using computerized program. P-value <0.05 or was taken as the criterion of significance.

RESULTS

The acute oral toxicity study of *Melia azedarach* showed no mortality upto 610 mg/kg. The phytochemical screening of *Melia azedarach* shows the presence of Alkaloids, Carbohydrates, Steroids, Tannins, Flavonoids and Glycosides **(Table 1)**.The effect of ethanol extract of *Melia azedarach* an serum transaminases, alkaline phosphates, bilirubin and total protein level in CCl_4 intoxicated rats are summarized in **Table 2**. There was a significant increase in bilirubin level, SGOT, SGPT and ALP, in CCl_4-intoxicated group compared to the normal control group **(Graph-1, 2, 3, and 4**). The total protein levels were significantly decreased to 5.4 g/dl in CCl_4 intoxicated rats from the level of 7.1 g/dl in normal group **(Graph 5).** On the other hand the groups with received both *Melia azedarach* leaf extract (500mg/kg, p.o) and CCl_4 **(Group III)** and CCl_4+Silymarin (25mg/kg, p.o) **(GROUP IV)** showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level **(Table 2)**. Group III and Group IV treated also reduced the level of bilirubin to 1.08 and 1.05 mg/dl respectively from the level of 3.29 mg/dl in the untreated group.

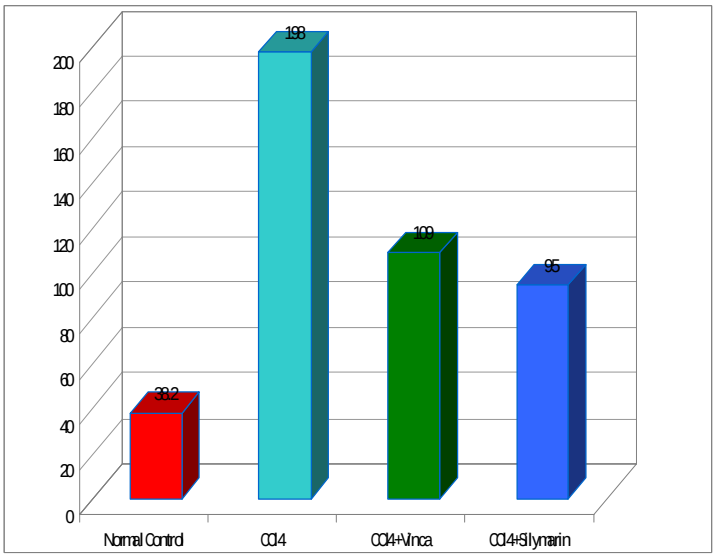
Table 1.Preliminary Phytochemical Screening.

Sl. No	Constituents	Ethanol Extract
1	Alkaloids	+
2	Steroids	+
3	Tannins	+
4	Phenols	+
5	Flavonoids	+
6	Glycosides	+
7	Saponins	+
8	Terpenes	+
9	Reducing Sugar	-
10	Anthraquinone	+

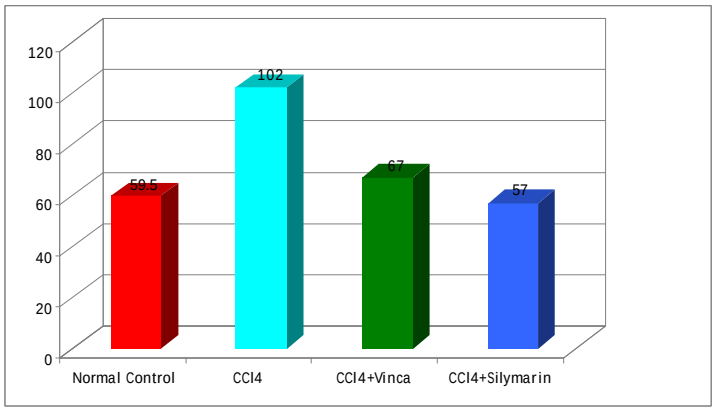
Table 2. Effect of *Melia azedarach* on some serum chemical parameters of paracetamol intoxicated rats.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Bilurubin (mg/dl)	Total protein (g/dl)
Normal control	38.2± 1.50	59.5±1.21	155± 1.50	0.17± 0.42	7.1± 0.30
CCl_4	198±16.77°	102± 10.5	253± 11.5	3.29± 0.33	5.4± 0.37
CCl_4+Extract	109± 2.66	67±2.21	186±1.21	1.08±1.2	6.98±0.21
CCl_4+Silymarin	95±2.25	57±1.11	163±2.21	1.05± 0.27	7.0± 0.37

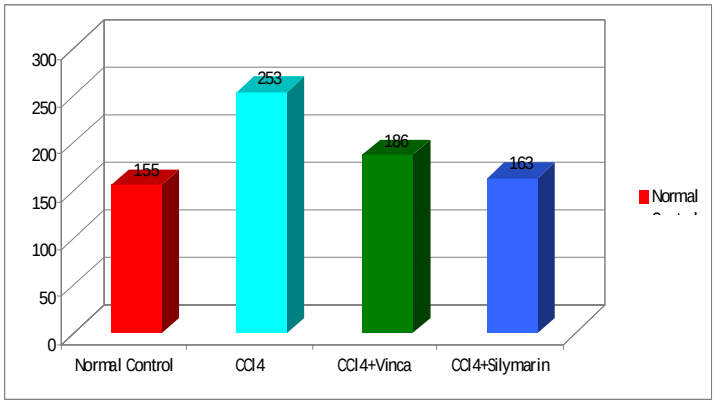
Values are mean ± S.E.M. number of rats = 6.



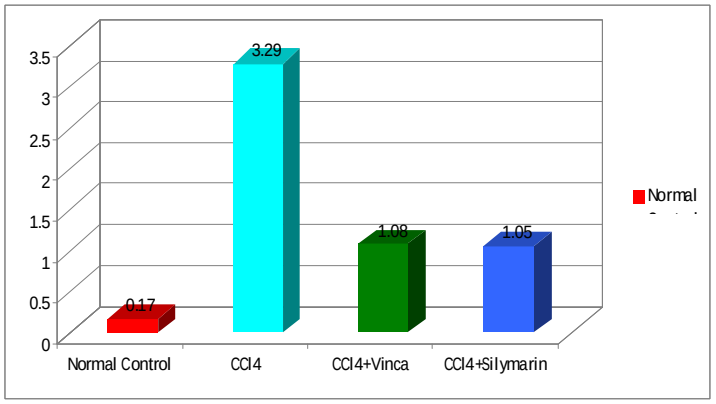
GRAPH 1: BLOOD SERUM SGOT (U/L)



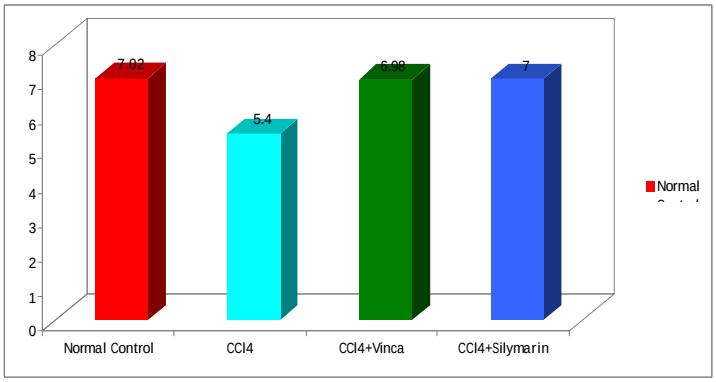
GRAPH 1I: BLOOD SERUM SGPT (U/L)



GRAPH 1II: BLOOD SERUM ALP(U/L)



GRAPH 1V: BLOOD SERUM BILURUBIN(mg/dl)



GRAPH V: BLOOD SERUM TOTAL PROTEIN (mg/dl)

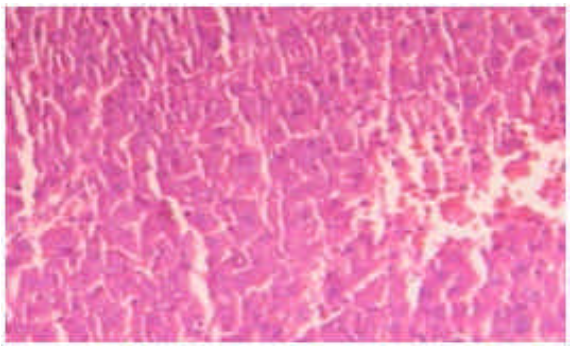


Fig1: Section of liver of control group

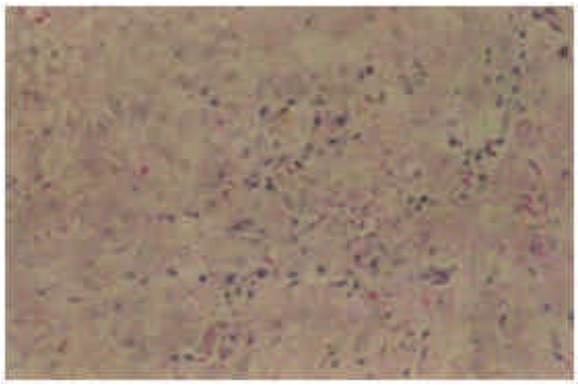


Fig 2: Section of the liver of CCl_4 treated group

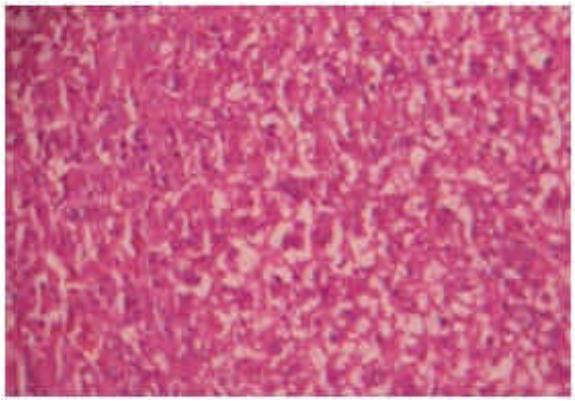


Fig 3: Section of liver of CCl_4 and *Melia azedarach* treated group

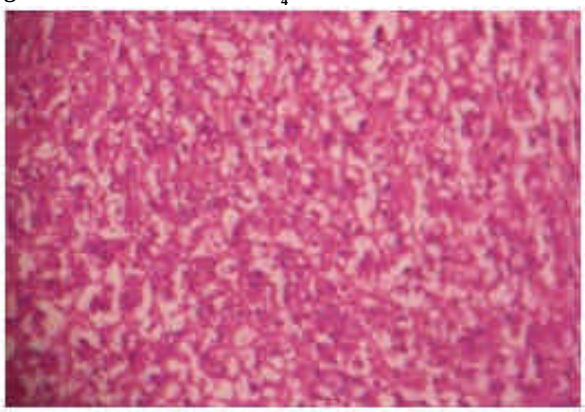


Fig 4: Section of liver of CCl_4 and silymarin group

DISCUSSION

The liver can be injured by many chemicals and drugs. In the present study, CCl_4 was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant, CCl_4 produces a constellation of dose related deleterious effects in the liver .[25] During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration.[26] Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl_4 control group **(Fig 2)**. Ethanolic extract of *Melia azedarach* leaf extract (500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity **(Fig 3).** All the histological changes observed were in correlation with the biochemical and functional parameters of the liver.

CONCLUSION

In conclusion, the results of present study demonstrate that *Melia azedarach* leaf extract (500mg/kg, p.o) has potent hepatoprotective activity against CCl_4 induced liver damage in rats. The results also imply that the hepatoprotective effects of *Melia azedarach* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepataprotective effect.

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REFERENCE:S

1. Ward FM, Daly MJ (1999). Hepatic disease. In: Walker R, Edwards C, editors. Clin. Pharm. Ther.. Churchill Livingston: New York; pp. 195-212.
2. Pang S, Xin X, Stpierre MV (1992). Determinants of Metabolic Disposition. Rev. Pharmacol. Toxicol., 32: 625-626.
3. Ross MH, Romrell LJ, Kaye GI (1996). Histology a text and atlas. Wilian and Wilkin: Baltimore, p. 245.
4. Wolf P L, Biochemical diagnosis of liver disease, Indian J Clin Biochem, 14 (1999) 59.
5. Recknegel R O, Glende E A, Dolak J A & Waller R L, Mechanism of carbon tetrachloride toxicity, Pharma Ther, 43 (1989) 139.
6. Junnila M, Rahko T, Sukra A & Linderberg L A, Reduction of carbon tetrachloride induced hepatotoxic effects by oral administration of betaine in male. Hans–Wistar rats: A morphometric histological study, Vet Pathol, 37(2000) 231.
7. Pandit S, Sur T K, Jana Udebnath P K, Sen S & Bhattacharya D, Prevention of carbon tetra chloride – induced hepatotoxicity in rats by Adhatoda vasica leaves, Indian J Pharmcol, 36 (2004) 313.
8. Clawson G A, Mechanism of carbon tetrachloride hepatotoxicity, Pathol Immunol Res, 8 (1989) 104.
9. Wealth of India, raw material Vol-IV (L.M) Page no.323. [10a] Chemical constituents Page 324-325.
10. Corpinella MC, Miranda M, Almiron WR, Ferrayoli CG, Almedia FL, Palacios SM. (In vitro pediculicidal and ovicidal activity of an extract and oil from fruit of melia azedarach L). J Am Acad Delmatol, 2007; 56(2):250 -6. Epub 2006 Decu. PMID: 17147968.
11. Wandscheer CB, Duque JE, Da Silva MA, Fukuyama Y. Wohlke JL, Adelmann J, Foutana JD. (Larvicidal action of ethanolic extracts from fruits endocarps of melia azedarach and Azadirachta indica against the dengue mosquito. Aedes Aegypti. Toxicon. 2004 Dec. 15; 44(8):829-35.
12. Descalzo AM, Coto C. (Inhibition of the pseudorabies virus (scis herpesvinyl) by an and vital agent isolated from the leaves of Melia azedarach. Rev. Argent microbial 1989 Jul-Dec. 21(3-4):133-40,
13. Choudhary DN, Singh JN, Verma SK, Singh BP. (Antifertility effects of leaf extracts of some plants in male rats) Indian J Exp. Biol. 1990 Aug. 28(8) L714-6.
14. Trease GE and Evans WC. A text book of Pharmacognosy, 15th edition, 2002, Elsevier Company, Philadelphia, USA. pp.191-418.
15. Kokate, C.K., Purohith, A.P. &Gokhale, S.B. (1990). Pharmacognosy, Nirali Prakashan, Pune, 120.
16. Khandelwal, K.R. (2006). Practical Pharmacognosy techniques and experiments, 16 Edition, Nirali Prakashan, 149-156.
17. Litchfield JT, Wilcoxon FA. Simplified method of evaluating dose effect exp eriments. J Pharmacol Exp Ther 1949; 96:99-133.
18. Brijesh KT, Khosa RL (2008). Evaluation of hepatoprotective activity of Sphaeranthus indicus flower heads extract. J. Nat. Remedies, 8/2: 173-178.
19. Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
20. Walter K, Schutt C (1974). Acid and alkaline phoshatases in serum. In: Verlag Chemic Weinheim, In: Hans Ulrich Bergmeyer (Ed.), Method Enzymatic Anal. Academic Press Inc., New York, 2: 856-864.
21. Malloy HT, Evelyn KA (1937). The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem., 119: 481-490.
22. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin-phenol reagent. J Biol Chem 1951; 193:265-275.
23. Luna LG (1999). Manual in histology and staining method. McGraw Hill: New York, p. 96.
24. Krajian AA (1963). Tissue cutting and staining. In: Frankel, S., Reitman,S.(Eds.), Gradwohl's Clinical Laboratory Method and Diagnosis. The CV. Mosby Co., Saint Louis, USA, p. 1639.
25. Leo MA, Arai M (1982). Hepatotoxicity of vitamin A and CCl_4. Gastroenterology, 82: 194-205.
26. Deb AC (1998). Fundamental of Biochemistry. 7th Ed. New Central Book Agency: Kolkata.

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