Cardiotonic activity of Petrolium Ether and alcoholic extract of Seeds of *Cassia Tora* Linn.

Neelima Janardan[1]*, Dheeraj Ahirwar[1], M. P. Singh[1], Prashant Tiwari[1]

1. School of Pharmacy, C.E.C. Bilaspur, Chhattisgarh, India

Summary

Cassia Tora Linn (Caesalpiniaceous) is a small annual herbs of under shrub growing as common weed in Asian countries .It have been used as a liver protective, hypertensive activity & many medicinal properties such as antimicrobial, antihepatotoxic & antimutagenic activities attributed to this plant. Phytochemical studies had revealed the presence of flavonoids, glycosides, Saponin, bufadenolide, anthraquinone glycosides, phenol, and steroids. The present study was undertaken to evaluate cardiotonic activity of alcoholic and petroleum ether extract of *Cassia Tora* Linn seeds by using isolated guinea pig heart perfusion technique. Calcium free Ringer Locke solution was used as a vehicle for administration of alcoholic and pet ether extract of *Cassia Tora* Linn. as a test extract and digoxin as a standard. A significant increase in height of force of contraction (positive inotropic effect) and decrease in heart rate (negative chronotropic effect) at a very low concentration (0.25 mg/ml) was observed with test extract as same dose like standard digoxin. The present results indicated that a significant increase in height of force of contraction with decrease in heart rate was observed as the dose of both the text extract increased however, alcoholic extract of *Cassia Tora* Linn. Produced little more positive inotropic effect then pet. Ether extract. The present preliminary studies confirm the cardiotonic activity of *Cassia Tora* Linn seeds which is similar to digoxin. Further studies can confirm the reduced toxicity & this will be the advantage of *Cassia Tora* Linn over digitalis.

Keywords: Cardiotonic activity, Digoxin, Calcium frees Ringer lock solution, isolated guinea pig heart.

Introduction

Cardiac disease is an important cause of premature death in industrialized countries. It is estimated that cardiac disease will emerge as single largest Contributor to morbidity in India accounting for nearly one third of total deaths in near future. Cardiac glycosides and catecholamines have been used as main therapeutic agents in the treatment of congestive cardiac failure. [1] Despite continuing advances in understanding the basic pharmacology of cardiac glycosides, digitalis intoxication remains a common clinical problem. It necessitates research for new nature based drugs which increase cardiac muscle contractility with a broad therapeutic index. The essential organ of the human body i.e. heart when fails to work leads to sudden death. Since the potent cardiotonic drug i.e. the digoxin which is of the plant origin has a long list of ADR and toxicity, it is a need of hour to develop and standardise cardiotonic drugs of herbal origin. [2-8]. *Cassia Tora* Linn (Caesalpiniaceae) is generally distributed throughout India, Sri Lanka, West China and tropics. It is known as *Charota* (Hindi), Foetid Cassia (English), and Jui *Ming Zi* (Chinese). In India it occurs as wasteland rainy season weed, grows in dry soil throughout tropical parts and high hill of elevations up to 1,800 m as well as in plains. [9]. Seeds extract contain two anthraquinone glycosides (chrysophanol-tetraglucoside, chrysophanol-triglucoside, rubrofusarin-6-gentiobioside, rubrofusarin –glycoside). [10]. The seed extract is

also reported for its hypotensive activity. [11]. Previous phytochemical investigation on the seeds of *C. tora* has resulted in isolation of several anthraquinone, naphthopyrone derivatives [12-15]. However, no pharmacological studies regarding cardiotonic activity have been carried out on the seeds of *C. tora* to date. In the present study was carriedout in petroleum ether and alcoholic extract of the seeds of *C. tora.*

Material and methods:

Drug **:** Extract of Cassia tora L.
Chemical **:** Digoxin, Ringer Locke solution
Animal **:** Guinea pigs
Instruments **:** Sherington Rotating Drum

Preparation of extracts:

Plant materials were dried in sun and shade. The dried seeds were crushed to coarsely powdered by wood-grinder. The powdered material (300 gm) was defatted with petroleum ether (60-80°C) and then extracted with benzene, chloroform, methanol and then macerate with water using cold maceration procedure (for 24 hours). The extract was concentrated for further studies on water bath at 40°C.[16]. The extract test drug was dissolved in sterile water to obtain appropriate concentration of 0.25, 0.5, 1, 2 and 4 mg/ml and pure sample of digoxin (as reference standard) was dissolved in sterile water to make solutions of concentration 0.25, 0.5, 1 as experimental setup (Table1). Guinea pig were sacrificed and the hearts were exposed. The inferior venacava was traced and cannulated for perfusing the heart with the ringer Locke solution (The composition of the ringer locke solution in grams: NaCl-9; KCl (10% w/v in ml) 4.2; CaCl2 (10% w/v in ml)-2.4; NaHCO3-0.5; Glucose- 1) [17]. The basal cardiac contraction was recorded on a smoked kymographic drum after the administration of ringer's Locke solution. The drugs and extracts were administered through the cannula. The average basal heart rate and the contraction amplitude were 220 beats/min and 9 mm respectively. The effects obtained with the drugs and extracts were transposed to the respective percentage of the basal values. Graded dose response was recorded for each extract (0.25, 0.5 and 1mg). The Guinea pig heart was washed with the ringer solution after every administration of extracts and drugs till it was brought back to the normal state.

Table 1- Effects of alcoholic and pet. Ether extracts from Cassia tora Linn. on guinea pigs hearts. The average basal heart rate- 220 beats/min. Contraction amplitude- 9mm.

Expt.Set up	**Conc.of extract/Digoxin mg/ml .**	**Dose (ml.)**	**HR**	**HFC**
1	Extract 0.25 (Alcoholic)	0.1	180	9
		0.2	180	10
		0.4	175	9

2	0.5	0.1 0.2 0.4	180 175 165	10 11 11
3	1	0.1 0.2 0.4	160 150 150	12 12 13
4	Extract 0.25 (Pet ether)	0.1 0.2 0.4	172 180 181	8 9 9
5	0.5	0.1 0.2 0.4	165 168 172	10 11 10
6	1	0.1 0.2 0.4	154 168 178	9 10 11
7	Digoxine 0.25	0.1 0.2 0.4	175 173 170	13 13 15
8	0.5	0.1 0.2 0.4	165 170 168	12 14 17
9	1	0.1 0.2 0.4	160 155 150	14 18 20

(HR- Heart Rate, HFC- Height of force of contraction.

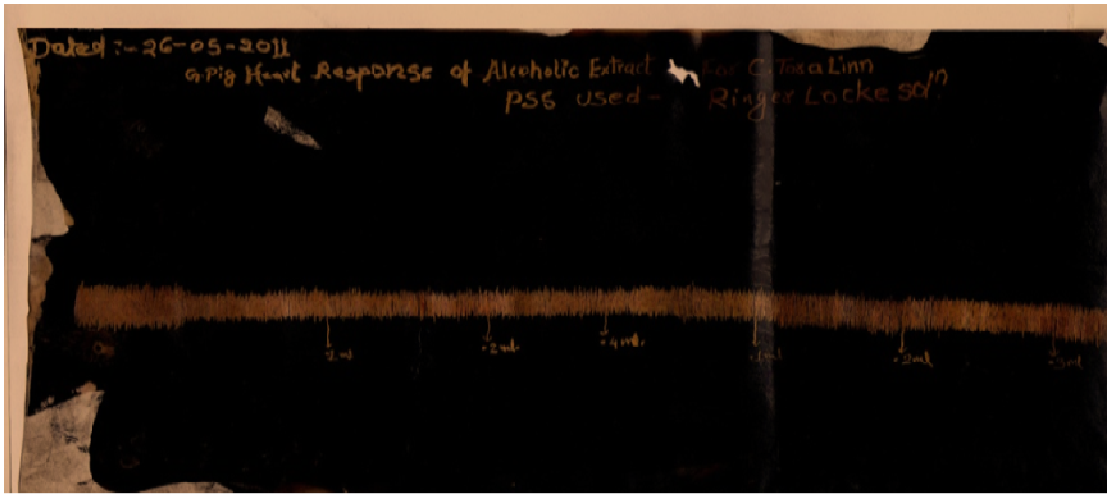

Fig. 1- (Effect of alcoholic extract of *cassia tora* Linn at (.25mg/ml, 0.5mg/ml, 1mg/ml)

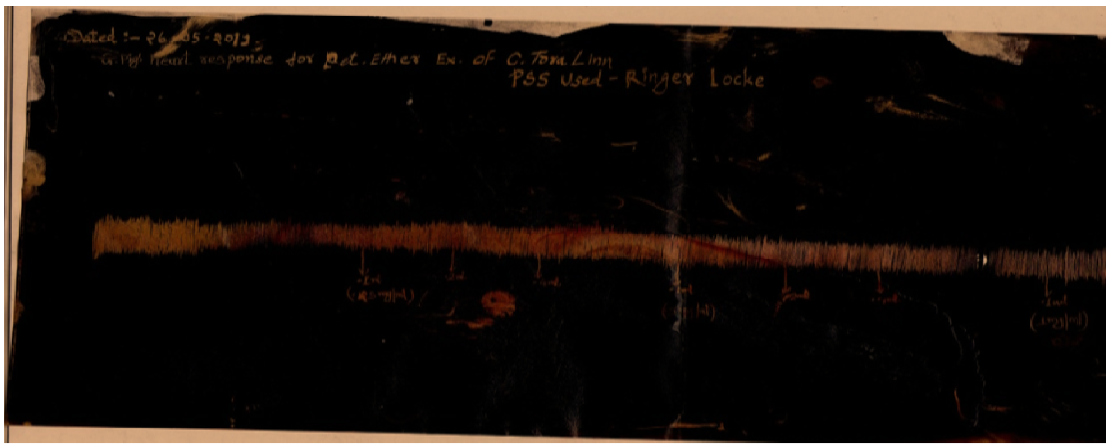

Fig. 2- (Effect of Pet.ether extract of *cassia tora* Linn at (.25mg/ml, 0.5mg/ml, 1mg/ml)

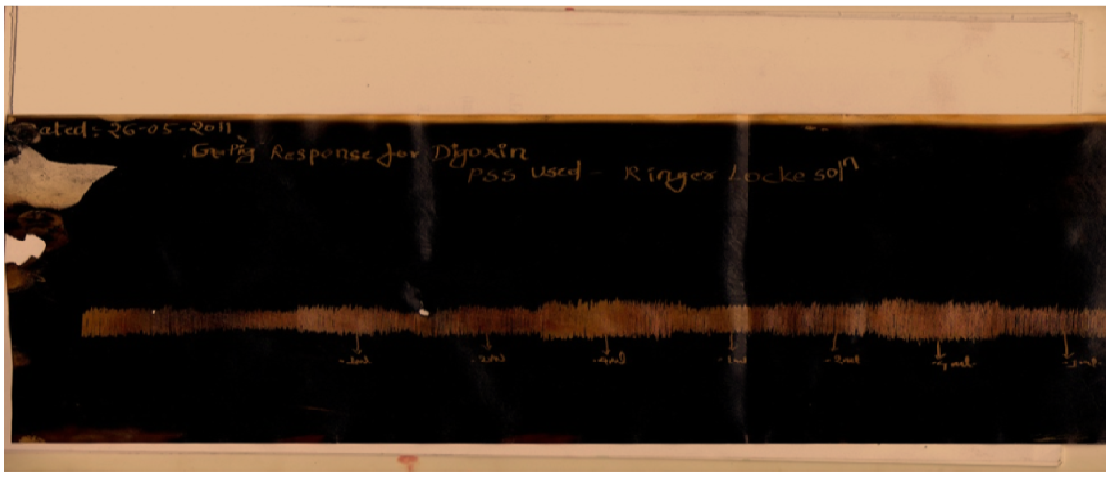

Fig. 3- (Effect of Digoxin at (.25mg/ml, 0.5mg/ml, 1mg/ml)

Effect of alcoholic extract and petroleum ether extract in camparision to Digoxin in different concentration

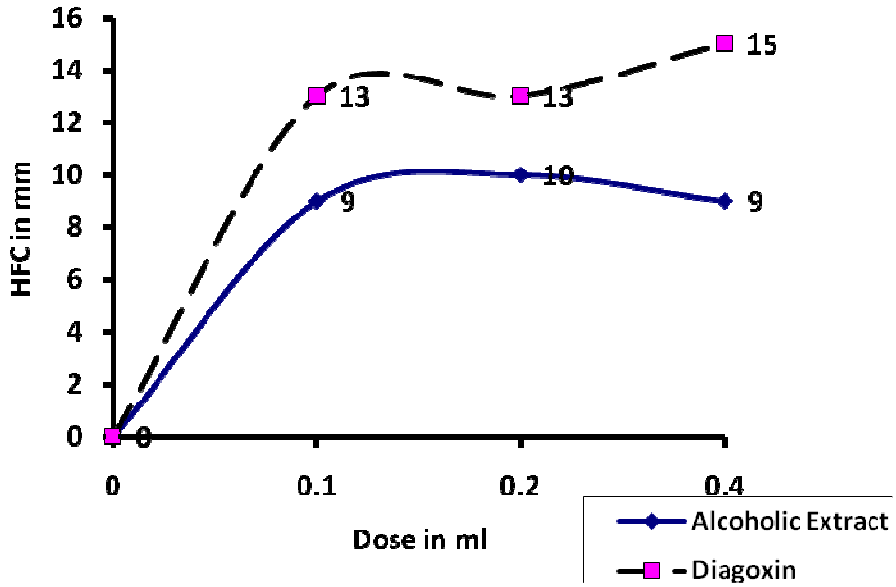

Fig. 4- (Effect of alcoholic extract of *cassia tora* Linn at 0.25mg/ml)

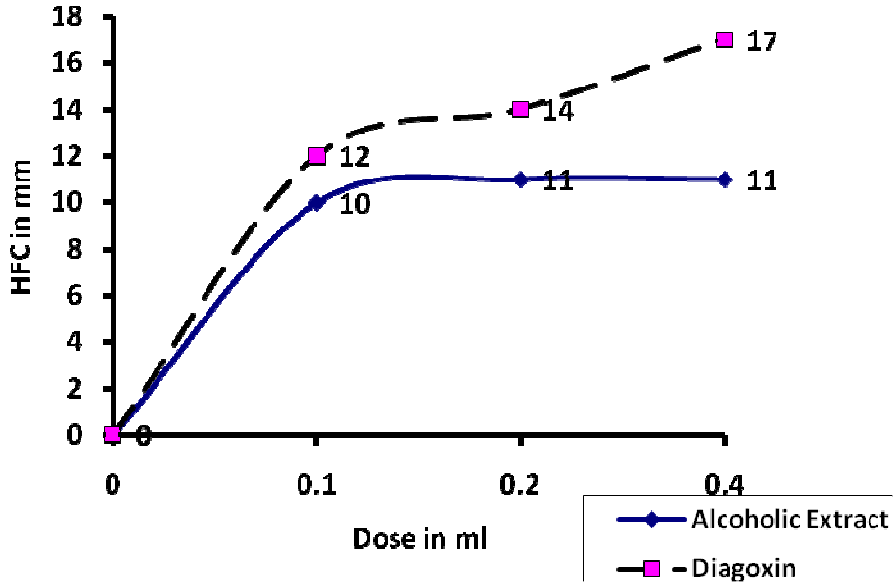

Fig. 5- (Effect of alcoholic extract of *cassia tora* Linn at 0.50mg/ml)

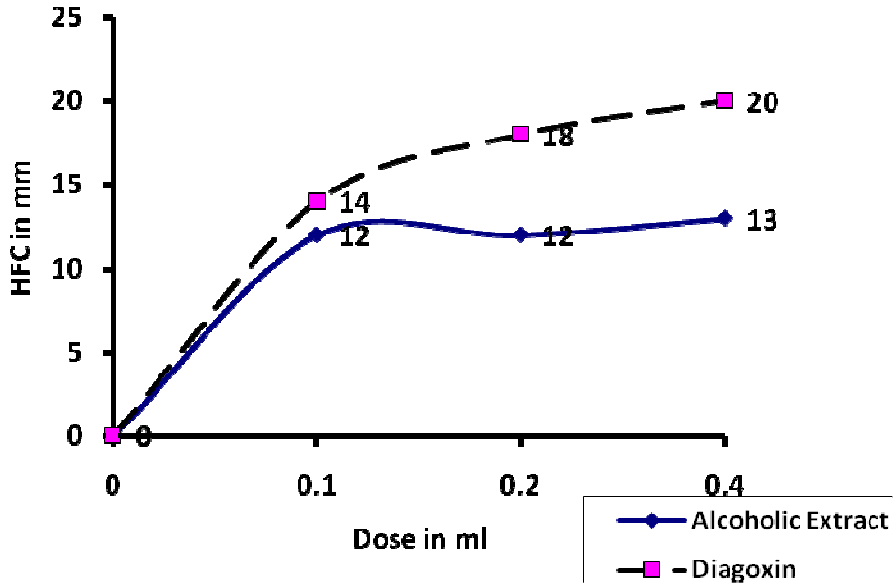

Fig. 6- (Effect of alcoholic extract of *cassia tora* Linn at 1.0mg/ml.)

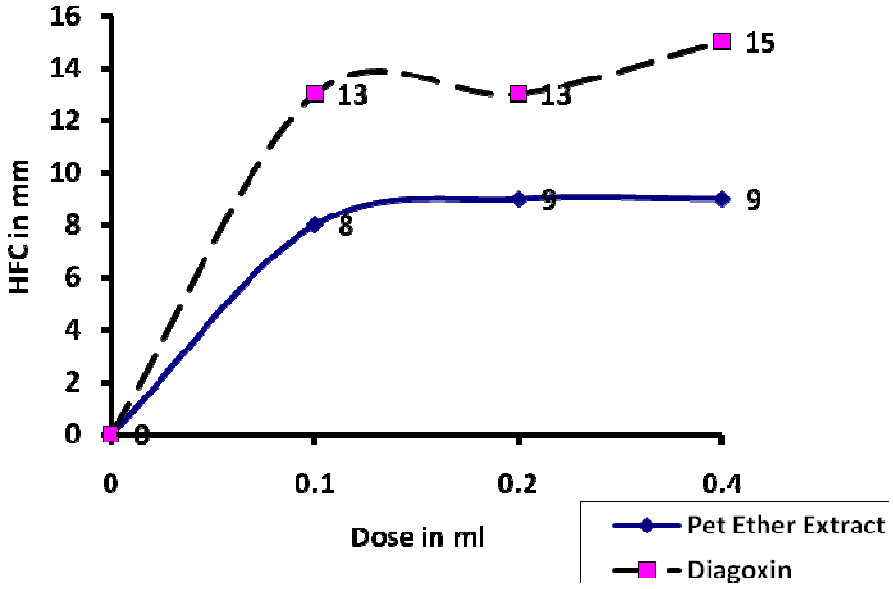

Fig. 7- (Effect of Pet.ether extract of *cassia tora* Linn at 0.25mg/ml.)

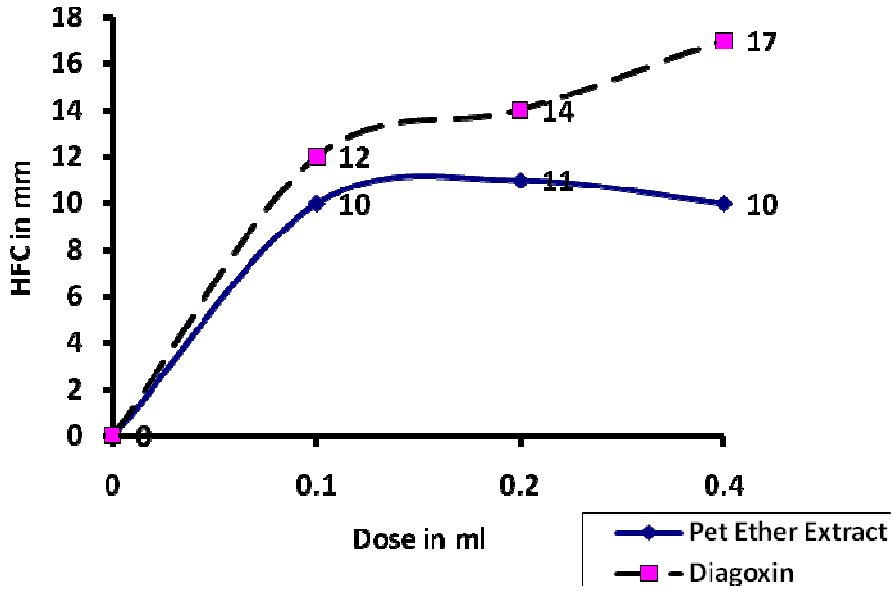

Fig. 8- (Effect of Pet.ether extract of *cassia tora* Linn at 0.5mg/ml.)

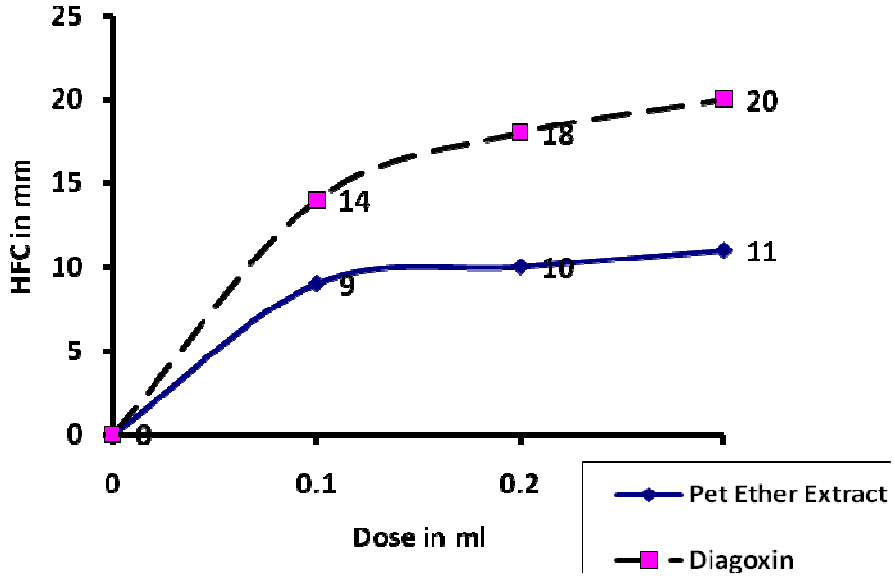

Fig. 9- Effect of Pet.ether extract of *cassia tora* Linn at 1.0mg/ml.)

Statistical analysis-

Table 2- Effect of *Cassia Tora* LINN extracts on heart rate on isolated guinea pigs heart perfusion technique.

S.no.	Treatment	HR	HR	HR
		0.25 (mg/ml)	0.5 (mg/ml)	1 (mg/ml)
1	Digoxin	172.66 ± 1.4	167.6 ± 1.4	155 ± 2.88
2	Alcoholic extract	178.33 ± 1.6^{ns}	173.33 ± 4.4^{ns}	153.3 ± 3.33 ^{ns}
3	Pet. Ether Extract	178 ± 3.03 ^{ns}	168.33 ± 2.02 ^{ns}	165.66 ± 6.12 ^{ns}

Table 3- Effect of *Cassia Tora LINN* extracts on force of contraction on isolated guinea pigs heart perfusion technique.

S.no.	Treatment	HFC	HFC	HFC
		0.25 (mg/ml)	0.5 (mg/ml)	1 (mg/ml)
1	Digoxin	13.66 ± .66	14.33 ± .4	17.3 ± 1.76
2	Alcoholic extract	9.33 ± .33***	10.66 ± .33***	12.66 ± .33**
3	Pet. Ether extract	8.16 ± .33***	10.66 ± .33***	10 ± .57**

All the values in MEAN ± SEM, Compaired the values of Digoxin with two different extract using DUNNETT Test and found Significant if $P \leq 0.05$ Very -significant if $P \leq 0.01$, Extreamly significant if $P \leq 0.001$, ns - not significant, (**) - very significant, (***) - extremely significant.

Result and Discussion

Pharmacological effect was studied by using ringer Locke solution and isolated guinea pig heart. Incremental dosage of alcoholic and pet. Ether extract of *Cassia Tora* Linn was taken for positive inotropic and negative chronotropic effect. The similar concentration of solution of Digoxin produced positive inotropic and chronotropic effect. The cardiotonic action was studied by the effects of alcoholic and pet ether extracts at various concentration given in (Table-1) and as shown in kymographs. (Fig-1, 2, 3). From the observations, it was revealed that at a very high dilution (0.25 mg/ml) the alcoholic extract of *Cassia Tora* Linn showed decrease in heart rate and increase in height of force of contraction effect, but the same concentration of pet. Ether extract showed increase in heart rate and increase in height of force of contraction. At concentration (0.05 mg/ml) of drug (alcoholic extract) showed decrease in heart rate with positive inotropic effect and pet. Ether with same concentration showed positive inotropic with increase in heart rate. While at a low concentration (0.25 mg/ml, 0.5mg/ml) Digoxin showed increase in height of force of contraction and negative chronotropic effects. (fig- 4, 5 & 7, 8) indicates that alcoholic and pet. Ether both the extracts reveal the therapeutic index and margin of safety like Digoxin. From the graphs (fig- 4, 5, 6, 7, 8, 9) it reveals that both extract and Digoxin showed dose dependant activity. However, alcoholic extract of *Cassia Tora* Linn produced little more positive inotropic effect then pet. Ether extract and Digoxin produced more significant positive inotropic effects than both of the extract at the same doses.

Heart failure is a common serious condition associated with high morbidity and mortality [18]. The side effect of currently used therapy for congestive heart failure is well documented. Toxicity of Digoxin is high and margin of safety is low. Therapeutic index (1.5-3 mg) and fatalities have occurred occasionally about 25% patients develop one or more toxic effects with digitalis [19]. The results obtained reveal that the therapeutic efficacy of extract of *Cassia Tora* Linn is dose dependent and similar to that of Digoxin. Limitation of using Digoxin may be overcome by using an alcoholic and pet. Ether extract of Cassia *Tora* Linn which has been reveals a cardiotonic activity. It may be a safe alternative to Digoxin in the treatment of congestive cardiac failure. The present result indicated that alcoholic and pet. ether extract of *Cassia Tora* Linn showed similar therapeutic index like cardiac glycosides.

Acknowledgements

The authors are grateful to Mr. M. P. Singh for his assistance in the experimental set up for pharmacological activity and technical assistance.

References

1. Emilio L. Ghisalberti, Survey of Secondary Plant Metabolites with Cardiovascular activity, Pharmaceutical Biology, October 1998, 4(36):237-279.

2. Stephen, CP., Aaron, H, Herb drug interactions and compounding in clinical trial, J Herb Pharmacotherapy, 2002, 2(1): 23-36.
3. Harsh Mohan -Textbook of Pathophysiology, 4th edition, Jaypee Brothers Medical Publishers 2000: 278-325.
4. Goodman & Gillman, The Pharmacological Basis of Therapeutics, 9th edition, McGraw Hill Publications, 1996: 810- 820.
5. Remington: The Science and Practice of Pharmacy, 19th Edition, Mack Publishing Company: 956.
6. Tripathi KD, Essentials of Medical Pharmacology, 5th Edition, New Delhi, Jaypee Brothers Medical Publishers, 2003: 457-467.
7. Barar FSK, Essentials of Pharmacotherapeutics, 1st edition, S.Chand Publications, 1985: 253-254
8. Satoskar RS, Bhandarkar SD, Ainpure SS, Pharmacology and Pharmacotherapeutics, 16th Edition, Mumbai, Popular Prakashan, 1997: 352-355.
9. Varier PS, Indian Medicinal Plant, Orient Logmen Pvt. Ltd., Hyderabad, 1995, Vol. 4, p. 149.

10. Shibata, S., Morishita, E., Kaneda, M., Kimura, Y., Takido, M., Takahashi, S., 1969. Chemical studies on the oriental plant drugs.XX. The constituents of *Cassia tora* L. 1. The structure of torachrysone. Chemical and Pharmaceutical Bulletin (Tokyo) 17, 454-457.

11. Koo, A., Chan, W.S., Li, K.M., 1976b. A Possible reflex mechanism of hypotensive action of extract from *Cassia tora* seeds. American Journal of Chinese Medicine 4, 249-255.

12. Ansari H. S. (2004). Plant profile of *Cassia tora*, *The Ayurvedic Plants,* Delhi Satguru Publication, 2004, 334-339, ISBN 81-7030-797.

13. Maity T.K., Mandal S.C., Mukharjee P.K., Saha K., Das J., Pal M. and Saha B.P., (1997) Evaluation of Hepatoprotective Potential of *Cassia tora* Leaf Extract, *Natural Product Science,* 3(2): 122-126.

14. Mukharjee P.K., Saha K., Das J., Pal M. and Saha B.P. (1998). Antifungal Screening of *Cassia tora* Linn. (Fam. Leguminosae), *Phytotherapy Research,* 10(6): 521-522.

15. Lee G.Y., Jang D.S., Lee Y.M., Kim J.M. and Kim J.S. (2006). Naphthopyrone glucosides from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation. *Arch Pharm Res.,* 29(7):587-90.

16. Khandelwal K.R., "Practical Pharmacognosy", Nirali Prakashan, Pune, Ed. 13, pp-157-161, 2005.
17. Kale S.R., "Practical Pharmacology and Toxicology", Nirali Prakashan, Pune, Ed. 12, pp-23, 2006.
18. Satoskar R.S, Bhandarkar S.D & Nirmala N Rege, "handbook of pharamacology & pharamacotherpeutics" Revised 19th edition (Popular Prakashan Pvt. Ltd. Popular Press Bldg, Mumbai) 435, 2005.
19. Thripathi K D, cardiac glycosides and drugs for CHF, "Essentials of medical pharmacology" 4th edition (Jaypee Brothers Medical Publishers Pvt. Ltd. New Delhi) 497, 2002.