ANTIPYRETIC ACTIVITY OF HYDRO-METHANOLIC EXTRACT OF *TRACHYSPERMUM AMMI* LINN. SEEDS IN RABBITS

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ABSTRACT: Antipyretic activity of hydro-methanol extract of *Tachyspermum ammi* Linn. seeds at dose 250mg/Kg and 500mg/Kg was determined in experimental animals. Yeast induced pyrexia method was used. Paracetamol (150 mg/Kg, p.o.) was used as standard drug. Rectal temperatures of rabbits were measured before the administration of extract, vehicle and paracetamol, and repeated at one-hour interval up to six hours by digital thermometer. Phytochemical analysis was carried out on extract to determine the existing groups. The extract dose 500 mg/Kg significantly reduces the elevated temperature. Phytochemical screening showed that flavonoids, alkaloids, phenols, anthraquinones, glycosides, tannins, phenols and saponins are present in the extract. The results showed hydro-methanol extract of *Trachyspermum ammi* L. exhibited a significant antipyretic effect and its efficacy was similar to standard antipyretic drug Paracetamol.

Key words: Antipyretic activity, medicinal plants, hydro-methanol extract, medicinal plants and rabbits.

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INTRODUCTION

Plant based medicine is used all over the world from the dawn of human civilization. Plants contain chemical constituents which produce therapeutic effect in several diseases. The finding of medicinal plants takes part in the improvement of health issues. Medicinal plants like neem, arjuna, aswagandha, tulsi, etc. traditionally used in pyrexia. The extract of *Acacia catechu*, *Bauhinia racemosus*, *Cleome viscosa* etc. reported to have antipyretic effect in experimental animals (Sharma *et al.,* 2010).

Trachyspermum ammi L. (*T. ammi*) is grassy annual plant. It is aromatic plant. It has Umbelliferae family. It is widely cultivated some area of India, Iran, Egypt and Pakistan. Its flowers are white and its fruit is brown in colour. The *T. ammi* is wide spread cooking spice and frequently used in India for therapeutic purpose (Asif *et al.,* 2014).

It contains alkaloids, carbohydrates, steroids, protein, fixed oils, glycosides, saponin, tannins, flavonoids, thymene, cumene, iron, lysine, starch, calcium and, essential oils like thymol, pcymene, c-terpinene.

It has antimicrobial, antihypertensive, hypolipidaemic, hepatoprotective, bronchodilating, antispasmodic, gastro-protective, anti-inflammatory, antitussive, and anthelmintic properties of *T. ammi* seeds extract.

The antipyretic and febrifugal activites (Umadevi *et al.,* 1990; Vedavathy *et al.,* 1995) are also mentioned in the Ayuverdic and Unani literature but it is not evaluated scientifically. Hence, we investigated the antipyretic efficacy of hydro-methanol extract of the *T. ammi* seeds in experimental animals and compared the effect with the standard drug Paracetamol.

MATERIALS AND METHODS

Identification of plants used in the study: *Trachyspermum ammi* sample was purchased from local market of the Bahawalpur and was identified by botanist Dr. Shazia Anjum, Director, Cholistan Institue of Desert Studies, The Islamia University of Bahawalpur. The voucher specimen *T. ammi* 3413/CIDS/IUB was deposited in herbarium of Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur.

Preparation of plant extract: The *T. ammi* L. seeds were washed to remove external dirt and undesired material. It was dried in shade for 72 hrs. One kilogram of powdered seed was taken in 5 liter glass beaker and added two liter of the 70% methanol. The material was macerated for 72 hour. During soaking occasional shaking and stirring was carried out. The soaked material

was filtered by muslin cloth after 72 hour and then filtered by whatman number one filter paper. The scum was extracted three times with fresh solvent and filtrate extract was combined. The filtrate was concentrated under reduced pressure at 40°C, and was made free from solvent, by rotary evaporator. The obtained extract was weighed. The yield was (9.3% w/w). It was stored in the refrigerator (-8°C), for analysis.

Phytochemical analysis: Screening test was carried out on extract for quantitative analysis using standard method (Khan *et al.*, 2011).

Test for alkaloids: In 2 ml hydro-methanolic filtrate, 1.5 ml of HCl1% added. The filtrate was heated on water bath than added some drops of Dragendroff reagent. Orange precipitate means alkaloids are present.

Test for saponins: Little amount of extract was taken in test tube and places it on boiling water. It was then cooled. Extract was wobbling strenuously to froth. If no froth formed mean saponins are not present. If froth less than 1 cm formed mean saponins are weakly present. Froth formed were 1-2 cm high mean extract is positive for saponins.

Flavonoids analysis: Fatty material was removed by petroleum from dried extract. 80% ethanol was taken in a beaker and was put in it Defatted residue. It was then filtered.

To three mL of filtrate, four mL of the 1% potassium hydroxide in test tube was mixed. Dark yellow color indicated that flavonoid is present.

Analysis for tannins: To distilled water 0.3 g of extract was added and then filtered. 1% alcoholic ferric chloride was added in the filtrate in the test tube. If tannins present then color will be green, black or purple color.

Analysis for phenols: One mL of 1% ferric chloride was mixed in two mL of extract. If blue or green color appears mean phenols were present.

Analysis cardiac glycosides: Two mL chloroform and H_2SO_4 is added carefully to 0.3gm of extract, at the bottom layer was formed. Reddish-brown color at edge showed the presence of steroidal ring.

Experimental animals: Healthy rabbits weighing 1000-1200gm were aquaired from the animal house, of Islamia University of Bahawalpur. The rabbits were placed in animal house of the Department of Pharmacy. They were fed on usual rabbit's diet. The animals were kept at controlled temperature of 22-25 °C and light/dark 12hrs/ 12hrs cycle for five days before the start of study. All the rabbits were kept without food for one hour before the start of drug treatment. Water and food was started after the administration of drug.

The animals were grouped as:

a) Control groups

i. Solvent group I (-Ve Control): receiving distilled water.

ii. Paracetamol group II (+Ve Control): receiving standard antipyretic agent Paracetamol.

b) Experimental groups

i. Group III: receiving extract dose of 250mg/Kg body weight.

ii. Group IV: receiving extract dose of 500mg/Kg body weight.

Drug, test agents and reagents: Paracetamol, normal saline, methanol, baker yeast, distilled water were issued in the study.

Antipyretic activity

Yeast induced pyrexia method: Antipyretic efficacy was investigated by the procedure stated by (Bose *et al.*, 2007). Rabbits were grouped in to four. Each group consisted of six rabbits. Rectal temperature was obtained with thermometer before administrating yeast suspension. To induce pyrexia 3mL/ Kg/bodyweight of 10% w/v yeast suspension subcutaneously was injected to the rabbits. Temperature was measured after 18 hours, of the administration of yeast suspension. The rabbits showing 0.5˚C to 1.5˚C rise in the temperature were selected for the study. Distilled water was given to Group I which is negative control. Paracetamol (standard drug) dose 150mg/kg body weight was given orally to group II which is positive. Group III and IV given extract dose of 250mg/Kg body weight and 500 mg/Kg body weight, respectively. The rectal temperature was measured for 6 hours at the interval of one hour.

Statistical analysis: The result and data of the study were examined statistically by using SPSS 17. One way ANOVA was used for multiple comparisons followed by LSD post hoc test.

RESULTS AND DISCUSSION

Phytochemical screening showed tannins, flavonoids, alkaloids, saponins, cardiac glycosides and phenols were present in the extract.

The administration of *T. ammi* extract dose 250 mg/Kg body weight and dose 500 mg/kg body weight and standard drug decrease the body temperature to 39.11 ± 0.09, 38.70 ± 0.06, 38.57 ± 0.06, respectively after three hrs. The 500 mg/Kg extract dose significantly ($p<0.0001$) reduce body temperature in comparison to control. After 6hrs temperature become not changed and remain at 38.15± 0.02 and 38.28 ± 0.06 in standard Paracetamol treated group and in extract treated groups 500mg/Kg, respectively.

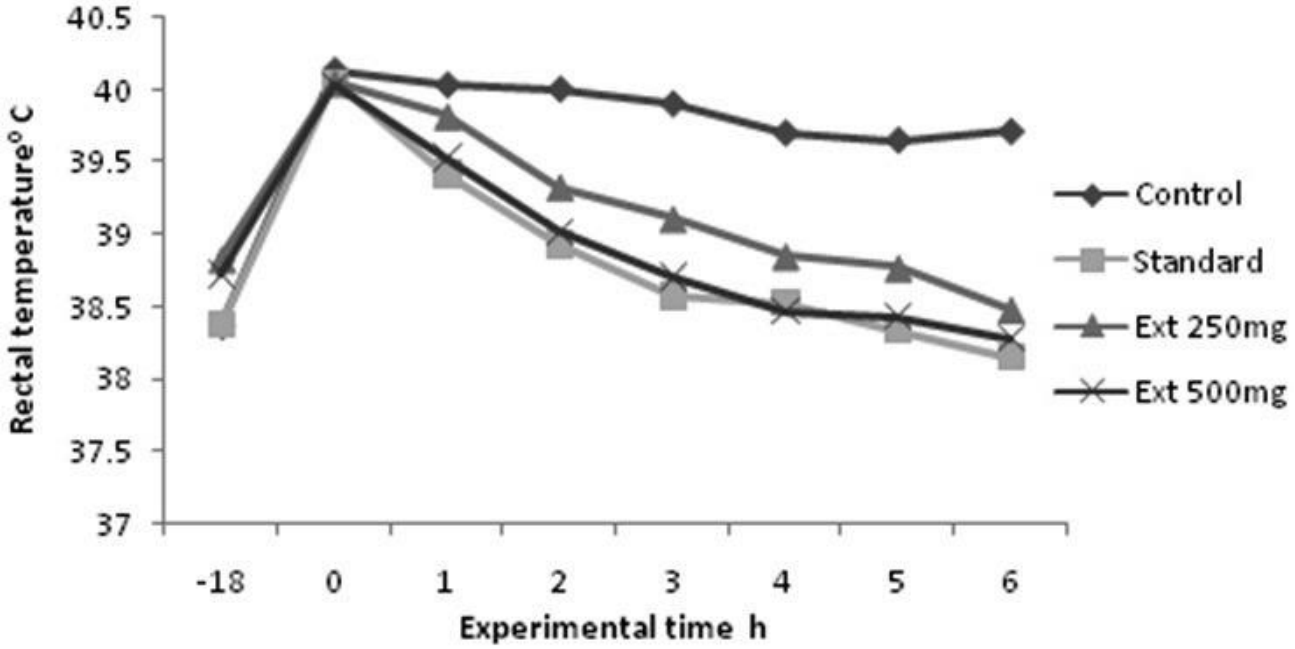

Figure-1: Antipyretic effect of *Tachyspermum ammi* extract. -18: Temperature before giving yeast injection; 0: Temperature before the administration of drug

Table-1: Results of phytochemical investigation of phytoconstituents present in the hydro-methanol extract of *Trachyspermum ammi* seeds.

Test Sample	Alkaloids	Tannins	Flavonoids	Saponins	Phenols	Cardiac glycosides
***Trachyspermum ammi* seeds**	+	+	++	++	++	+

(+) Present; (-) Absent; (++) Highly present

Table-2: Antipyretic effects of hydro-methanol extract of *Tachyspermum ammi* seeds.

	Treatment	Rectal Temperature °C		Rectal temperature°C after drug administration					
Groups	**Dose mg/Kg**	**-18hr Before yeast injection**	**0hr After yeast injection**	**1st hr**	**2nd hr**	**3rd hr**	**4th hr**	**5th hr**	**6th hr**
Control	_	38.37 ±0.06	40.13 ±0.02	40.03 ±0.02	40.00 ±0.00	39.86 ±0.03	39.70 ±0.07	39.65 ±0.06	39.7 ±0.06
Paracetamol	150mg/Kg	38.38 ±0.07	40.05 ±0.04	39.42 ±0.04**	38.93 ±0.04**	38.57 ±0.06**	38.53 ±0.06**	38.33 ±0.04**	38.15 ±0.02**
Extract	250mg/Kg	38.83 ±0.04	40.05 ±0.04	39.82 ±0.04	39.33 ±0.14	39.11 ±0.09	38.85 ±0.06**	38.77 ±0.06**	38.48 ±0.04**
Extract	500mg/Kg	38.72 ±0.17	40.03 ±0.04	39.53 ±0.09*	39.02 ±0.10**	38.70 ±0.06*	38.47 ±0.08**	38.43 ±0.06**	38.28 ±0.06**

Values are defined as means ±SEM

N=6. * *P < 0.001,* ** *P<0.0001*

Fever is caused by infection, inflammation, graft rejection, cancer or any other disease state. Natural defense mechanism is present in the body which fights against the infectious agent. Whenever body is attack by any foreign body or infectious agent, formation of the mediators takes place. The mediators raised the formation of the prostaglandin E2 (PEG2). The prostaglandin stimulates the hypothalamus which regulates body

temperature. Therefore the body temperature increases. The nervous feedback mechanism is present in the body. Whenever temperature of the body rises, it will dilate blood vessels and increases sweating. This phenomenon is happening to decrease the body temperature. High grade fever causes tissue catabolism, disease progression and dehydration (Sultana *et al.,* 2013).

Mostly antipyretic drugs blocks COX-2 enzymes to decrease temperature by preventing PGE2 synthesis (Sultana *et al.,* 2013). Furthermore, synthetic drugs also irreversibly inhibit COX-2. Studies revealed that they have toxic effect on the body, whereas COX-2 inhibitors from natural source possess little side effects. Search for herbal compounds with potential antipyretic efficacy received drive now days, as current antipyretics, have adverse effect on different organs of the body (Sultana et al *et al.,* 2015).

Antipyretic drugs such as acetylsalicylic acid reduce body temperature by inhibiting the synthesis of prostaglandin in hypothalamus. Similarly, Paracetamol give antipyretic effect by inhibiting the cyclooxygenase (COX) iso enzyme in brain (Begum *et al.,* 2011).

The extract of *T. ammi* showed antipyretic efficacy and the antipyretic effect might be, due to reticence of prostaglandin synthesis (Sultana *et al*., 2013). Phytochemical analysis showed alkaloids, saponins, tannins, flavonoids and phenolic compounds are present in seed extract. Alkaloids like bolidine and baicalin possess the capability to reduce raised temperature by inhibiting prostaglandin E2 production and tumor necrosis factor (Backhouse *et al.,* 1994).

Conclusion: The study showed hydro-methanol extract of *T. ammi* exhibited significant antipyretic activity in rabbits and its effects was similar to standard drug paracetamol. The antipyretic activity may be because of secondary metabolites alkaloid or flavonoids components of extract. The study therefore, showed that hydro-methanol extract of *T. ammi* seeds exhibited significant antipyretic effect.

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REFERENCES

Backhouse N., C. Delporte, M. Givernau, B.K. Cassels, A. Valenzuela and H. Speisky (1994). Anti inflammatory and antipyretic effects of boldine. Inflamm. Re. 42(3): 114-117.

Begum T.N., H. Mohamed, I. Muhammad and V.A. Arumugam (2011). Antipyretic activity of azima tetracantha in experimental animal. Int. J. Cur. Biomed. Phar. Res.1(2): 41 – 44.

Bose A., S. Mondal, K. Jayanta, J.K. Gupta, T. Gosh, G.K. Dash and S. Sudam (2007). Analgesic, anti-inflammatory and anti-pyretic activities of the ethanolic extract and its fraction of Cleome rutidosperma. Fitoterpia, 78: 515-520

Khan A.M., Q.A. Rizwana and U. Faizan (2011). Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. J. Med. Plants Res. 5(25) : 6017-6023.

Khan, F.A., I. Hussain, F. Shahid, A. Majed and R. Muhammad (2011). Phytochemical screening of some Pakistanian medicinal plants. Mid. E. J. Sci. Res. 8 (3): 575-578.

Sharma, J.P., A. Srivastava, S.P. Thakur, P.K. Barpete and S. Singh (2010). Herbal medicine as antipyretic: A comprehensive review. Int. J. Pharm. Life Sci. 1(1):18-22.

Sultana, S., H.M. Asif, N. Akhtar and K. Ahmad (2015). Medicinal plants with potential antipyretic activity: A review. Asian. Pac. J. Trop. Dis. 5(1): 202-208.

Sultana, S., N. Akhtar and H.M. Asif (2013). Phytochemical screening and antipyretic effects of hydro-methanol extract of Melia azedarach leaves in rabbits. Bangladesh J. Pharmacol. 8: 214-217.

Sultana, S., N. Akhtar and H.M. Asif (2013). Phytochemical screening and Antipyretic effects of Hydromethanol extract of Melia azedarach leaves in rabbits. Bangla J. Pharmacol. 8: 214-217.

Umadevi, I. and M. Daniel (1990). Phenolics of some fruit spices of the Apiaceae. Nat. Acad. Sci. Let. 13(12): 439-441.

Vedavathy, S. and D.N. Rao (1995). Herbal folk medicine of Tirumala and Tirupati region of Chittoor district, Andhra Pradesh. Fitoterapia. 66(2):167-171.

Zahin, M., I. Ahmad and F. Aqil (2010). Antioxidant and antimutagenic activity of Carum copticum fruit extracts. Toxicol. Vitro. 2 (24): 1243-1249.