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Evaluating Carthamus Oxyacantha For 5α-Reductase Inhibition: Therapeutic Perspectives In BPH

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common condition in older men, marked by the enlargement of the prostate gland due to increased growth of stromal and epithelial cells. Among elderly men, BPH is the fourth most frequently diagnosed condition and the primary cause of lower urinary tract symptoms. Histological data indicate that BPH affects over half of men by age 60 and nearly 90% by age 85. This condition is strongly linked to dihydrotestosterone (DHT), an androgen derived from testosterone through the action of the enzyme 5α-reductase, which drives prostate growth. Although the exact mechanisms behind BPH are not fully clarified, DHT and other androgens are considered key contributors, making the inhibition of DHT production a valuable approach for reducing prostate growth.
In this study, we conducted in vitro tests to assess the 5α-reductase inhibitory potential of *Carthamus oxyacantha* extracts. Prostate homogenates were used to prepare enzyme solutions, and enzyme concentration was measured with the Bradford reagent. The inhibitory activity was evaluated using a standard testosterone curve, with peak areas monitored by HPLC at 254 nm, using a methanol(70:30) mobile phase at a flow rate of 1 ml/min. Optimal enzyme concentration was determined by adjusting enzyme levels while maintaining constant substrate concentrations.
The findings showed that the n-hexane fraction of the methanolic extract of *Carthamus oxyacantha* displayed significant 5α-reductase inhibitory activity, with an IC50 value of 0.301 mg. Other fractions and extracts, however, did not exhibit substantial inhibitory effects. These results suggest that *Carthamus oxyacantha*, particularly its n-hexane fraction, may offer therapeutic potential for BPH treatment through effective inhibition of 5α-reductase.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a highly prevalent condition in aging men, leading to an enlarged prostate due to the proliferation of stromal and epithelial cells. This condition often results in lower urinary tract symptoms (LUTS) that significantly impact quality of life. BPH is recognized as one of the most common disorders in elderly men, affecting over 50% of men by age 60 and nearly 90% by age 85. The androgen dihydrotestosterone (DHT), synthesized from testosterone by the enzyme 5α-reductase, is a key factor in promoting prostate growth. Thus, inhibition of 5α-reductase, and consequently DHT production, has become a primary therapeutic strategy in managing BPH symptoms and slowing prostate enlargement (Khan, *et al.*, 2018). (Shah *et al.*, 1997).
Conventional treatments for BPH, including synthetic 5α-reductase inhibitors like finasteride, are effective in reducing prostate size but may cause adverse effects, such as sexual dysfunction and hormonal imbalance, that limit their long-term use. This has sparked growing interest in identifying natural 5α-reductase inhibitors with fewer side effects and comparable efficacy. *Carthamus oxyacantha*, a medicinal plant used in traditional systems, has shown promise in various pharmacological activities and is being explored for its potential role in managing androgen-dependent conditions (Naik, *et al.*, 2020).
This study focuses on evaluating the 5α-reductase inhibitory activity of *Carthamus oxyacantha*, with an emphasis on understanding its potential application as a natural therapeutic agent for BPH. Through in vitro experiments, we examined the ability of different extracts and fractions of *Carthamus oxyacantha* to inhibit 5α-reductase. The results of this study could offer valuable insights into the potential of *Carthamus oxyacantha* as a plant-based alternative in BPH treatment, contributing to the search for safer and more accessible therapies for this common age-related condition. (Sharma, M. *et al.*, 2019).

MATERIALS AND METHODS

PLANT MATERIAL

Plant leaves and flowers of *Carthamus oxyacantha* were collected from forested areas surrounding Nauradehi in the Bundelkhand region, Madhya Pradesh, India. The collected specimens were subsequently authenticated, and voucher samples were deposited in the herbarium of the Department of Botany at Dr. Harisingh Gour University, Sagar, Madhya Pradesh, India.

PREPRATION OF EXTRACTS

The leaves and flowers of *Carthamus oxyacantha* were separately dried at 30°C in a shaded environment to prevent degradation. The dried plant materials were then coarsely ground using an electric grinder and extracted with a 70% methanol-water mixture in a Soxhlet apparatus. The resulting extracts underwent solvent recovery under reduced pressure, yielding a 4.8% (w/w) extract, using a rotary vacuum evaporator. This was followed by freeze-drying with a lyophilizer to obtain a powdered methanolic extract.

The freeze-dried methanolic extract was then suspended in 200 ml of distilled water and subjected to partitioning with n-hexane (4 x 200 ml) using a separatory funnel. The n-hexane layer was concentrated by rotary evaporation and dried. The remaining aqueous phase was sequentially partitioned with chloroform and butanol following the same protocol. After the final partitioning, the aqueous fraction was concentrated by distillation and dried, as described by Partin et al. (1991).

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***IN VITRO* STUDIES**

To evaluate the potential of the extracts in mitigating prostate hyperplasia, their activity against 5α-reductase, the pivotal enzyme involved in the pathogenesis of prostate hyperplasia, was investigated (Resnick et al., 1983). In vitro assays were conducted to assess the 5α-reductase inhibitory effects of the extracts, their respective fractions, and the standard drug finasteride. The concentration of testosterone in the reaction mixtures was quantified using high-performance liquid chromatography (HPLC). A detailed description of the methodology is provided in the subsequent sections.

Preparation of Enzyme Solution

Approximately 200 mg of prostate tissue was acquired from a local hospital in Sagar and processed to extract the enzyme source. The tissue was finely chopped and homogenized in 10 ml of a buffer containing 20 mM sodium phosphate (pH 6.5), 0.32 mM sucrose, and 1 mM EDTA. The homogenate was centrifuged at 4000 rpm for 15 minutes, and the resulting supernatant was used as the enzyme source. The protein concentration in this supernatant was measured using the Bradford assay (Agrawal et al., 2012).

Protein Estimation Procedure:

Preparation of Bradford Reagent:

Coomassie Brilliant Blue G-250 (10 mg) was dissolved in 5 ml of 95% ethanol, to which 10 ml of concentrated o-phosphoric acid was added. The solution was brought up to 20 ml with distilled water, diluted in a 1:4 ratio with distilled water, and filtered using Whatman No. 1 filter paper before use (Bradford, 1976).

Standard Curve Using Bovine Serum Albumin (BSA)

A BSA stock solution at 100 µg/ml in phosphate buffer was prepared, from which aliquots with concentrations of 20 µg/ml to 200 µg/ml were made. Each aliquot was combined with 4 ml of Bradford reagent for a final volume of 5 ml, incubated for 5 minutes, and the absorbance was measured at 590 nm with a UV spectrophotometer (GBC Cintra, Australia).

This procedure allowed accurate protein quantification, essential for the subsequent assessment of enzyme activity.

Table 1: ***Standard solution of BSA was prepared in phosphate buffer***

S.No	Concentration of protein (µg/ml)	Absorbance (at 595 nm)	Regressed Value	Statistical Analysis
1	50	0.0755	0.069	
2	100	0.1015	0.104	
3	150	0.1308	0.139	
4	200	0.1625	0.174	**Equation of straight line**
5	250	0.1933	0.209	Y=0.0007x+0.034
6	300	0.2258	0.244	**Correlation Coefficient**
7	350	0.2555	0.279	R^2=0.996
8	400	0.2909	0.314	
9	450	0.3342	0.349	
10	500	0.3686	0.384	

Estimation in Enzyme Solution
The enzyme solution (1 ml) was incubated with 4 ml of Bradford reagent for 5 minutes. The absorbance was recorded as 0.3072, and the concentration was determined to be 390.28 µg/ml using the standard curve of BSA (Nahata *et al.*, 2017)

Standard curve of testosterone
A standard solution of testosterone at a concentration of 100 µg/mL was prepared in ethanol. Aliquots ranging from 5µg/mL to 50 µg/mL were prepared from this stock solution, and a standard curve was constructed using HPLC (AT10, Shimadzu Corp., Kyoto, Japan). The peak area was measured at 254 nm using a mobile phase of methanol and water (70:30) at a flow rate of 1 ml/min (Carbajal, *et al.*, 2004)

Table 2: Standard curve for Testosterone Table 5.2: Standard curve for Testosterone

S. N.	Concentration	Peak Area	Regressed Value	Statistical Analysis
1	5	178184	198523	
2	10	342851	355463	
3	15	478791	512403	**Equation of straight line**
4	20	698278	669343	
5	25	865281	826283	y = 31388x + 41583
6	30	1004387	983223	**Correlation Coefficient**
7	35	1164182	1140163	
8	40	1323870	1297103	R^2=0.995
9	45	1394418	1454043	
10	50	1597286	1610983	

Preparationof Test Materials
A 1 mg/mL extract solution was prepared using 95% ethanol, with gentle heating applied when necessary. An EDTA solution (10 mg/mL) was prepared in distilled water, and a finasteride solution (10 µg/mL) was made in ethanol.

Determination of Optimum Enzyme Concentration
To determine the optimal enzyme concentration, the substrate concentration was kept constant while varying the enzyme concentration. A 1 mM testosterone solution was prepared in ethanol. The reaction mixture (1 mL) was made by combining 0.1 mL of the testosterone solution, varying amounts of the enzyme solution (ranging from 0.1 to 0.9 mL), and sodium phosphate buffer (20 mM). The reaction mixture was incubated at 37°C for 1 hour. After incubation, the reaction was halted by adding 2 mL of ethyl acetate. The mixture was vigorously vortexed for 1 minute, and the ethyl acetate layer was separated. After evaporating the solvent to dryness, the residue was dissolved in 2 mL of methanol. The remaining testosterone in the methanolic solution was analyzed by HPLC (AT10, Shimadzu Corp., Kyoto, Japan). The column was eluted with an isocratic mobile phase of methanol(70:30) at a flow rate of 1.0 mL/min. The optimal enzyme concentration that effectively inhibited the conversion of testosterone to DHT was found to be 0.6 mL (Nahata et al., 2014).

Table 3:OPTIMUM CONCENTRATION OF ENZYME

S.N.	VOLUME OF ENZYME (mL)	PEAK AREA	CONCENTRATION OF TESTOSTERONE
1	**0.1**	***680425***	***23.76***
2	**0.2**	***634539***	***22.13***
3	**0.3**	***582616***	***20.29***
4	**0.4**	***502616***	***17.46***
5	**0.5**	***426719***	***14.77***
6	**0.6**	***376530***	***12.99***
7	**0.7**	***322379***	***11.07***
8	**0.8**	***295721***	***10.12***
9	**0.9**	***249974***	***8.50***
10	**1.0**	***319171***	***10.96***

Determination of Inhibitory Concentration of Extract
The reaction mixture (1.5 ml) was prepared by mixing 0.1 ml of EDTA solution, 0.1 to 0.5 ml of the extract solution (depending on the experimental group), 0.6 ml of enzyme solution, and 20 mM sodium phosphate buffer, bringing the final volume to 1.5 ml. The mixture was incubated at 37°C for 60 minutes, and the reaction was terminated by adding 2 ml of

ethyl acetate. After vortexing for 1 minute, the ethyl acetate layer was separated and evaporated to dryness. The resulting residue was dissolved in methanol and the final volume was adjusted to 2 ml with methanol. The testosterone content remaining in the methanolic solution was quantified using HPLC. The column was eluted with a methanol(70:30) mobile phase at a constant flow rate of 1.0 ml/min (Nahata et al., 2012; Nandecha et al., 2010).

Table 4: Inhibitory Concentration of methanolic Extract of *Carthamus oxycantha*

S.No.	Concentration of extract	Peak Area	RTC*	Regressed Value	Statistical Data
1	0.1	95247	1.709	1.653	y=12.07x+0.446
2	0.2	125478	2.672	2.86	
3	0.3	175894	4.279	4.067	R^2=0.988
4	0.4	204784	5.199	5.274	

*Relative testosterone concentration

Table 5: Inhibitory Concentration of n-Hexane soluble Fraction of *Carthamus oxycantha*

S.No.	Concentration of extract	Peak Area	RTC*	Regressed Value	Statistical Data
1	0.1	128754	2.8	3.06	
2	0.2	219975	5.7	5.57	y=25.1x+0.55
3	0.3	312877	806	8.08	
4	0.4	361873	10.2	10.59	R^2 = 0.984

*Relative testosterone concentration

Table 6: Inhibitory Concentration of *Chloroform* Soluble Fraction of *Carthamus oxycantha*

S.No.	Concentration of extract	Peak Area	RTC*	Regressed Value	Statistical Data
1	0.1	70148	0.91	0.807	y=6.172x+0.19
2	0.2	83541	1.33	1.424	
3	0.3	101478	1.90	2.041	R^2=0.974
4	0.4	128745	2.77	2.658	

*Relative testosterone concentration

Table 7: Inhibitory Concentration of Ethyl acetate soluble Fraction of *Carthamus oxycantha*

S.No.	Concentration of extract	Peak Area	RTC*	Regressed Value	Statistical Data
1	0.1	72847	0.99	0.898	
2	0.2	85743	1.40	1.486	y=5.877x-0.311
3	0.3	102576	1.94	2.074	
4	0.4	128735	2.77	2.661	R^2=0.974

*Relative testosterone concentration

Table 8: Inhibitory Concentration of n-butanol soluble fraction of *Carthamus oxycantha*

S.No.	Concentration of extract	Peak Area	RTC*	Regressed Value	Statistical Data
1	0.1	728.74	0.99	0.944	
2	0.2	82478	1.30	1.292	y=3.476x+0.597
3	0.3	87458	1.46	1.639	
4	0.4	107585	2.1	1.987	R^2=0.926

*Relative testosterone concentration

Table 9: Inhibitory Concentration of Water Soluble Fraction of *Carthamus oxycantha*

S. No.	Concentration of extract	Peak area	RTC*	Regressed Value	Statistical analysis
1	0.1	67645	0.63	0.819	
2	0.2	77584	1.14	1.109	y=2.900x+0.529
3	0.3	81547	1.27	1.399	
4	0.4	96874	1.76	1.689	R^2=0.948

*Relative testosterone concentration

Table 10: Inhibitory Concentration of Finasteride

S. No.	Concentration of extract	Peak area	RTC*	Regressed Value	Statistical analysis
1	0.1	4.74	3.81	3.812	
2	0.2	9.01	10.03	10.032	Y=6.220x+2.408
3	0.3	15.5	16.252	16.252	
4	0.4	23.31	22.472	22.472	R^2=0.983

*Relative testosterone concentration

RESULTS

The IC50 value, representing the concentration of the test compound required to reduce the conversion of 1 mM testosterone by 50%, was calculated through regression analysis and reported to be (Pandit et al., 2008).:

Extract/Fraction*(Carthemus. oxycantha)*	IC_{50} Value
Methanolic extract	1.15±0.11mg
n-hexane soluble fraction	0.552±0.09mg
Chloroform soluble fraction	2.30±0.13 mg
Ethyl acetate soluble fraction	2.397±0.12 mg
n-butanol soluble fraction	3.97±0.22 mg
Water soluble fraction	4.78±0.23 mg
Finasteride	2.7±0.13 mg µg

Discussion

Testosterone is converted into the more potent dihydrotestosterone (DHT) by the enzyme 5α-reductase type 2, which is present in prostate homogenates. When methanolic extracts and their fractions from *Carthamus oxyacantha* were introduced into the reaction mixture, a significant increase in the levels of unchanged testosterone was observed, indicating that the extract inhibited 5α-reductase activity. This suggests that *Carthamus oxyacantha* effectively blocks the enzyme, leading to higher levels of testosterone remaining unconverted. In vitro studies demonstrated the 5α-reductase inhibitory potential of *Carthamus oxyacantha* extracts, with comparisons made to the standard drug finasteride. The IC50 values, representing the concentration required to inhibit 50% of the conversion of testosterone to DHT, revealed that both the methanolic extract and the n-hexane fraction exhibited significant inhibition of the enzyme compared to finasteride. Lower IC50 values indicate higher potency, as they represent the reduced concentration needed to achieve the desired level of inhibition (Nahata et al., 2017). Based on these results, *Carthamus oxyacantha* extracts were selected for further in vivo investigations.

Conclusion

The findings from this study suggest that the n-hexane fraction of the methanolic extract of *Carthamus oxyacantha* effectively inhibits the 5α-reductase enzyme. This makes it a promising candidate for the treatment of conditions related to 5α-reductase activity, highlighting its potential as an alternative therapeutic option for managing prostate-related disorders.

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