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Research Article

Anti-Inflammatory Activity of Ethanolic Extract of *Canthium dicoccum*

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Abstract

Ethanolic extract of *Canthium dicoccum* whole plant was studied for its Anti-inflammatory activity in various animal experimental models. Wistar albino rats were used to study anti-inflammatory activity of ethanolic extract of *C.dicoccum* whole plant at doses 250mg/kg p.o and 500mg/kg p.o against the standard Diclofenac 25mg/kg p.o in various models of anti-inflammatory activity viz.Carrageenan induced paw oedema, Formalin induced paw oedema, fresh egg white induced paw oedema and cotton pellet induced granuloma model. The plant extract at doses 250mg/kg and 500mg/kg significantly inhibited inflammation at $P<0.05$ in all the experimental models. Test dose at 500mg/kg, offered more protection against inflammation when compared to standard in carrageenan paw oedema model. In the formalin induced model a progressive inhibition of inflammation from the 4th day was observed with both the study compound and the standard Diclofenac. From the study it can be thus concluded that the ethanolic extract of C.dicoccum has Anti-inflammatory property and can be an effective alternative to NSAIDS like Diclofenac.

1. INTRODUCTION

Inflammation was characterized two thousand years ago by Celsus by the four Latin words: Rubor, calor, tumor and dolor[1]. It is a process in which the body immediately responses to an injurious stimulus evoked by a wide variety of noxious agents like infections, antibodies, or physical injuries. This response is a complex process which includes activation of white blood cells, release of immune system chemicals such as complements and cytokines, and production and release of inflammatory mediators and prostaglandins[2] (Cotran et al. 2001). In some situations and diseases, the inflammatory response may lead to severe adverse consequences without apparent benefit. Inflammatory responses occur in three distinct phases[3]:

1. Acute phase: transient local vasodilation and increased capillary permeability
2. Delayed, Subacute phase: infiltration of leukocytes and phagocytic cells
3. Chronic proliferative phase: tissue degeneration and fibrosis occurs.

Canthium dicoccum also known as nalla balusu (telugu), nallamandharam (tamil) in India belongs to the family Rubiaceae. The plant is found in deccan peninsula, maharastra southwards, and extending from bihar eastwards to assam and Meghalaya. It is an unarmed shrub, grows upto 3m tall. In India the bark is used for fever and is also applied as plasters, decoction of the root is used in diarrhea. Bark powder with sesame oil is used in rheumatic pains. Used in inflammation, during night boiled leaf extract is taken for 2 months[4]. The plant is reported to contain ursolic acid, quercetin, rutin (K Subramani 2010), 7-O-(6-O-benzoyl-β- -glucopyranosyl)-rutin, spathulenol (20.76 %), caryophyllene oxide (19.25 %), cedren-13-ol (10.62 %) and ledene oxide (5.24 %)[5].

The objective of the present research work is to study the anti-inflammatory activity of ethanolic extract of *Canthium dicoccum* (ECD).

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2. MATERIALS AND METHODS

2.1 Chemicals

Diclofenac Voveran-D (Novaritis), carrageenan (Sigma aldrich Banglore), formaldehyde, ethanol and diethyether (rankem ltd, New Delhi)

2.2 Collection of Plant Material and Preparation of Extract

The whole plant *Canthium dicoccum* used for the present study was collected from the Chittoor district of Andhra Pradesh. The plant was identified, confirmed and authenticated by botanist Dr. K. Madhava Chetty, Assistant professor, department of Botony, Sri Venkateswara University, Tirupati. The dried material was then pulverized separately into coarse powder by a mechanical grinder, and was then extracted with 99% ethanol in a soxhlet extracting apparatus.

2.3 Animals

Wistar albino rats (150-170g) were obtained from Sainath Agency Hyderabad. They were used for both acute toxicity and Anti-Inflammatory study. Rats were kept in cages and allowed for 7 days acclimatization (12 hr light and dark cycles) prior drug administration and supplied with standard rat pellet diet and water ad libitum. All the animals which were used in this study were taken care of ethical consideration, with an approval from the institutional ethical committee, SICRA preclinical lab, Andhra Pradesh (registration number: 769/2011/ CPCSEA).

2.4 Acute Toxicity Study

Acute toxicity study was conducted in female rats according to OECD guidelines (guideline 423, adopted on 17th December 2001). Ethanolic extract of plant material in single dose was administered orally using a stomach tube at graded doses upto 2000mg/kg and were checked for toxic signs and mortality. Animals were observed individually after dosing once during the first 30 minutes, 4hrs, 24 hours, and then daily thereafter, for a total of 14 days.

2.5. Anti-Inflammatory Activity

2.5.1 Carrageenan-induced paw oedema model

Acute inflammation was induced by carrageenan induced paw oedema model (Winter et al. 1962). The rats were starved overnight after which they were divided into four groups (A-D) of 6 animals each (n=6). All the rats in all the four groups (A-D) were given 0.1ml of 1% carrageenen suspension into the plantar surface of the right hind paw 1h after the drug treatment.

Group A (control): [untreated 10ml/kg saline p.o + 0.1ml of 1% carrageenan suspension]

Group B (standard): [diclofenac 25mg/kg p.o + 0.1 ml of 1% carrageenan suspension]

Group C (Test 1): [ECD 250mg/kg p.o + 0.1ml of 1% carrageenan suspension]

Group D (Test 2): [ECD 500mg/kg p.o + 0.1ml of 1% carrageenan suspension]

a) The linear circumference of the paw was measured after 0 h and 3 h of carrageenan injection using a loop of thread tied round the paw. The length of the thread was then measured on a ruler and rounded off to the nearest centimetre.
b) Paw volume was measured by digital plethysmometer.

The percentage inhibition was calculated according to the formula:

Percentage inhibition = $\{1 - [(C_1\text{-}C_0)_{test} / (C_1\text{-}C_0)_{control}]\} \times 100$

C_0 = Mean paw size at 0 h after carrageenan injection,

C_1 = Mean paw size at 3 h after carrageenan injection

2.5.2. Fresh egg white induced paw oedema model

In this model paw oedema was induced by subplantar injection of 0.05 ml of fresh undiluted egg white. Grouping, treatment method and recording of results were as that of carrageenan induced paw oedema model.

2.5.3. Formalin induced paw oedema model

Grouping of animals is as mentioned in method 1. In this model paw oedema in all the four groups (A-D) was induced by subplantar injection of 50µl of 2% formalin in normal saline on 1st and 3rd day. Drugs were administered for a period of 10 days[6]. Oedema was measured by plethysmometer before formalin induction (0 day) and at 3h on 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th day of formalin induction.

Percentage inhibition = $[1- V_t/ V_c] \times 100$

Where V_t = paw volume of test on particular day at 3h

V_c = paw volume of control on particular day at 3h

2.5.4. Cotton pellet granuloma model

Grouping of animals is as mentioned in method 1. Rats were anesthetized with diethylether and 20 mg of sterile cotton pellet was subcutaneously inserted in the groin region in all the four groups (A-D). Rats were treated for five consecutive days from the day of cotton pellet implantation. The animals were anesthetized with diethylether on the 6th day, and the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C for constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Weight of Exudate = weight of wet cotton pellet - weight of dry cotton pellet

Weight of Granuloma = weight of dry cotton pellet – weight of cotton pellet before implantation

2.6 Statistical Analysis

Results are expressed as mean ± SEM. Data was analysed using the one-way Analysis of Variance (ANOVA) followed by the Dunnette`s test using graphpad prism software. The *P* value < 0.05 when compared to group A was considered significant.

3. RESULTS AND DISCUSSIONS

3.1 Acute Toxicity Study

In acute toxicity study, ECD did not show any mortality and toxic signs upto the dose of 2000mg/kg b.w. At 2000mg/kg group diarrhea was observed in a few animals at 30min of drug administration but later till the 14th day no sign of diarrhea was seen and all the animals survived.

3.2 Anti-inflammatory Activity

Inflammation is a protective response which is intended to eliminate the initial cause of cell injury as well as the necrotic cells and tissues resulting from the original insult[7, 8]. The leukocytes attracted to the inflamed area are responsible for specifically eliminating the noxious agent[9]. Anti-inflammatory activity of ECD was studied in carrageenan induced paw oedema, fresh egg white induced paw oedema, formalin induced paw oedema and cotton pellet granuloma model in albino rats. Carrageenan and egg white induced paw oedema represents acute models of anti-inflammatory activity, where as formalin induced and cotton pellet granuloma represents chronic models of anti-inflammatory activity. In all the models the test ECD significantly inhibited inflammation at P<0.05 and the results were comparable to the standard Diclofenac.

3.2.1 Carrageenan induced paw oedema

In this model paw oedema was measured by loop of thread and digital plethysmometer. In both of these cases ECD at low and high doses significantly inhibited inflammation at P<0.05 when compared to group A. 250mg/kg ECD showed 43.86% inhibition when measured by loop of thread & 60.9% inhibition when measured using digital plethysmometer. Similarly 500mg/kg ECD showed 75.59% and 92.1%inhibition respectively. The standard Diclofenac 25mg/kg showed 65.94% using loop of thread and 86.7% using digital plethysmometer. The variations of %inhibition using loop of thread and digital plethymometer accounts to the differences in manual and instrumental errors. The ranking for anti-inflammatory activity can be presented in the order:

ECD 500mg/kg > Diclofenac > ECD 250mg/kg

3.2.2 Fresh egg white induced paw oedema:

250mg/kg ECD showed 79% inhibition when measured by loop of thread & 63.2% inhibition when measured using digital plethysmometer. Similarly 500mg/kg ECD showed 91% and 78.2% respectively. The standard Diclofenac 25mg/kg showed 92.5% using loop of thread and 89.5% using digital plethysmometer. The ranking for anti-inflammatory activity can be presented in the order:

Diclofenac > ECD 500mg/kg > ECD 250mg/kg

3.2.3 Formalin induced paw oedema

In this model paw oedema is measured using digital plethysmometer. ECD and Diclofenac showed an increase in %inhibition from day 4th. Formalin is induced on 1st and 3rd day. On these particular days ECD 500mg/kg has shown greater %inhibition with 4.5% and 18.4% respectively when compared to the standard Diclofenac at 3.1% and 13.6% respectively. On the 9th day diclofenac and ECD high dose, both have shown an equal of 36.8% inhibition, but where as on the 10th day Diclofenac has shown 56.7% inhibition and ECD 500mg/kg has shown 55.7% inhibition. The ranking for anti-inflammatory activity can be presented in the order:

On 1st and 3rd day: ECD 500mg/kg > Diclofenac > ECD 250mg/kg

On 10th day: Diclofenac > ECD 500mg/kg > ECD 250mg/kg

3.2.4 Cotton pellet induced granuloma

ECD effectively reduced cotton pellet induced granuloma, suggesting its activity in proliferative phase of inflammation.

Table- 1.1: Anti-inflammatory activity of ECD in carrageenan induced paw oedema (n=6)
(a) Measured by loop of thread

GROUPS	DOSE (mg/kg)	PAW OEDEMA (cm) [mean±SEM]		% INHIBITION
		0h	3h	
A	10 ml/kg saline	2.133±0.02	3.133±0.02	-
B	DIC 25 mg/kg	2.117±0.01	2.35±0.02***	65.94%
C	ECD 250 mg/kg	2.133±0.02	2.517±0.03***	43.86%
D	ECD 500 mg/kg	2.15±0.02	2.317±0.03***	75.59%

*Values are expressed as mean±SEM (Standard Error Mean); ***P < 0.001 indicating extremely significant when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum*

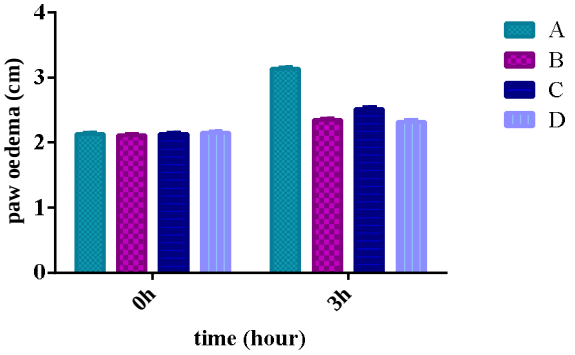


Figure 1.1: Anti- inflammatory activity of ECD in carrageenan induced paw oedema

Table 1.2: Anti-inflammatory activity of ECD in carrageenan induced paw oedema (n=6)
(b) Measured by digital plethysmometer

GROUPS	DOSE (mg/kg)	PAW OEDEMA (ml) [mean±SEM]		% INHIBITION
		0h	3h	
A	10 ml/kg saline	1.87±0.027	2.882±0.194	-
B	DIC 25 mg/kg	1.872±0.033	1.737±0.079**	86.7%
C	ECD 250 mg/kg	1.862±0.037	2.258±0.032*	60.9%
D	ECD 500 mg/kg	1.868±0.04	1.948±0.025**	92.1%

*Values are expressed as mean±SEM (Standard Error Mean); *P < 0.05 indicating significant, **P < 0.01 indicating very significant, when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum*

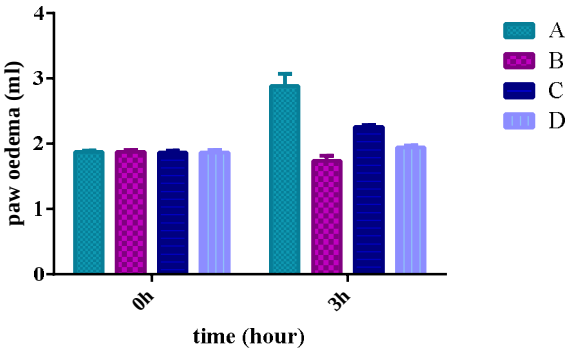


Figure 1.2: Anti- inflammatory activity of ECD in carrageenan induced paw oedema

Table- 2.1: Anti-inflammatory activity ECD in fresh egg white induced paw oedema (n=6)
(a) Measured by loop of thread

GROUPS	DOSE (mg/kg)	PAW OEDEMA (cm) [mean±SEM]		% INHIBITION
		0h	3h	
A	10 ml/kg saline	2.117±0.02	3.233±0.06	-
B	DIC 25 mg/kg	2.183±0.03	2.267±0.04***	92.5%
C	ECD 250 mg/kg	2.133±0.04	2.367±0.03***	79%
D	ECD 500 mg/kg	2.167±0.04	2.267±0.03***	91%

*Values are expressed as mean ± SEM (Standard Error Mean); ***P < 0.001 indicating extremely significant, when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum.*

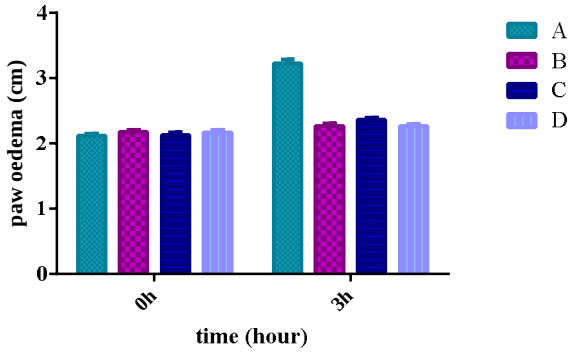


Figure 2.1: Anti-inflammatory activity of ECD in fresh egg white induced paw oedema

Table- 2.2: Anti-inflammatory activity ECD in fresh egg white induced paw oedema (n=6)
(a) Measured by digital plethysmometer

GROUPS	DOSE (mg/kg)	PAW OEDEMA (ml) [mean±SEM]		% INHIBITION
		0h	3h	
A	10 ml/kg saline	1.507±0.141	3.47±0.14	-
B	DIC 25 mg/kg	1.562±0.153	1.76±0.25***	89.9%
C	ECD 250 mg/kg	1.578±0.190	2.3±0.31*	63.2%
D	ECD 500 mg/kg	1.572±0.103	2±0.021***	78.2%

*Values are expressed as mean±SEM (Standard Error Mean); ***P < 0.001 indicating extremely significant, *P < 0.05 indicating significant, when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum*

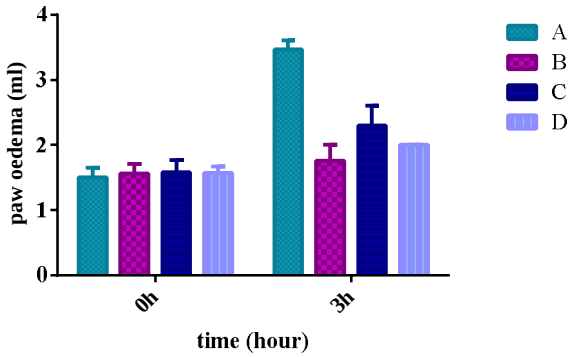


Figure 2.2: Anti-inflammatory activity of ECD in fresh egg white induced paw oedema

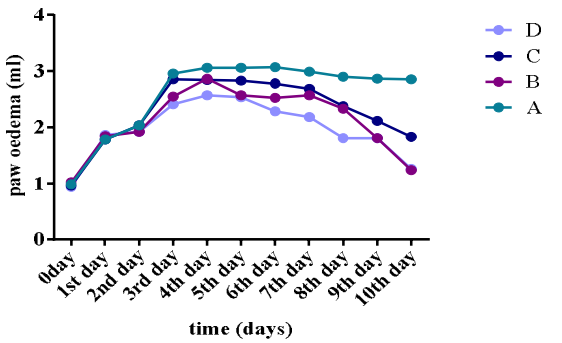


Figure 3.3: Anti-inflammatory activity of ECD in formalin induced paw oedema model

Table 3: Anti-inflammatory activity of ECD in formalin induced paw oedema model (n=6)

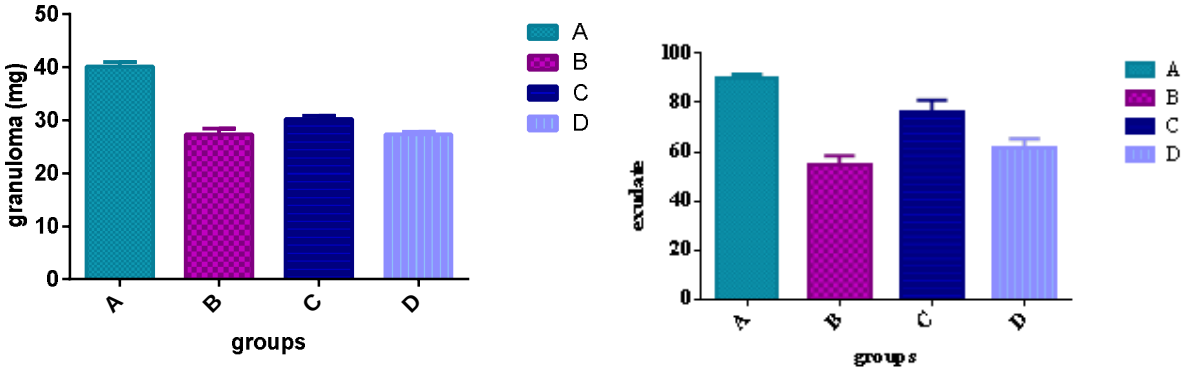
DAYS	PAW OEDEMA (ml) [Mean ± SEM]				% INHIBITION		
	Group A	Group B	Group C	Group D	B	C	D
0	0.98±0.117	1.018±0.115	0.96±0.091	0.938±0.065	-	-	-
1st	1.783±0.065	1.84±0.049	1.788±0.047	1.865±0.024	3.1%	-	4.5%
2nd	2.035±0.094	1.923±0.076	2.035±0.025*	1.917±0.022**	5.5%	-	4.1%
3rd	2.957±0.05	2.553±0.143	2.857±0.048**	2.412±0.109*	13.6%	3.3%	18.4%
4th	3.057±0.092	2.863±0.106*	2.843±0.048	2.57±0.066**	6.3%	7%	15.9%
5th	3.062±0.202	2.57±0.062	2.83±0.039	2.538±0.102	16%	7.5%	17.1%
6th	3.068±0.192	2.527±0.055	2.783±0.039	2.285±0.121*	17.6%	9.2%	25.5%
7th	2.988±0.1	2.568±0.056*	2.685±0.053	2.182±0.103***	24%	10.1%	26.9%
8th	2.897±0.058	2.03±0.077**	2.377±0.131*	1.813±0.047***	30%	17.9%	37.4%
9th	2.87±0.025	1.81±0.07***	2.12±0.146**	1.813±0.048***	36.8%	26.1%	36.8%
10th	2.857±0.028	1.237±0.113***	1.835±0.11***	1.263±0.195***	56.7%	35.7%	55.7%

*Values are expressed as mean±SEM (Standard Error Mean); *P < 0.05 indicating significant, **P < 0.01 indicating very significant, ***P < 0.001 indicating extremely significant when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum*

Table 4: Anti-inflammatory activity of ECD in cotton granuloma model (n=6)

GROUPS	DOSE (mg/kg)	WEIGHT OF GRANULOMA (mg) [mean±SEM]	% INHIBITION	WEIGHT OF EXUDATE(mg) [mean±SEM]	% INHIBITION
A	10ml/kg saline	40.17±0.792	-	90±1.612	-
B	25mg/kg	27.33±1.116***	31.9%	54.83±3.619***	39%
C	250mg/kg	30.3±1.62**	24.6%	76.3±4.702**	15.2%
D	500mg/kg	28.12±0.49***	30%	59.1±4.055***	34.3%

*Values are expressed as mean±SEM (Standard Error Mean); **P < 0.01 indicating significant, ***P < 0.001 indicating extremely significant, when compared to group A; n= number of rats; DIC: diclofenac; ECD: ethanolic extract of Canthium dicoccum*



(a) Graph for granuloma (b) Graph for exudate

Figure 4: Anti-inflammatory activity of ECD in cotton granuloma model

4. CONCLUSION

From the present study it is clear that the plant extract is comparable to the standard Diclofenac. And the present study is providing a scientific proof for its traditional claim of its use in inflammation and rheumatoid pains. It can be thus concluded that the ethanolic extract of *Canthium dicoccum* has Anti-inflammatory property and can be an effective alternative to NSAIDS like diclofenac.

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REFERENCES

1. Gerhard VH, Drug Discovery and Evaluation. Pharmacological Assays, 2nd edition, Springer- Verlag Berlin, New York, 2002, 725.
2. Johnny OO, Olubukola TA, et al "Studies on the Anti-inflammatory and Analgesic properties of *Jatropha curcas* leaf extract" *Acta Vet. Brno*, 2011, 80: 259-262.
3. Goodman and Gilman's the pharmacological basis of therapeutics, 11th edition, McGraw-Hill, the United States of America, 2006, 671.
4. Karunyal JS and Andrews B "Traditional medicinal plant wealth of Pachalur and Periyur hamlets Dindigul district, Tamilnadu" *Indian J Traditional Knowledge*, 2010, 9(2): 264-270.
5. Rajarajeswari N, Ramalakshmi.S, et al "GC-MS Analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum* (Gaertn.) Teijsm&Binn" *J.Chem.Pharm.Res.*, 2011, 3(3): 792-798.

6. Patel PD, Patel NJ, et al "In-Vivo evaluation of *Pleurotus sajorcaju* mycelium extract for Anti-inflammatory activity" *Pharmacologyonline*, 2011, 2: 784-789.
7. Asim KG, Manasi B, et al "Anti-inflammatory activity of root of *Alpinia galanga* Wild" *Chron Young Sci*, 2011, 2(3): 139-143.
8. Kumar V, Abbas KA, Fausto N, Aster CJ Robins and Cotran: Pathologic basis of disease, 8th edition, Sauders Elsevier, Philadelphia, 2010, 43.
9. Riede UN, Werner M, Color atlas of Pathology; Pathologic principles and associated diseases sequela, Georg Thieme Verlag, Germany, 2004, 194.