Annexure -VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st /2nd /3rd/Final Final
2. UGC Reference No.F. 42-536 / 2013 (SR) dt. 20.03.2013
3. Period of report: from 01.04.2013 to 31.03.2014
4. Title of research project Anti-arthritic activity ..... study.
5. (a) Name of the Principal Investigator Antony Gomes
   (b) Deptt. Physiology
   (c) University/College where work has progressed University of Calcutta
6. Effective date of starting of the project 02.05.2013
7. Grant approved and expenditure incurred during the period of the report:
   a. Total amount approved Rs. 11,71,800/– (Total amount released = 6,37,800/-)
   b. Total expenditure Rs. 6,24,761/–

c. Report of the work done: (Please attach a separate sheet)
   i. Brief objective of the project Appendix 1
   ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication Appendix 2
   iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons No. Because project was terminated at an early date
   iv. Please indicate the difficulties, if any, experienced in implementing the project No

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet. N.A.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission. Appendix 3

vii. Any other information which would help in evaluation of work done on theproject. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any Appendix 4

24/4/18

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

UGC Sponsored research project "Anti arthritic potential of krait venom: An experimental study (Ref. No. F. No. 42 - 536/2013 (SR) dt. 20.3.2013)

Principal Investigator

24/4/18

SIGNATURE OF THE CO-INVESTIGATOR

Dr. Subir Chandra Dasgupta
Professor & Head (W.B.S.E.S.)
Department of Zoology
Maulana Azad College
Govt. of West Bengal

24/4/18

DY.REGISTRAR/PRINCIPAL

(Seal)

Deputy Registrar
University of Calcutta

Forwarded
24/4/18

SECRETARY
Faculty Councils for P.G. Studies in Sc. Tech. & Engg Ag. & Vet. Sc.
Calcutta University

Forwarded
Somnath Gangopadhyay
24/4/2018

PROFESSOR & HEAD
Dept. of Physiology
University of Calcutta
92, APC Road, Kolkata-9

Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project: Anti-arthritic activity of ---- experimental study
2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: Antony Gomes, Dept. of Physiology, University of Calcutta
3. NAME AND ADDRESS OF THE INSTITUTION: Dept. of Physiology, Univ. of Calcutta, 92 APC Road, Kolkata-9
4. UGC APPROVAL LETTER NO. AND DATE: F.42-536/2013 (SR) Dt. 20/03/2013
5. DATE OF IMPLEMENTATION: 02.05.2013
6. TENURE OF THE PROJECT: 1 year (Upto 30.03.2014)
7. TOTAL GRANT ALLOCATED: ~~6,37,800/-~~ 11,71,800/-
8. TOTAL GRANT RECEIVED: 6,37,800/-
9. FINAL EXPENDITURE: 6,24,761/-
10. TITLE OF THE PROJECT: Anti-arthritic activity of ---- experimental study
11. OBJECTIVES OF THE PROJECT: ~~Appendix~~ 1
12. WHETHER OBJECTIVES WERE ACHIEVED: Yes, Appendix 2
    (GIVE DETAILS)
13. ACHIEVEMENTS FROM THE PROJECT: Appendix 4
14. SUMMARY OF THE FINDINGS: Appendix 3
    (IN 500 WORDS)
15. CONTRIBUTION TO THE SOCIETY: Appendix 5
    (GIVE DETAILS)
16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT: Yes, thesis submitted
17. NO. OF PUBLICATIONS OUT OF THE PROJECT: 3
    (PLEASE ATTACH)

24/4/18

(PRINCIPAL INVESTIGATOR)
Principal Investigator
UGC Sponsored research project "Anti arthritic potential of krait venom: An experimental study"
(Ref. No. F. No. 42 - 536/2013 (SR) dt. 20.3.2013)

24/4/18

(CO-INVESTIGATOR)
Dr. Indra Chandra Dasgupta
Professor & Head (W.B.S.E.S.)
Department of Zoology
Maulana Azad College
Govt. of West Bengal

24/4/18

(DY.REGISTRAR/PRINCIPAL)

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University of Calcutta

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24/4/2018

SECRETARY
Faculty Council for P.G. Studies in Sc. Tech. & Eng. Ag. & Vet. Sc.
Calcutta University

Forwarded
Soumitra Gangopadhyay
24/4/2018
Professor
Dept. of Physiology
University of Calcutta
92, APC Road, Kolkata-9

BRIEF OBJECTIVE OF THE PROJECT

Preface:

Arthritis is a common pathophysiological condition creating global problem for the aged people. This joint disorder is associated with pain, inflammation and stiffness. Two major types of arthritis that demands concern are osteoarthritis (OA) and rheumatoid arthritis (RA). OA results due to erosion of cartilage while RA is an autoimmune disease. Currently available treatments for arthritis include non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), glucocorticoids and biological response modifiers. All these treatments have their own limitations like cost factor, side effects. Therefore search for alternative therapy using natural products are ventured throughout the world. Snake venoms were identified as an important agent among the natural products to treat inflammatory disorders. Gomes *et al.*, 2010 reported anti-arthritic activity of Indian monocellate cobra (*Naja kaouthia*) venom on FCA-induced experimental arthritis. In traditional medicinal system of China, snake bile obtained from *Bungarus fasciatus* was used to treat rheumatic pain (Dharmananda, 1997). BF-CT1 isolated from *Bungarus fasciatus* venom showed anti-cancer activities by inducing apoptosis of cancer cells and arresting cell cycle progression at the check points (Bhattacharya *et al.*, 2013). But there was no report on anti-arthritic activity of Banded krait (*Bungarus fasciatus)* venom. The present research work is an effort to investigate the anti-arthritic activity of *Bungarus fasciatus* venom in experimental animals.

Objectives of the project:

1. Anti-arthritic property of *Bungarus fasciatus* venom (BFV).
2. Purification, characterization and evaluation of anti-arthritic activities of the *Bungarus fasciatus* venom fraction in experimental animals.
3. Analysis of the toxicity profile of the venom fraction.
4. Analysis of the molecular mechanism of the venom fraction against arthritis.

Appendix 2

WORK DONE AND RESULTS ACHIEVED

METHODS

A. Chemicals and reagents

All the chemicals and reagents used were of analytical grade unless otherwise stated.

B. Experimental animals

Wistar male albino rats (120±10 g) were used for the experiments and purchased from the approved animal breeders. They were housed at controlled temperature (25±2^{0}C), with light conditions (12 h light and 12 h dark cycle) and relative humidity (65±5%). The animals were given standard pellet food and water *ad libitum*. The animal ethical clearance was obtained before conducting any expt. AEC no. IAEC-III/ Proposal/ AG-02/2012 dated 07.06.2012.

C. Collection of snake venom

Lyophilized *Bungarus fasciatus* venom (BFV) was purchased from Calcutta Snake Park, Kolkata, India. Venom concentration was expressed in terms of dry weight/protein equivalent (Lowry *et al.*, 1951)

D. Anti-arthritic activity of BFV

a) Development of experimental rheumatoid arthritis

Rheumatoid arthritis (RA) was developed by injecting 0.05 ml emulsion of Freund's complete adjuvant (FCA) in olive oil (1:1 v/v) into the subplantar region of right hind paw of the rats. Same volume of saline will be injected in the left hind paw of the rats (Newbould, 1963).

b) Treatment protocol

Animals were divided into four groups (n=6), Gr.1: Sham control, Gr.2: RA control, Gr.3: Standard drug (Indomethacin) treated (0.25 mg.kg $^{-1}$ × 5 days alternatively, p.o.), Gr.4: BFV treated (30 µg.100g $^{-1}$ × 14 days, i.p.). At day 14, urine was collected for measurement of urinary

parameters. At day 16, blood was collected, serum was isolated for analysis of biochemical and inflammatory parameters.

c) Anti-arthritic activity study

1. **Physical parameters :** In order to determine degree of swelling, paw and ankle diameters were measured using electronic digital calipers (Mitutoyo, Japan) in an interval of 5 days from day 0 after FCA induction.

2. Biochemical analysis of urinary and serum parameters : Urinary glucosamine (Elson *et al.* 1933) and Urinary Hydroxyproline (Neuman and Logan, 1960) were measured spectrophotometrically. Serum ACP and ALP levels were measured by the method of Karnovsky *et al,* 1971.

3. **Measurement of serum pro-inflammatory markers :** Serum pro-inflammatory cytokines TNF-α, IL-1β, IL-17, Cathepsin-k and MMP-1 levels were measured by using ELISA reader (BioTek, ELx800).

E. Protein purification & characterization of active fraction from BFV

a) **Purification of BFV-active fraction :** Lyophilized BFV (60 mg) was dissolved and heat treated at 70-80°C for 30 min to precipitate out heat-labile proteins, followed by centrifugation at 1200 rpm for 15 min. Supernatant was collected and applied to CM-cellulose column (100×20 mm) and equilibrated with 0.02 M phosphate buffer. Maintaining a flow rate of 25 $ml.h^{-1}$, fractions were collected with increasing concentrations of NaCl (0.02-1.0 M) and subjected to protein estimation (Lowry *et al.*, 1951).

b) Characterization of BFV-active fraction

SDS-PAGE : Homogeneity of the active fraction was checked by SDS-PAGE on 15% acrylamide gel electrophoresis (Laemmli *et al.*, 1970).

HPLC : The active fraction was further purified by RP-HPLC using Protein pak 60 column (Shimadzu HPLC system, Japan, Model LC 20AD).

F. Anti-rheumatic activity of BFV-active fraction (BFV-F47)

a) **Development of experimental rheumatoid arthritis :** Rheumatoid arthritis (RA) was developed by injecting 0.05 ml emulsion of Freund's complete adjuvant (FCA) in olive oil (1:1 v/v) into the subplantar region of right hind paw of male the rats. Same volume of saline will be injected in the left hind paw of the rats (Newbould, 1963).

b) **Treatment protocol :** Animals were divided into four groups (n=6). Gr.1: Sham control, Gr.2: RA control, Gr.3: Standard drug (Indomethacin) treated (0.25 mg. kg^{-1} × 5 days alternatively, p.o.), Gr.4: BFV-active fraction (BFV-F47) treated (30 µg.100 g^{-1} × 14 days, i.p.). At day 14, urine was collected for measurement of urinary parameters. At day 16, blood was collected, serum was isolated for analysis of biochemical and inflammatory parameters.

1. **Anti-arthritic activity study**
2. **Physical parameters:** Paw and ankle diameters were measured on day 0, 2, 5 and 10 by using electronic digital calipers (Mitutoyo, Japan).

2. Biochemical analysis of urinary and serum parameters : Urinary glucosamine (Elson *et al.* 1933) and Urinary Hydroxyproline (Neuman and Logan, 1960) were measured. Serum ACP and ALP levels were measured according to Karnovsky *et al.* 1971. Serum calcium, creatinine and gamma glutamyl transpeptidase (γ-GT) levels were measured by using biochemical kits (Merck, India).

3. **Measurement of serum pro-inflammatory cytokines :** Serum interleukins TNF-α, IL-1β, CINC-1, IL-17, MMP-1 and VEGF levels were measured by using ELISA kits.
4. **Statistical analysis :** Data expressed in terms of mean ± SEM (n=6). One way ANOVA was used for determination of significant differences between groups. $p<0.05$ was considered to be statistically significant.

RESULTS

A. Anti-arthritic activity of BFV

a. **Effect of BFV on physical parameters :** Paw and ankle diameters were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV (30µg.

$100g^{-1}$) treatment significantly reduced paw and ankle diameters; by 54.94% and 53.74% respectively whereas indomethacin treated group showed 62.28% and 55.44% restoration of paw and ankle diameters respectively as compared to RA control group 2 rats.(Fig1 & 2)

b.

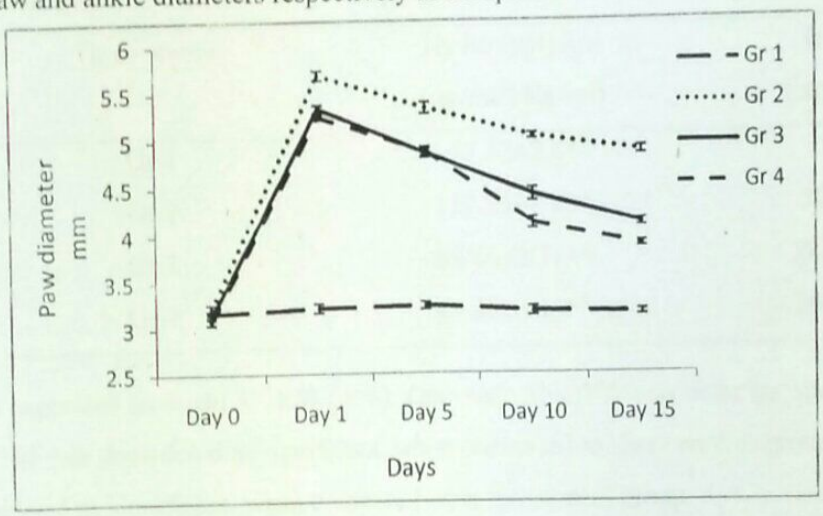


Fig 1: Effect of BFV on paw diameter of FCA induced rats

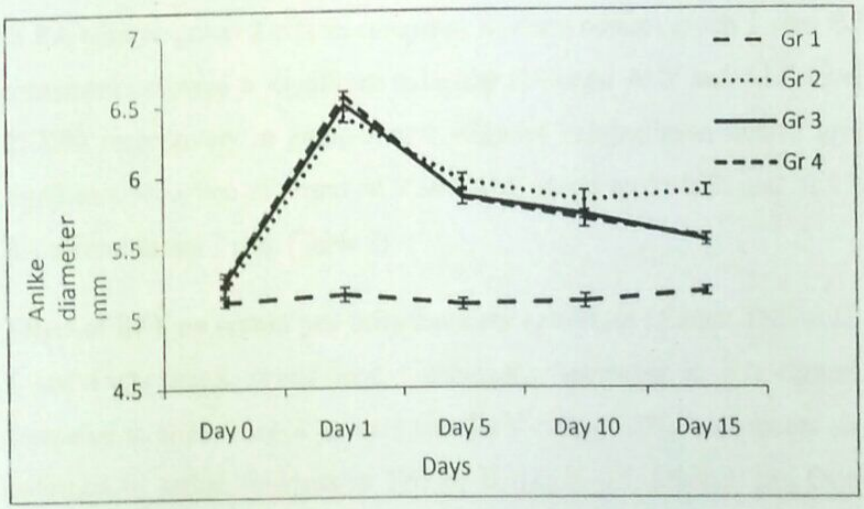


Fig 2: Effect of BFV on ankle diameter of FCA induced rats

c. **Effect of BFV on urinary parameters** : Urinary hydroxyproline and glucosamine levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV (30µg. $100g^{-1}$) treatment showed significant reduction in urinary hydroxyproline and glucosamine levels by 46.48% and 47.41% respectively in group 3 rats; whereas

Indomethacin treated group 4 rats showed significant reduction of the above parameters by 58.71% and 51.94% respectively as compared to RA control group 2 rats.(Table 1)

Table 1: Effect of BFV on urinary parameters of FCA-induced arthritic rats

Gr of animal	Hydroxyproline (µg/ml/18hr/rat)	Glucosamine (µg/ml/18hr/rat)
Gr.1	52.32±2.53	101.02±2.13
Gr.2	152.25±2.87*	504.98±5.96*
Gr.3	62.86±2.51**	242.68±4.62**
Gr.4	81.49±3.13**	265.55±4.51**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *$p<0.05$ was considered as significant, when compared to sham control group and **$p< 0.05$ was considered as significant, when compared with RA control group.

d. **Effect of BFV on serum parameters :** Serum ACP, ALP levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV (30µg. $100g^{-1}$) treatement showed a significant reduction in serum ACP and ALP levels by 48.38% and 27.12% respectively in group 3 rats; whereas Indomethacin treated group 4 rats showed significant reduction of serum ACP and ALP levels by 55.08% and 31.17% as compared to RA control group 2 rats. (Table 2)

e. **Effect of BFV on serum pro-inflammatory cytokines :** Serum TNF-α, IL-1β, IL-17, MMP-1 and Cathepsin-K levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV (30µg. $100g^{-1}$) treatment showed a significant reduction in serum interleukins TNF-α, IL-1β, IL-17, MMP-1 and Cathepsin-K levels by 47.04%, 54.63%, 48.54%, 41.48% and 43.55% respectively when compared to RA control group 2 rats. Whereas Indomethacin treated group 4 rats showed significant reduction of the above parameters by 49.84%, 59.92%, 64.57%, 37.09% and 54.95% respectively as compared to RA control group 2 rats. (Table 3).

Table 2: Effect of BFV on serum parameters

ANIMAL GROUP	ACP (µmole of PNPP/min)	ALP (µmole of PNPP/min)
Gr.1.	12.29±1.13	60.26±2.63
Gr.2.	38.98±2.75*	194.78±4.41*
Gr.3.	17.51±1.51**	134.07±2.75**
Gr.4.	20.12±2.05**	141.96±3.13**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *p<0.05 was considered as significant, when compared to sham control group and **p< 0.05 was considered as significant, when compared with RA control group.

Table 3: Effect of BFV on serum pro-inflammatory cytokines

ANIMAL GROUP	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-17 (pg/ml)	MMP-1 (pg/ml)	Cathepsin-K (pg/ml)
Gr.1	27.57±0.73	11.16±0.61	12.11±0.78	266.27±6.39	18.97±1.07
Gr.2	61.48±1.08*	32.71±0.99*	37.37±2.19*	667.27±10.23*	65.44±2.71*
Gr.3	30.84±1.29**	13.11±0.62**	13.24±1.02**	419.73±9.44**	29.48±1.91**
Gr.4	32.56±1.31**	14.84±0.69**	19.23±1.22**	390.51±7.33**	36.94±1.52**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *p<0.05 was considered as significant, when compared to sham control group and **p< 0.05 was considered as significant, when compared with RA control group.

B. Purification and characterization of BFV active fraction.

a. Ion Exchange Chromatography

Stepwise elution of BFV through increasing gradient of NaCl (0.02-1 M in 0.02 M phosphate buffer pH 7.2, at room temperature) resulted in 4 protein peaks. All the picks were subjected to anti-arthritic study. It was found that the fourth peak, eluted by 0.5 M NaCl, showed anti-arthritic activity, termed as BFV-F47. (Fig 3)

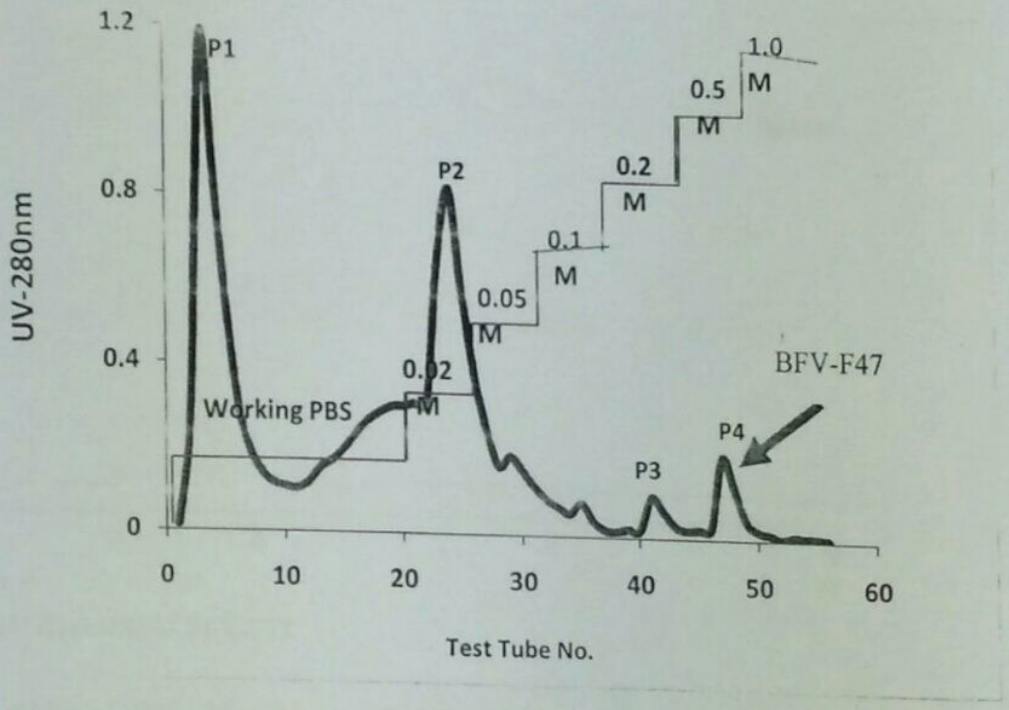
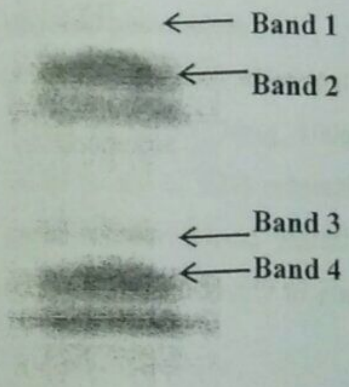


Fig 3: Ion- Exchange chromatography in CM-cellulose of BFV

b. SDS-PAGE



15% gel exhibited that the active fraction contains 3-4 distinct bands. (Fig 4)

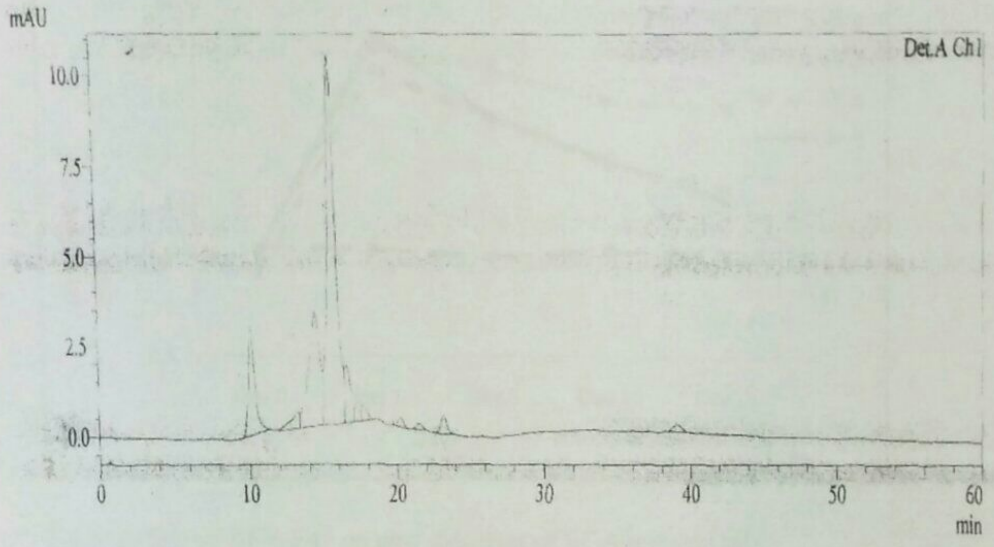


Fig 4: SDS-PAGE profile of BFV-F47

Fig 5: HPLC profile of BFV-F47 on Protein pak C18 column

c. HPLC

RP-HPLC using Protein pak 60 column exhibited 4 peaks with retention time of 10.147, 14.460, 16.132 and 23.562 minutes respectively. (Fig 5)

C. Anti-arthritic activity of BFV-active fraction (BFV-F47)

f. Effect of BFV-F47 on physical parameters

Paw and ankle diameters were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV-F47 (30µg. $100g^{-1}$) treatment significantly reduced paw and ankle swelling by 36.80 % and 65.02% respectively in group 3 rats; Whereas whereas Indomethacin treated group 4 rats showed significant reduction of the paw and ankle diameters by 48.11% and 67.28% respectively as compared to RA control group 2 rats. (Fig 6 & 7).

Intensity

Time

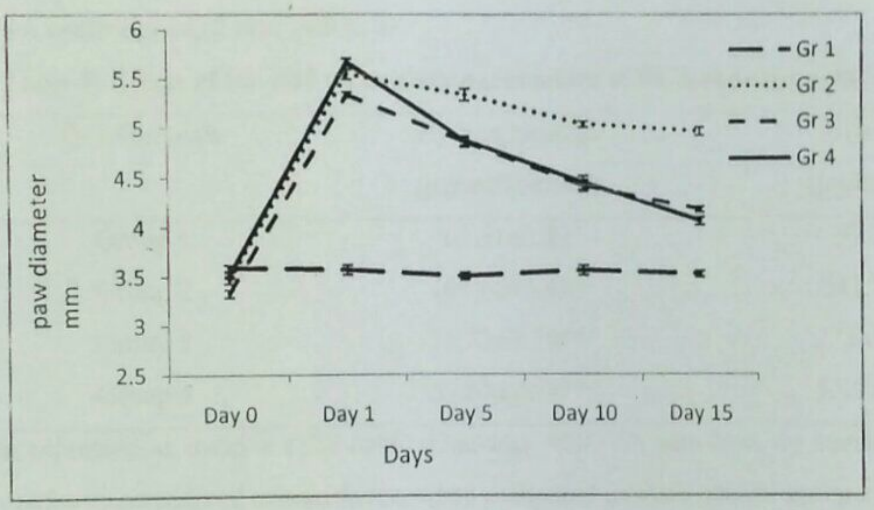


Fig 6: Effect of BFV-F47 on paw diameter of FCA induced rats

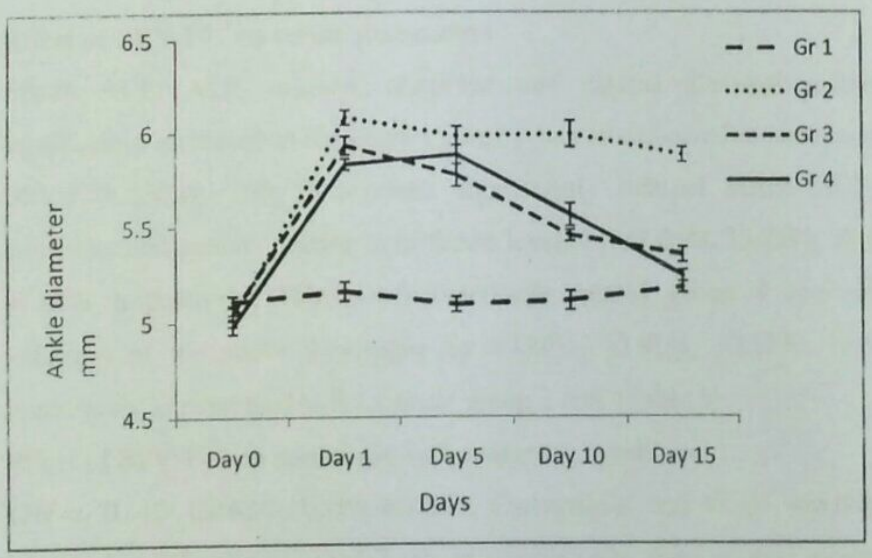


Fig 7: Effect of BFV-F47 on ankle diameter of FCA induced rats

g. Effect of BFV-F47 on urinary parameters

Urinary hydroxyproline and glucosamine levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV-F47 (30µg. $100g^{-1}$) treatment significantly reduced urinary hydroxyproline and glucosamine levels by 49.22% and 48.55% respectively; whereas indomethacin treated group showed reduction in urinary

hydroxyproline and glucosamine levels by 53.95% and 59.83% respectively in comparison to RA control group 2 rats. (Table 4)

Table 4: Effect of BF-F47 on urinary parameters of FCA-induced arthritic rats

Gr of animals	Hydroxyproline (µg/ml/18hr/rat)	Glucosamine (µg/ml/18hr/rat)
Group 1	65.51±2.21	97.27±2.74
Group 2	164.66±5.48*	681.35±4.65*
Group 3	75.83±3.39**	273.69±3.96**
Group 4	83.62±2.53**	350.55±4.71**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *$p<0.05$ was considered as significant, when compared to sham control group and **$p< 0.05$ was considered as significant, when compared with RA control group.

h. Effect of BFV-F47 on serum parameters

Serum ACP, ALP, calcium, creatinine and gamma glutamyltransferase levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV-F47 (30µg. $100g^{-1}$) treatment significantly reduced serum ACP, ALP, calcium, creatinine and gamma glutamyltransferase levels by 57.05%, 33.29%, 28.61%, 30.77% and 47.84% respectively; Whereas Indomethacin treated group 4 rats showed significant reduction of the above parameters by 49.80%, 33.91%, 30.57%, 29.67% and 36.31% respectively as compared to RA control group 2 rats. (Table 5)

i. Effect of BFV-F47 on serum pro-inflammatory cytokines

TNF-α, IL-1β, CINC-1, IL-17, MMP-1, Cathepsin-K and VEGF levels were significantly increased in RA control group 2 rats as compared to sham control group 1 rats. BFV-F47 (30µg. $100g^{-1}$) treatment significantly reduced serum interleukins TNF-α, IL-1β, CINC-1, IL-17, MMP-1, Cathepsin-K and VEGF levels by 47.26%, 39.47%, 56.25%, 41.91%, 43.87%, 44.95% and 48.93% respectively. Whereas Indomethacin treated group 4 rats showed significant reduction of the above parameters by 48.66%, 28.89%, 52.73%, 36.53%, 41.99%, 51.99% and 45.36% respectively as compared to RA control group 2 rats. (Table 6 & 7)

Table 5: Effect of BFV-F47 on serum biochemical parameters

Gr of animals	ACP (µmole of PNPP/min)	ALP (µmole of PNPP/min)	Calcium (mg/dl)	Creatinine (mg/dl)	γ-GT (U/L)
Gr.1	10.47±0.84	69.06±1.87	8.7±0.13	0.55±0.02	8.56±0.43
Gr.2	65.12±2.52*	151.19±2.99*	11.22±0.42*	0.91±0.03*	31.23±0.87*
Gr.3	32.69±2.27**	99.92±2.33**	7.79±0.21**	0.64±0.02**	19.89±0.55**
Gr.4	29.97±1.57**	100.85±2.44**	8.01±0.11**	0.63±0.01**	16.29±0.59**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *p<0.05 was considered as significant, when compared to sham control group and **p< 0.05 was considered as significant, when compared with RA control group.

Table 6: Effect of BF-F47 on serum proinflammatory cytokines

Gr of animals	Cathepsin-K (pg/ml)	VEGF (pg/ml)	IL-17 (pg/ml)
Gr.1	55.59±1.83	68.38±1.87	14.93±0.99
Gr.2	147.32±2.98*	249.66±3.14*	33.64±2.75*
Gr.3	70.72±2.29**	136.41±3.11**	21.35±1.10**
Gr.4	81.10±1.77**	127.50±2.47**	19.54±1.39**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *p<0.05 was considered as significant, when compared to sham control group and **p< 0.05 was considered as significant, when compared with RA control group.

Table 7: Effect of BFV-F47 on serum pro-inflammatory cytokines

Gr of animals	TNF-α (pg/ml)	IL-1β (pg/ml)	CINC-1 (pg/ml)	MMP-1 (pg/ml)
Gr.1.	10.96±0.67	25.19±0.85	162.21±2.03	334.12±5.82
Gr.2.	34.83±1.73*	41.12±1.34*	384.89±4.43*	685.26±7.08*
Gr.3.	17.88±1.06**	29.24±1.54**	181.94±2.12**	397.51±5.35**
Gr.4.	18.37±0.74**	24.89±0.77**	168.39±3.46**	384.67±7.13**

Data expressed as mean ± SEM (n=6). One-way ANOVA was done for statistical analysis and *$p<0.05$ was considered as significant, when compared to sham control group and **$p< 0.05$ was considered as significant, when compared with RA control group.

Paper published :

- **Susmita Ghosh**, Partha Pratim Saha, Subir C Dasgupta, Antony Gomes. Antinociceptive, anti-inflammatory and anti-arthritic activities of *Bungarus fasciatus* venom in experimental animal models. **Indian Journal of Experimental Biology, 54(9): 569-576** (2016). **Impact Factor: 1.165**
- Antony Gomes, **Susmita Ghosh**, Sourav Ghosh, Kalyani Saha, Partha P Saha, Subir C Dasgupta, Aparna Gomes. Anti-osteoarthritic activity of Bungarus fasciatus venom fraction (BF-F47) involving molecular markers in the rats. **Toxicon, 118, 43-46 (2016)** Impact factor: 2.78
- **Susmita Ghosh**, Kalyani Saha, Subir Chandra Dasgupta and Antony Gomes. In vitro and In vivo Anti-Arthritic and Anti-Inflammatory Activity of *Bungarus Fasciatus* Venom. **Journal of Toxins, 2(1), 5-10 (2015).**

Appendix 3

Summary of the work done for the period (01.04.2013-31.03.2014)

- Lyophilized *Bungarus fasciatus* venom was checked for its anti-rheumatic activity. BFV (30µg. $100g^{-1}$) treatment significantly restored urinary hydroxyproline and glucosamine levels, serum markers ACP and ALP levels as well as serum cytokines TNF-α, IL-1β, IL-17, MMP-1 and Cathepsin-K levels as compared to arthritic control rats.
- An active protein fraction (BFV-47) was purified from BFV by IEC using CM cellulose yielded 4 peaks with increasing gradient of NaCl. 4^{th} peak, i.e. fraction no. 47 showed anti-arthritic activity.
- 15% SDS-PAGE gel electrophoresis of BFV-F47 showed 4 bands.
- RP-HPLC of BFV-F47 using Protein pak 60 column exhibited 4 peaks.
- BFV-F47 (30µg. $100g^{-1}$) treatment significantly restored urinary hydroxyproline and glucosamine levels, serum markers ACP, ALP, Calcium, Creatinine, γ-GT levels as well as serum cytokines TNF-α, IL-1β, CINC-1, IL-17, MMP-1, Cathepsin-K and VEGF levels as compared to arthritic control rats.
- Further studies on BFV-F47 is warrented.

Plan of work 2^{nd} year (01.04.2014 to 31.03.2015)

1. Further purification of the active compound (BFV-F47)
2. Characterization of the active compound (BFV-F47)
   a. CD-Spectrometry
   b. UV-Vis
   c. Spectrofluorometry
   d. MALDI
3. Anti-osteoarthritic activity of BFV-F47
4. Acute and chronic toxicity studies
5. ***** 2^{nd} yr work (as mentioned above) has been completed without financial assistance from UGC.**

Appendix 4

ACHIEVEMENTS

- **Papers presented**

1. Paper presented (poster) "Indian snake venom as anti-arthritic agent" in UGC Sponsored National Conference on Biodiversity: Interrelationship between Flora, Fauna and Human", 29-30th September, 2013, organized by Mrinalini Datta Mahavidyapith, Birati. Abstract ID- PP10
2. Paper presented (poster) "Anti-arthritic activity of *Bungarus fasciatus* venom fraction" in 3rd Annual Conference of the Toxinological society of India and 1st International Conference on "Biology of Natural Toxins", 19-21 December 2013, BITS Pilani K K Birla Goa Campus, Goa, India. Abstract ID- PP28
3. Paper presented (poster) "Indian snake venom and arthritis: recent developments" in UGC Sponsored National Seminar on "Prospect of Biotechnology in Rural Bengal", 17-18 January, 2014, Uluberia College, Uluberia, Howrah-711315.
4. Paper presented (poster) "Anti-osteoarthritic activity of Banded krait venom fraction (F47) in experimental animals" in 1st annual conference of CBS- India and International Conference on Chemical Biology "Disease mechanisms and therapeutics (ICCB-2014)", 6-8 February, 2014, CSIR-IICT, Hyderabad. Abstract ID- PP66

- **Paper published :**
- **Susmita Ghosh**, Partha Pratim Saha, Subir C Dasgupta, Antony Gomes. Antinociceptive, anti-inflammatory and anti-arthritic activities of *Bungarus fasciatus* venom in experimental animal models. **Indian Journal of Experimental Biology, 54(9): 569-576 (2016). Impact Factor: 1.165**
- Antony Gomes, **Susmita Ghosh**, Sourav Ghosh, Kalyani Saha, Partha P Saha, Subir C Dasgupta, Aparna Gomes. Anti-osteoarthritic activity of Bungarus fasciatus venom fraction (BF-F47) involving molecular markers in the rats. **Toxicon, 118, 43-46 (2016) Impact factor: 2.78**
- **Susmita Ghosh,** Kalyani Saha, Subir Chandra Dasgupta and Antony Gomes. In vitro and In vivo Anti-Arthritic and Anti-Inflammatory Activity of *Bungarus Fasciatus* Venom. **Journal of Toxins, 2(1), 5-10 (2015).**

- **Manpower trained**

  Susmita Ghosh (Project Fellow for this UGC project) registered for Ph.D. under Dept. of Zoology, University of Calcutta, vide. Ph.D. Reg. No. 3077 Ph.D. (Sc.) Proceed/ 2014 dated 07.05.2014.

  Susmita Ghosh, submitted her Ph.D thesis in 15 Feb 2018 thesis entitled "Banded Krait venom & venom fraction induced anti arthritic activity in experimental models" under Prof A Gomes, through Dept of Zoology, Calcutta university (her Ph.D viva-voce examination is pending).

  I would like to request UGC to release her arrear fellowship as per our claim in the audit report.

Annexure – XI

Final Report Assessment / Evaluation Certificate
(Two Members Expert Committee Not Belonging to the Institute of Principal Investigator)
(to be submitted with the final report)

It is certified that the final report of Major Research Project entitled "Anti arthritic potential of krait venom: An experimental study" by Dr./Prof. Antony Gomes Dept. of Physiology has been assessed by the committee consisting the following members for final submission of the report to the UGC, New Delhi under the scheme of Major Research Project.

Comments/Suggestions of the Expert Committee:-

The report submitted by Principal Investigator is satisfactory.

Name & Signatures of Experts with Date:-

Name of Expert	University/College name	Signature with Date
1. Prof (Dr) S K Tripathi	Dept of Clinical & Exp Pharmacology, School of Tropical medicine, Kolkata 700 072	19/05/2018. Prof. & Head, Dept. Of Clinical & Exp. Pharmacology, School of Tropical Medicine, C. R. Ave., Kolkata-700 073
2. Dr Rupak K. Bhadra	Chief Scientist, Head-Infectious Diseases & Immunology, CSIR-IICB, Kolkata 700032	19/05/18 Dr. Rupak K. Bhadra Ph.D., FNASc, FAScT Chief Scientist Infectious Diseases & Immunology Div. CSIR-Indian Institute of Chemical Biology 4, Raja S.C. Mullick Road, Kolkata-700032, India

It is certified that the final report has been uploaded on UGC-MRP portal on .....

It is also certified that final report, Executive summary of the report, Research documents, monograph academic papers provided under Major Research Project have been posted on the website of the University/College.

21/6/18

(DY Registrar/Principal)

Seal

Deputy Registrar
University of Calcutta