UNIVERSITY GRANT COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DEHLI - 110 002

Final Report of the work done on the Major Research Project (Report to be submitted within six weeks after compilation of each work)

1. Project report no.
1st /2nd /3rd /final : Final

2. UGC reference no. : F. No. 36-208/2008(SR)
Dated 26/03/2009

3. Period of the project : From 01/05/2009 to 30/04/2012

4. Title of research project : "Myofibrillar Compositional Study of Skeletal Muscles in Salivariadenectomised Male and Female Offsprings of Salivariadenectomised Mother"

5. a) Name of the

Principal investigator : Dr. (Smt.) M. V. Walvekar
Department of Zoology
Shivaji University
Kolhapur – 416004
(MS) INDIA

b) Dept. /university /college where the work has progressed : Department of Zoology,
Shivaji University,
Kolhapur-416004

6. Effective date of starting the project: 01/05/2009

7. Grant approved and expenditure incurred during the period of the report:

a) Total amount approved: Rs. 9, 37, 800/-

b) Grant released as first installment: Rs. 6, 98, 800/-

c) Grant released as second installment: Rs. 1, 78, 800/-

d) Total grant sanctioned: Rs.8, 76, 800/-

e) Total expenditure: Rs. 8, 75, 004/-

f) Total balance remained: Rs. 1, 796/-

8. Report of the work done: (please see appendix A)

9. OBJECTIVES OF THE PROPOSAL:

1. To find out the effect of salivary gland secretions on the development and differentiation of skeletal muscles (gastrocnemius, soleus and rectus abdominus) of male and female offsprings of sham operated mother in mice.
2. To find out the effect of salivary gland secretions on the development and differentiation of skeletal muscles of male and female offsprings of sialoadenectomised and salivariadenectomised mother.

10. Has the progress been according to original plan of work and towards achieving the objectives if not, state reason: Yes

11. Please indicate the difficulties, if any, experienced in implementing the project: No difficulties in implementing the project.

12. If project has not been completed, please indicate approximate time by which it is likely to complete: Not applicable.

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13. If the project has been completed, please enclose the summary of the findings of the study: (please see appendix A)

14. Any other information which would help in the evaluation of the work done on the project. At the completion of project, the second report should indicate the output, such as:

a) Manpower trained:

Project fellow appointed in this project has attended five workshops.

1. Workshop on "Analytical Instrumental Techniques" at University Science Instrumentation Center USIC (CFC), Shivaji University, Kolhapur.
2. Workshop on "Advanced Genetic Engineering" at Department of Biotechnology Engg., KIT's College of Engg. Kolhapur.
3. Workshop on "Recent Advances in Applied Zoology" at Department of Zoology, Shivaji University, Kolhapur.
4. National Workshop on "Biological control of pests" at Department of Zoology, Shivaji University, Kolhapur.
5. Workshop on "Research Writings, Ethics, Plagiarism and Publishability" at The New College, Kolhapur.

b) Ph.D. awarded:

Project fellow has been registered for Ph. D. and the work is in progress.
(Title of Ph. D. work: "Studies on effect of Sialoadenectomy on sublingual gland of male albino mice *Mus musculus* L")

c) Publications of the results:

a. Paper presented in conference:

1. Research paper entitled "Sialoadenectomy Effect on Skeletal Muscles of Female Mice (*Mus musculus)*" presented in the "**National conference on**

13. If the project has been completed, please enclose the summary of the findings of the study: (please see appendix A)

14. Any other information which would help in the evaluation of the work done on the project. At the completion of project, the second report should indicate the output, such as:

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(Title of Ph. D. work: "Studies on effect of Sialoadenectomy on sublingual gland of male albino mice *Mus musculus* L")

c) Publications of the results:

a. Paper presented in conference:

1. Research paper entitled "Sialoadenectomy Effect on Skeletal Muscles of Female Mice (*Mus musculus)"* presented in the "**National conference on**

Zoology & Nanotechnology" from 8th to 10th February, 2010 at Department of Zoology Goa University, Goa.

2. Research paper entitled "Lactate dehydrogenase activity and isoenzyme patterns in the developing skeletal muscles of sialoadenectomised female mice" presented in the "**National conference on Global Warming and Conservation Strategies"** on 22nd and 23rd December, 2010 at Lal Bahadur Shastri College, Satara (Maharashtra).
3. Research paper entitled "Myofibrillar protein content from skeletal muscles of sialoadenectomised male mice" presented in the **"International Symposium on Current Trends in Endocrine and Reproductive Health and 29th Meeting of the Society for Reproductive Biology and Comparative Endocrinology (SRBCE)"** at Department of Studies in Zoology, University of Mysore, Mysore. 10th – 12th February 2011
4. .Research paper entitled "Alterations in lactate dehydrogenase of skeletal muscles in salivariadenectomised mice" presented in the **"National conference on recent trends in life sciences with special reference to Animal Physiology, Biotechnology and Biodiversity"** at Department of Zoology, S.G. M. College Karad. 19th -20th October, 2011

b. Conferences/Seminar Attended:

1. National seminar on "Recent Trends and Techniques in Biosciences" at Department of Zoology, ACS college, Palus, Sangli. 30th November- 1st December, 2011.
2. National seminar on "Preparation of Skillful Research Papers at National and International Scenario" at Department of Zoology, Shivaji University, Kolhapur. 6th February, 2012.

c. Papers Published:

1. Research paper entitled "Sialoadenectomy Effect on Skeletal Muscles of Female Mice (*Mus musculus)* "has been published in ***"Journal of Cell and Tissue Research"*** Vol. 10(2) 2207-2211 (2010). **ISSN: 0974- 0910.**

2. Research paper entitled "Sialoadenectomy Effect on Blood Glucose Level and Skeletal Muscle Glycogen from Male and Female Mice" has been published in ***Journal of Experimental Zoology India.*** Vol. 14 (1), pp. 145-148, (2011). **ISSN 0972-0030. Citation index= 1.**

d. Papers Communicated:

1. Research paper entitled "Lactate dehydrogenase activity and developing skeletal muscles of sialoadenectomised female mice". Communicated for publication in Current Science.
2. Research paper entitled "Myofibrillar protein content from skeletal muscles of sialoadenectomised male mice". Communicated for publication in Journal of current bio medical and pharmaceutical Research.
3. Research paper entitled "Alterations in lactate dehydrogenase of skeletal muscles in salivariadenectomised mice". Communicated for publication in Asian Journal of microbiology, Biotechnology and Environmental sciences.
4. Research paper entitled "Siaoladenectomy effect on gastrocnemius muscle of male mice". Communicated for publication in Journal of Cell and Tissue Research.

d) Other impact if any: No.

Principal Investigator

Dr.M.V.Walvekar
Principal Investigator(U.G.C.Scheme)
Department of Zoology
Shivaji University,Kolhapur-416004(India)

Registrar/Principal

Registrar,
Shivaji University, Kolhapur

Appendix A

a) Work done so far and results obtained:

Submandibular glands of mouse and other species contain heterologous group of polypeptides, growth factors, such as epidermal growth factor (EGF), mesodermal growth factor (MGF), nerve growth factor (NGF), rennin, kallikreins, etc. These growth factors promote cell proliferation, regulate tissue differentiation and modulate organogenesis. Also it has been found that macromolecular fraction from mouse salivary glands markedly enhances growth of mesenchymal tissues *in vitro.*

The various organs of the body have long been known to be influenced by these growth factors. Atterdi in 1965 studied effect of a fraction of submandibular gland of mouse on tissues of mesodermal origin in vitro.

After 1965 no body had tried to study effect of salivary gland secreted polypeptides on any mesodermal tissue muscle. So our attempt is to study whether the polypeptides secreted by mother's salivary gland affect the skeletal muscles of offsprings. To study this effect we have decided to do the sialoadenectomy and salivariadenectomy of mother.

Methodology:

Material:

Male and female albino mice (*Mus musculus*) were used for present investigation. The animal ethics committee had approved the protocol of animal experiments. The mice were allowed to breed in the air-conditioned animal house in the department. The mice were housed in aluminum cages having dimension of 10"x 8"x 12" inches in groups of 3 to 4 with rice husk bed. They were fed with Amrut Rat/ Mice fed, which was obtained from Pranav Agro Industries Ltd. Sangli and water given *ad Libitum.*

Sialoadenectomy and Salivariadenectomy:

Sialoadenectomy is the removal of submandibular gland and salivariadenectomy is the removal of both submandibular and sublingual glands, while from both sides. For the study of effect of maternal sialoadenectomy and salivariadenectomy on the skeletal muscles (gastrocnemius, soleus, rectus abdominus) sialoadenectomy and salivariadenectomy was carried out in about 3 months old pregnant female (weighing 38to 42gm) on the 10^{th} day of their gestation period. Operation was carried out between 9 a.m. to 10 a.m. Operated females were maintained in animal house with proper care. Control females were sham operated. The mother's were divided into following groups.

1. Control mother
2. Sialoadenectomised mother
3. Salivariadenectomised mother

The male and female offspring's obtained from each group were further divided into sialoadenectomised and salivariadenectomised offsprings. Sialoadenectomy and salivariadenectomy was carried out on 20^{th} day after birth. After operation they were grouped according to the required age groups for the study as 40 days, 60 days, 80 days and 100 days from birth. Then the animals were sacrificed on their respective age and the gastrocnemius, soleus and Rectus abdominus muscles were dissected out and used for following estimations and electrophoresis.

Methods:

a. Biochemical estimations done in above groups of mice muscles are-

1) Estimation of Blood glucose: (Folin and Wu, 1920)

This method is based on the principle that protein free blood filtrate when treated with alkaline copper sulphate, using special

tube for preventing reoxidation, cuprous oxide was formed which on treatment with phosphomolybdic acid gives blue color. It is compared with standard color produced by glucose. Color intensity was measured at 420 nm by adjusting the colorimeter to zero with blank.

2) Estimation of Glycogen: (Pfleiderer, 1957)

Glycogen is determined by hydrolysis to glucose which is then estimated chemically.

3) Estimation of Protein: (Lowry et. al, 1951)

Protein estimation was carried out by Lowry's method (Lowry et.al., 1952) using Bovine serum albumin as standard.

4) Estimation of Lactate Dehydrogenase: (Sevela and Tovorek, 1989)

This method is based on the measurement of the pyruvate formation rate during the lactate oxidation; reaction is assisted by lactate dehydrogenase of the sample. Pyruvate reacts with 2, 4-dinitrophenylhydrazine in an alkaline medium to yield hydrazone of red brown color whose intensity was presumed to be proportional to the ketoacid concentration. Measurement of the absorbance was taken for sample solution against blank solution on spectronic 20 colorimeter, at 520-560 nm wavelengths.

5) Estimation of Alkaline Phosphatase: (Linhardt, K. and Walter, K. 1965)

P-nitrophenyl phosphate is used as the substrate for the determination of activity of alkaline phosphatase. After 30 mins incubation the alkaline phosphatase activity was inhibited by NaOH the P-nitrophenyl liberated by the phosphatase forms a yellow anion.

The phosphatase activity is directly proportional to the amount of P-nitrophenyl liberated per unit time. The optical density of the sample was noted on the spectronic 20 colorimeter, adjusting zero with control at 400nm wavelength.

b. Electrophoretic separations done in above groups of mice muscles are-

1) Electrophoretic separation of Lactate Dehydrogenase: (Ornstein 1964, Davis 1964, Andrew 1981).

This method is based on the property of migration of charged particles under the influence of electric field. It is single phase system and depends upon the relative mobilities of ions under identical electric conditions.

2) Electrophoretic separation of muscle protein: (Ornstein 1964, Davis 1964, Andrew 1981).

Electrophoretic separation of muscle protein from three muscles Gastrocnemius, Soleus and Rectus abdominus by using Ornstein (1964), Davis (1964), Andrew, (1981). The proteins were separated by using SDS –PAGE. The muscles were incubated overnight in 10% glycerol/ 5% 2- mercaptoethanol / 2.3 % SDS / 62.5mM Tris HCL buffer pH (6.8). After incubation solution centrifuged and supernatant was used as sample. The sample was loaded on 10% polyacrylamide. The electrophoresis buffer was 0.1 % SDS / 25mM Tris glycine buffer. After electrophoresis gels were stained with Comassie G250.

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Results:

Estimation Results:

Effect of salivary secretions was seen on three different skeletal muscles viz, gastrocnemius, soleus and rectus abdominus muscles during development i.e. in 40^{th}, 60^{th}, 80^{th} and 100^{th} days offsprings by using different parameters like- blood glucose level, glycogen content, protein content, lactate dehydrogenase activity and alkaline phosphatase activity of control, sialoadenectomised and salivariadenectomised male and female offsprings of control mother, sialoadenectomised mother and salivariadenectomised mother.

1) **Control Mother: Control, sialoadenectomised and salivariadenectomised offsprings.**

The blood glucose level in sialoadenectomised male and female mice was decreased in all groups i.e. 40, 60, 80 and 100 days as compared to control male and female mice. While there was highly significant decrease in blood glucose level in salivariadenectomised mice as compared to control and sialoadenectomised mice. Whereas there was increase in blood glucose level from juvenile to adult in the control, sialoadenectomised and salivariadenectomised mice. It was observed that there was decrease in blood glucose level in female mice when compared with male mice.

The glycogen content, protein content, lactate dehydrogenase (LDH) activity and alkaline phosphatase activity was increased from gastrocnemius, soleus and rectus abdominus muscles in sialoadenectomised male and female mice in all groups i.e. 40, 60, 80 and 100 days as compared to control male and female mice. While there was significant increase in all above parameters in salivariadenectomised mice as compared to control and sialoadenectomised mice. It was also observed that the

glycogen content was more in soleus muscle whereas protein, LDH and alkaline phosphatase was increased significantly in rectus abdominus muscle as compared to other muscles. The result shows that all parameters were increased from juvenile to adult in both male and female mice and there was significant increase in male mice in all groups than in female mice.

2) **Sialoadenectomised mother: Sialoadenectomised and salivariadenectomised offsprings.**

The blood glucose level was decreased in sialoadenectomised and salivariadenectomised male and female offsprings as compared to control, sialoadenectomised and salivariadenectomised male and female offsprings of control mother. Whereas the decrease was highly significant in salivariadenectomised male and female offsprings as compared to offsprings of control mother and sialoadenectomised mother.

The glycogen content, protein content, lactate dehydrogenase (LDH) activity and alkaline phosphatase activity was increased from gastrocnemius, soleus and rectus abdominus muscles in all groups i.e. 40, 60, 80 and 100 days of sialoadenectomised and salivariadenectomised male and female offsprings as compared to sialoadenectomised and salivariadenectomised male and female offsprings of control mother. But in salivariadenectomised offsprings of salivariadenectomised mother the increase was much more as compared to other groups.

3) **Salivariadenectomised mother: Sialoadenectomised and salivariadenectomised offsprings.**

In sialoadenectomised and salivariadenectomised male and female offsprings of salivariadenectomised mother the blood glucose level was decreased in all groups whereas the glycogen, protein, LDH and

alkaline phosphatase was increased as compared to control mother offsprings and sialoadenectomised offsprings and the increase was significant.

Electrophoresis results:

1. **Electrophoretic separation of lactate dehydrogenase (LDH):**

The electrophoretic separation of LDH from all three muscles viz, gastrocnemius, soleus and rectus abdominus in 40, 60, 80 and 100 days reveals that at juvenile stage i.e. 40 days and 60 days all five bands i.e. LDH-I, II, III, IV and V were clearly separated and at adult stage i.e. 80 days and 100 days all five bands were separated but band IV^{th} was not clearly separated from V^{th} band. In salivariadenectomised male and female offsprings of salivariadenectomised mother the IV^{th} and V^{th} band were very much intensely stained than the all other groups. The electrophoretic separation of LDH reveals that LDH was much more increased in rectus abdominus muscles than the gastrocnemius and soleus muscles.

2. **Electrophoretic separation of protein:**

The electrophoretic separation of protein from skeletal muscles was revealed by SDS-polyacrylamide gel electrophoresis and compared with standard protein marker (broad range) and the myofibrillar proteins are determined as Myosin heavy chain or MHC (2, 00,000 da), Actin or A (95, 000 da), Tropomyosin or TM (65, 000- 70, 000 da), Trophonin I or TN (22, 000- 24, 000 da), Trophonin T (37, 000- 40, 000 da) and Myosin light chains or MLC (20, 000- 15, 000 da).

Electrophoretic separation of protein from all the three skeletal muscles i.e. gastrocnemius, soleus and rectus abdominus in

salivariadenectomised and sialoadenectomised mother male and female offsprings was increased significantly as compared to control mother's sialoadenectomised and salivariadenectomised male and female offsprings from all groups i.e. 40, 60, 80 and 100 days old mice and the increase was more in rectus abdominus muscle as compared to other two muscles.

The results also shows that at later stage of life i.e. at 80 and 100 days the staining intensity of trophonin and myosin light chain bands was increased significantly in sialoadenectomised and salivariadenectomised offsprings in all three types of muscles in all groups.

Conclusion:

In all above groups there was decrease in blood glucose level and increase in all other parameters. The most significant results were obtained in male offsprings than in female offsprings which proves that salivary secretion of male mice mainly the mesodermal growth factors and immunologically reactive glucagon (IRG) affects the development of skeletal muscles in sialoadenectomised mice and more significantly in salivariadenectomised mice than the female mice.

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Utilization certificate

Certified that the grant of Rs. 8, 76, 800/- (Eight lakh seventy six thousand eight hundred only) received from the university Grants Commission under the scheme of support for Major Research Project entitled "Myofibrillar Compositional Study of Skeletal Muscles in Salivariadenectomised Male and Female Offsprings of Salivariadenectomised Mother" vide UGC letter no. F.No.36-208/2008 (SR) dated 26th March, 2009. From the sanctioned amount Rs. 8, 75, 004/- (Eight lakh seventy five thousand and four only) has been fully utilized for the purpose for which it was sanctioned and accordance with the terms and conditions lay down by the University Grant Commission. The remaining balance of Rs. 1, 796/- (One thousand seven hundred and ninety six only) from non-recurring and recurring will be submitted to UGC.

Principal Investigator

Dr.M.V.Walvekar
Principal Investigator(U.G.C.Scheme)
Department of Zoology
Shivaji University,Kolhapur-416004(India)

Registrar

Registrar,
Shivaji University, Kolhapur

Statutory Auditor

For Sankpal Kulkarni & Associates
Chartered Accountants

(Shrirang Kulkarni)
Partner M. No. 108722

SANKPAL KULKARNI & ASSOCIATES * KOLHAPUR * Chartered Accountants

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Statement of expenditure in respect of major research Project

1. Name of Principal Investigator – Dr. (Smt.) M. V. Walvekar
   Assistant Professor,
   Department of Zoology,
   Shivaji University,
   Kolhapur, 416004

2. Department of University - Zoology Department
   Shivaji University,
   Kolhapur, 416004

3. UGC Approval No. and date - F.No.33-208/2008 (SR),
   26 March 2009

4. Title of research project - "Myofibrillar Compositional Study of Skeletal Muscles in Salivariadenectomised Male and Female Offsprings of Salivariadenectomised Mother"

5. Effective date of starting the project: 01/05/2009

b) Staff: **Date of Appointment**: 25th June 2009

Sr. No.	Expenditure Incurred (Rs.)	Amount Approved (Rs.)	Expenditure Incurred in 2009-2010 (Rs.)	Expenditure incurred in 2010-2011	Expenditure incurred in 2011-2012 Upto-30/4/12	Total expenditure	Balance remained
1	Honorarium to PI (Retired Teachers) 10,000/-p.m.	----	----	----	----	----	----
2.	Project Associate Fellowship @, Rs.8, 000/- p.m.	2,73,333/-	65,333/-	1,04,000/-	1,04,000/-	2,73,333/-	nil
3.	Project Fellow Consolidated Salary, @ Rs. 6000/- p.m.	----	----	----	----	----	----

Expenditure done: (From 01/05/2009 to 30/04/2012)

Item	Total Amount Approved (Rs.)	Expenditure Incurred in 2009-2010 (Rs.)	Expenditure incurred in 2010-2011 (Rs.)	Expenditure incurred in 2011-2012 Upto-30/4/2012 (Rs.)	Total Expenditure of three years (Rs.)	Balance remained (Rs.)
Books and Journal	1. First installment: **6,98,800/-** 2. Second installment: **1,78,000/-**	10,789/-	2,935/-	nil	13,724/-	1, 276/-
Equipment		1, 92, 507/-	1, 72, 741/-	34, 236/-	3, 99, 484/-	516/-
Contingency		23, 950/-	12, 018/-	16, 471/-	52, 439/-	04/-
Field Work/Travel		2, 795/-	14, 359/-	nil	17, 154/-	nil
Hiring Services		nil	nil	nil	nil	nil
Chemicals and glassware		32, 113/-	21, 865/-	20, 092/-	74, 070/-	nil
Overhead		14, 933/-	14, 933/-	14, 934/-	44, 800/-	nil
Total	**8, 76, 800/-**	**3, 42, 420/-**	**3, 42, 851/-**	**1, 89, 733**	**8, 75, 004/-**	**1, 796/-**

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1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. It as result of check or audit objective, some irregularly is noticed, later date, action will be taken to refund, adjust or regularize the objected amount.

3. Payments@ revised rates shall be made with arrears on the availability of additional funds.

4. It is certified that the grant of Rs. 8, 76, 800/- (Eight lakh seventy six thousand eight hundred only) received from the University Grants Commission under the scheme of support for Major Research Project entitled "Myofibrillar Compositional Study of Skeletal Muscles in Salivariadenectomised Male and Female Offsprings of Salivariadenectomised Mother" vide UGC letter no. F.No.36-208/2008 (SR) dated 26th March, 2009. From the sanctioned amount Rs. 8, 75, 004/- (Eight lakh seventy five thousand and four only) has been fully utilized for the purpose for which it was sanctioned and accordance with the terms and conditions lay down by the University Grant Commission. The remaining balance of Rs. 1, 796/- (One thousand seven hundred and ninety six only) from non-recurring and recurring will be submitted to UGC.

Principal Investigator

Dr.M.V.Walvekar
Principal Investigator(U.G.C.Scheme)
Department of Zoology
Shivaji University,Kolhapur-416004(India)

Registrar / Principal
Registrar,
Shivaji University, Kolhapur

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Statement of expenditure incurred on Travel

Name of Principal Investigator: Dr. M. V. Walvekar
Assistant Professor,
Department of Zoology,
Shivaji University,
Kolhapur, 416004

Name of the place visited	Duration of the visit		Mode of the journey	Expenditure Incurred
	From	To		
Goa to Kolhapur	9 pm. on 14/06/2009	9 pm. on 15/06/2009	Own vehicle	Rs. 2,795/-
Kolhapur-Pune-Delhi and Delhi-Pune-Kolhapur	22/03/2011	25/03/2011	By air	Rs. 14, 359/-

Certified that the above expenditure is in accordance with the UGC norms for Major Research Project.

Principal Investigator

Dr.M.V.Walvekar
Principal Investigator(U.G.C.Scheme)
Department of Zoology
Shivaji University,Kolhapur-416004(India)

Registrar / Principal

Registrar,
Shivaji University, Kolhapur