Annexure III

University Grants Commission
Bahadur Shah Zafar Marg
New Delhi – 110001.

FINAL REPORT OF THE WORK DONE ON THE MINOR RESEARCH PROJECT.

1. Project report I/II/III : Final report.
2. UGC Reference Number : MRP (S)-138/08-09/KABA023/ UGC- SWRO dated 18-10-2010.
3. Period of the report from : 18-11-2010.
4. Title of the Research Project :"Effects of Arsenic and Selenium Interactions on Ovary and Estrogen Production".
5. A) Name of the Principal Investigator : Dr. Tejaswini. V. Nandi.
   B) Dept. of University / College : K.L.E's S.Nijalingappa College Dept of Zoology Rajajinagar Bangalore.
6. Effective date of the starting the project :
7. Grant approved and expenditure incurred during the period of the report.
   a. Total amount approved Rs. 1,30,000/- (65 released as first installment and Rs. 52,000/- released as second installment).
   b. Total expenditure of second installment : Rs. 54,570/-.
   c. **Report of the work done:**
   1. **Brief Objectives of the Project.**

- To assess and validate the effect of combined Arsenic Selenium on the Arsenic induces tumours in the ovary of mice by using histopathology.
- To assess and validate the effect of combined drug (Arsenic +Selenium) on the serum concentration of Estrogen in blood samples.

2. **Work done so far and results achieved and publications if any, resulting from the work (give details).**

- The project is being carried out in a lab. The first and the foremost task were to set up the animal house in a working conditions i.e designing the lab and setting the animal house.

- The reagents purchased are sodium selenite, As (III) (merk GmbH, Germany), Periodic acid, PB sodium salt, DPX, Glutaldehyde, gloves, surgical spirit, syringe, cotton, aluminium foil, reagents bottles, beakers, micropipettes, roter and blades and repair of microtome (Rocker), alcohol grades, DW, Cages, Female albino mice, NaoH etc, werepurchased were of highest purity, A.R grade, Thermometer, Special tags, Humidifier, Weighing machines, Coolers were provided.
- Animals and treatment – All of the procedures involving animals in this study, we have an animal ethical committee and registered animal house.
- Fifty four adult fertile female swiss albino mice were divided into nine groups.
- Various concentrations of As_2O_3 solutions were prepared.
- Set I-25 μ mol, 50 μ mol, 75 μ mol and 100 μ mol.
- Set II-15 μ mol, 30 μ mol, 45 μ mol and 60 μ mol were prepared.
- Animals were acclimatization for one month. They were provided with feed and water.
- Set I- received 0.2 ml of 25 μ mol As_2O_3, IP for three days / week, alternately 0.2 ml to 25, 50, 75, 100 ml.
- Set II received 0.2 ml of 30 μ mol As_2O_3, IP for three days / week, alternately 0.2 ml to 15, 30, 45, 60 ml.
- IX group was set III and it acted as control.
- Acclimatization of animals.
- Two months for As_2O^3 treatment.
- Sodium Selenite treatment.
- Observation and animal behavoiur and physiology pretreated animals with As_2O_3 – when we started treatment after a couple of days, the following changes were noticed among the mice.
- They become a sluggish, tired. There was weight loss for 22, 21, 20 gms etc. hair loss and impaired vision, formation of lesions.
- Light microscopy work revealed that conteal animals showed normal morphology of Graafian follicles, with prominent ova, when compared to Arsenic treated ovary when there was dissolution of Graafian follicles. The condition stated improving when treated with Selenium.

3. Has the progress been according to original plan of work and towards achieving the objectives? If not state reason.

   YES.

   The progress has been as planned from the commencement of the project.

4. Please indicate difficulties if any experienced in implementing the project.

   No such problems encountered.

5. 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 from the period may be sent to the commission on a separate sheet.

   It is completed.

6. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of the work done may also be sent to the commissioner.

   Enclosed.

OBJECTIVE.

T o determine the concentration of antigen by sandwich Elisa method.

PRINCIPLE.

ELISA or enzyme linked immunosorbent assay is a sensitive laboratory method used to detect the presence of antigens (Ag) or Antibodies (Ab) of interest in a wide variety of biological samples. This assay requires an immunosorbent, i.e antigen or antibody immobilized on solid surface such as the wells of microtitre plates or membranes.

In this method, two antibodies that can bind to two different epitopes on the same antigen are required. One of the antibodies is immobilized on a microtitre well and is referred to as capture antibody and other antibody is labeled with a suitable enzyme (eg, horse radish peroxidase (HRP), alkaline phosphatase (ALP) etc) and is referred to as labeled antibody. Sample (standard and test) containing the antigen is allowed to react with the immobilized antibody. After the well is washed, labeled antibody is added and allowed to react with the bound antigen. Unreacted labeled antibody is washed out and the enzyme bound to solid support is estimated by adding a chromogenic substrate. The colour developed is measured spectrophotometrically which is directly proportional to the antigen concentration.

The reaction sequence:

1. Coating of microtitre wells with capture antibody.
2. Blocking of the residual sites.
3. Addition of antigen.
4. After wash.
5. Addition of labeled antibody.
6. After wash.
7. Addition of substrate.

8. Conversion of substrate to coloured product.

Kit description:

In this kit, a standard antigen, three test antigens, capture antibody and HRP labeled antibody are supplied. Students will immobilize the capture antibody on the wells and to this add antigen (test antigen or varying amounts of standard antigen). The amount of antigen will be detected by addition of HRP labeled antibody, which in turn will be estimated using H_2O_2 as substrate and tetramethylbenzidine (TMB) as chromogen. HRP acts on H_2O_2 to release nascent oxygen, which oxidizes TMB to TMB as Oxide, a blue coloured product. The intensity of the colour will be measured using a spectrophotometer at 450nm. Hence, by using various amounts of the standard antigen in three test samples provided.

KT52: The kit is designed to carry out 4 sandwich ELISA experiments, to determine the concentration of antigen in the given three test samples.

Duration of the experiment: experiment is carried out over a span of 2 days, approximate time taken on each day is indicated below:

Day 1: 15 minutes (Coating wells with capture antibody)

Day 2: 5 hours (Assay and Results)

Materials provided:

The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 6 months of arrival.

Note: Samples of semi-log graph sheets are provided in the manual. Photocopy as required.

Materials	**Quantity KT52 (4 expts)**	**Store**
Standard Antigen (0.4 mg/ ml)*	50 µl	4^0C
100X Capture Antibody*	0.25 ml	4^0C
Test samples (1,2 and 3)*	50 µl	4^0C
1000X Antibody – HRP Conjugate	50 µl	4^0C
Blocking Buffer	25 ml	4^0C
10XTMB/H_2O_2	2.5 ml	4^0C
Coating Buffer	25 ml	4^0C
10XPBST	40 ml	4^0C

5XStop solution	50 ml	4^0C
Microtiter Wells	8 X 12	RT

Note: *Samples are supplied in lyophilized form and volume indicated on the label is after reconstitution.

Materials required:

Glassware: Measuring cylinder, Test tubes.

Reagent: Distilled water.

Other Requirements: Blotting paper, Micropipette, Tips. Spectrophotometer, Cuvette.

Note:

- Read the entire procedure before the experiment.
- Bring all the buffers to room temperature before the assay.
- Dilute only required amount of buffers to IX with distilled water.
- Use 24 wells per experiment.
- Reconstitute samples of capture antibody, standard antigen and test samples with distilled water; volume as mentioned on their respective labels. Store at 4^oC and use within 3 months.
- Blocking buffer: BSA in PBST.
- Coating buffer: Carbonate bicarbonate buffer.
- PBST: Phosphate Buffered Saline Tween.
- Stop solution: Sulphuric acid.

Tissue and blood collection

Animals were anaesthetized by intraperitoneal injection of sodium barbital. Uteri were quickly removed from the experimental animals and washed in 0.9% (w/v) cold normal saline, pat dried and weighed in an electrical monopan balance (Lutron GM-300 P). small representative tissue slices were processed for histological and immunohistochemical studies, RNA isolation and protein purification. Blood was collected from the heart and serum was isolated for ELISA.

Assay of serum estradiol, LH and FSH

ELISA (DRG International ELISA Kit) was performed for estimating the circulating levels of estradiol, LH and FSH. For assay of serum stradiol, 25 µl each of standard, control and treated serum samples were added to respective wells coated with anti-estradiol antibody and incubated with 200 µl of enzyme conjugate for 2 hours at room

temperature. Subsequently, 100 μl of substarte was added and incubated for 15 minutes at room temperature. Reactions were stopped using 50 μl of stop solution and the O.D was measured at 450 nm. Each sample was run in triplicate. For assa of serum FSH and LH, 25 μl of standard, control or treated serum samples were added to respective wells coated with anti-FSH and anti-LH antibodies and incubated with 100 μl of substrate was added to each well. After incubation for 10 minutes at room temperature, reactions were stopped using 50 μl of stop solution and the O>D measured at 450 nm. The intra-assay and inter-assay variations were found to be less than 9% and 10% respectively. Limit of detection for estradiol was 3.6 pg/ml, for LH was 0.45mlU/ml and for FSH was 0.28mlU/ml.

Annexure V

Statement of the Expenditure in Respect of Minor Research Project.

1. Name of the Principal Investigator : Dr. Tejaswini. V. Nandi.

2. Dept. of University / College : KLE's S.Nijalingappa College
Dept. of Zoology, Rajajinagar,
Bangalore – 10.

3. UGC approval No. and Date : MRP (S)-138/08-09/KABA023/
UGCSWRO, dated 13/10/2010.

4. Title of the Research Project : "Effects of Arsenic and Selenium
Interactions on Ovary and
Estrogen production".

5. Effective date of starting of the Project : January 2011.

6. a. Period of Expenditure : july 2011 – august 2012.

   b. Details of Expenditure :

Sl.no	Item	Amount approved.	Expenditure Incurred
I	**NON RECURRING GRANTS**.		
a.	Equipment	NIL	NIL
b.	Books and Journals		
II.	**RECURRING.**	**52.000/-**	**54,570/-**

It is certified that the grant of Rs. 52,000=00 (Rs. Fifty two thousand only) received so far from the UGC under the scheme of support for Minor Research

Project entitled Vide UGC letter no MRP (S) – 138/08-09/KABA 023/UGCSWRO dated 13 /10 / 2010 has been fully utilized for the purpose for which sanctioned and in accordance with the terms and conditions laid down by the UGC.

Principal Investigator Principal.

Statement of the Expenditure in Respect of Minor Research Project.

Details of the Expenditure.

Sl.No.	**Item.**	**Voucher No.**	**Amount.**
01	Chemicals	01	5,182=00
02	Sand wich ELISA kit, genomic DNA, Cuvettes, Quartz, Micropipette 1000 μl, 1-10 μl, 10-100 μl, 100-1000 μl.	02	33, 388=00
03	Hiring services till the completion of project work	03	10,500=00
04	Soft copy, hard copy and miscellaneous	04	2,500=00
05	Chartered accountant fees	05	3,000=00

Total = 54,570=00

(Rs. Fifty four thousand five hundred seventy rupees only)

Principal Investigator **Principal** **Statutory Auditor**

From,

Dr. Tejaswini. V. Nandi.
Dept of Zoology,
K.L.E's S.Nijalingappa College,
II Block, Rajajinagar,
Bangalore – 10.

To,
The Deputy Secretary,
University Grants Commission,
SWRO,
Palace Road, Bangalore.

Respected Sir,

Sub: Submission of final Minor Research Project Report.

Ref: MRP (S) – 138/08-09 KABA023/ UGC SWRO dated 18-11-2010.

Title: "Effects of Arsenic and Selenium Interactions on Ovary and Estrogen production".

With reference to above subject I am submitting here with an audited Utilization Certificate for Rs. 1,22,790/- , sanctioned Rs. 1,17,000/- as I and II installment.

I am also submitting statement of expenditure along with bills, vouchers etc for Rs. 1,22,790/-.

I thank you for providing me financial assistance to execute the Minor Research Project.

Principal Investigator

Annexure III

University Grants Commission
Bahadur Shah Zafar Marg
New Delhi – 110001.

FINAL REPORT OF THE WORK DONE ON THE MINOR RESEARCH PROJECT.

1. Project report I/II/III : Final report.
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3. Period of the report from : 18-11-2010.
4. Title of the Research Project :"Effects of Arsenic and Selenium Interactions on Ovary and Estrogen Production".
5. A) Name of the Principal Investigator : Dr. Tejaswini. V. Nandi.
   B) Dept. of University / College : K.L.E's S.Nijalingappa College Dept of Zoology Rajajinagar Bangalore.
6. Effective date of the starting the project : May 2009
7. Grants approved and expenditure incurred during the period of the report.
   d. Total amount approved Rs. 1,30,000/- (65 released as first installment and Rs. 52,000/- released as second installment).
   e. Total expenditure : Rs. 1,22,790/-.

REPORT OF THE WORK DONE.

i. Brief Objectives of the Project.

- To asses and validate the effect of combined Arsenic Selenium on the Arsenic induces tumours in the ovary of mice by using histopathology.
- To evaluate the Immuno Histochemical effect of combined drugs (Arsenic and Selenium) on the ovary of the mouse.
- To assess and validate the effect of combined drug (Arsenic +Selenium) on the serum concentration of Estrogen in blood samples.

ii. Work done so far and results achieved and publications if any, resulting from the work (give details).

- The project is being carried out in a lab. The first and the foremost task were to set up the animal house in a working conditions i.e designing the lab and setting the animal house.
- The reagents purchased are sodium selenite, As (III) (merk GmbH, Germany), Periodic acid, PB sodium salt, DPX, Glutaldehyde, gloves, surgical spirit, syringe, cotton, aluminium foil, reagents bottles, beakers, micropipettes, roter and blades and repair of microtome (Rocker), alcohol grades, DW, Cages, Female albino mice, NaoH etc, were purchased were of highest purity, A.R grade, Thermometer, Special tags, Humidifier, Weighing machines, Coolers were provided.
- Animals and treatment – All of the procedures involving animals in this study, we have an animal ethical committee and registered animal house.
- Fifty four adult fertile female Swiss albino mice were divided into nine groups.
- Various concentrations of As_2O_3 solutions were prepared.
- Set I-25 μ mol, 50 μ mol, 75 μ mol and 100 μ mol.
- Set II-15 μ mol, 30 μ mol, 45 μ mol and 60 μ mol were prepared.
- Animals were acclimatization for one month. They were provided with feed and water.
- Set I- received 0.2 ml of 25 μ mol As_2O_3, IP for three days / week, alternately 0.2 ml to 25, 50, 75, 100 ml.

- Set II received 0.2 ml of 30 μ mol As_2O_3, IP for three days / week, alternately 0.2 ml to 15, 30, 45, 60 ml.
- IX group was set III and it acted as control.
- December 2009 – Acclimatization of animals.
- January 4 – March 3 – Two months for As_2O_3 treatment.
- Observation and animal behavoiur and physiology pretreated animals with As_2O_3 – when we started treatment after a couple of days, the following changes were noticed among the mice.
- They become a sluggish, tired. There was weight loss for 22, 21, 20 gms etc. hair loss and impaired vision, formation of lesions.
- Light microscopy work revealed that conteal animals showed normal morphology of Graafian follicles, with prominent ova, when compared to Arsenic treated ovary when there was dissolution of Graafian follicles. The condition stated improving when treated with Selenium.

iii. Has the progress been according to original plan of work and towards achieving the objectives? If not state reason.

YES.

The progress has been as planned from the commencement of the project.

iv. Please indicate difficulties if any experienced in implementing the project.

No such problems encountered.

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 from the period may be sent to the commission on a separate sheet.

It is not yet completed. It takes about one year.

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of the work done may also be sent to the commissioner.

This information will be provided once the project is complete after another one year.

REPORT OF THE WORK DONE.

i. Brief Objectives of the Project.

- To assess and validate the effect of combined Arsenic Selenium on the Arsenic induces tumours in the ovary of mice by using histopathology.
- To assess and validate the effect of combined drug (Arsenic +Selenium) on the serum concentration of Estrogen in blood samples.

ii. Work done so far and results achieved and publications if any, resulting from the work (give details).

- The project is being carried out in a lab. The first and the foremost task were to set up the animal house in a working conditions i.e designing the lab and setting the animal house.
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- Set II received 0.2 ml of 30 μ mol As_2O_3, IP for three days / week, alternately 0.2 ml to 15, 30, 45, 60 ml.
- IX group was set III and it acted as control.
- Acclimatization of animals.
- Two months for As_2O_3 treatment.
- Sodium Selenite treatment.

- Observation and animal behaviour and physiology pretreated animals with As_2O_3 – when we started treatment after a couple of days, the following changes were noticed among the mice.
- They become a sluggish, tired. There was weight loss for 22, 21, 20 gms etc. hair loss and impaired vision, formation of lesions.
- Light microscopy work revealed that conteal animals showed normal morphology of Graafian follicles, with prominent ova, when compared to Arsenic treated ovary when there was dissolution of Graafian follicles. The condition stated improving when treated with Selenium.

vii. Has the progress been according to original plan of work and towards achieving the objectives? If not state reason.

YES.

The progress has been as planned from the commencement of the project.

viii. Please indicate difficulties if any experienced in implementing the project.

No such problems encountered.

ix. 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 from the period may be sent to the commission on a separate sheet.

It is completed.

x. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of the work done may also be sent to the commissioner.

Enclosed.

OBJECTIVE.

T o determine the concentration of antigen by sandwich Elisa method.

PRINCIPLE.

ELISA or enzyme linked immunosorbent assay is a sensitive laboratory method used to detect the presence of antigens (Ag) or Antibodies (Ab) of interest in a wide variety of biological samples. This assay requires an immunosorbent, i.e antigen or antibody immobilized on solid surface such as the wells of microtitre plates or membranes.

In this method, two antibodies that can bind to two different epitopes on the same antigen are required. One of the antibodies is immobilized on a microtitre well and is referred to as capture antibody and other antibody is labeled with a suitable enzyme

(eg, horse radish peroxidase (HRP), alkaline phosphatase (ALP) etc) and is referred to as labeled antibody. Sample (standard and test) containing the antigen is allowed to react with the immobilized antibody. After the well is washed, labeled antibody is added and allowed to react with the bound antigen. Unreacted labeled antibody is washed out and the enzyme bound to solid support is estimated by adding a chromogenic substrate. The colour developed is measured spectrophotometrically which is directly proportional to the antigen concentration.

The reaction sequence:

1. Coating of microtitre wells with capture antibody.
2. Blocking of the residual sites.
3. Addition of antigen.
4. After wash.
5. Addition of labeled antibody.
6. After wash.
7. Addition of substrate.
8. Conversion of substrate to coloured product.

Kit description:

In this kit, a standard antigen, three test antigens, capture antibody and HRP labeled antibody are supplied. Students will immobilize the capture antibody on the wells and to this add antigen (test antigen or varying amounts of standard antigen). The amount of antigen will be detected by addition of HRP labeled antibody, which in turn will be estimated using H_2O_2 as substrate and tetramethylbenzidine (TMB) as chromogen. HRP acts on H_2O_2 to release nascent oxygen, which oxidizes TMB to TMB as Oxide, a blue coloured product. The intensity of the colour will be measured using a spectrophotometer at 450nm. Hence, by using various amounts of the standard antigen in three test samples provided.

KT52: The kit is designed to carry out 4 sandwich ELISA experiments, to determine the concentration of antigen in the given three test samples.

Duration of the experiment: experiment is carried out over a span of 2 days, approximate time taken on each day is indicated below:

Day 1: 15 minutes (Coating wells with capture antibody)

Day 2: 5 hours (Assay and Results)

Materials provided:

The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 6 months of arrival.

Note: Samples of semi-log graph sheets are provided in the manual. Photocopy as required.

Materials	Quantity KT52 (4 expts)	Store
Standard Antigen (0.4 mg/ ml)*	50 µl	4^0C
100X Capture Antibody*	0.25 ml	4^0C
Test samples (1,2 and 3)*	50 µl	4^oC
1000X Antibody – HRP Conjugate	50 µl	4^oC
Blocking Buffer	25 ml	4^0C
10XTMB/H_2O_2	2.5 ml	4^0C
Coating Buffer	25 ml	4^0C
10XPBST	40 ml	4^0C
5XStop solution	50 ml	4^0C
Microtiter Wells	8 X 12	RT

Note: *Samples are supplied in lyophilized form and volume indicated on the label is after reconstitution.

Materials required:

Glassware: Measuring cylinder, Test tubes.

Reagent: Distilled water.

Other Requirements: Blotting paper, Micropipette, Tips.
Spectrophotometer, Cuvette.

Note:

- Read the entire procedure before the experiment.
- Bring all the buffers to room temperature before the assay.
- Dilute only required amount of buffers to IX with distilled water.
- Use 24 wells per experiment.
- Reconstitute samples of capture antibody, standard antigen and test samples with distilled water; volume as mentioned on their respective labels. Store at $4^{o}C$ and use within 3 months.
- Blocking buffer: BSA in PBST.
- Coating buffer: Carbonate bicarbonate buffer.
- PBST: Phosphate Buffered Saline Tween.
- Stop solution: Sulphuric acid.

Tissue and blood collection

Animals were anaesthetized by intra-peritoneal injection of sodium barbital. Uteri were quickly removed from the experimental animals and washed in 0.9% (w/v) cold normal saline, pat dried and weighed in an electrical monopan balance (Lutron GM-300 P). Small representative tissue slices were processed for histological and immune-histochemical studies, RNA isolation and protein purification. Blood was collected from the heart and serum was isolated for ELISA.

Assay of serum estradiol, LH and FSH

ELISA (DRG International ELISA Kit) was performed for estimating the circulating levels of estradiol, LH and FSH. For assay of serum estradiol, 25 µl each of standard, control and treated serum samples were added to respective wells coated with anti-estradiol antibody and incubated with 200 µl of enzyme conjugate for 2 hours at room temperature. Subsequently, 100 µl of substrate was added and incubated for 15 minutes at room temperature. Reactions were stopped using 50 µl of stop solution and the O.D was measured at 450 nm. Each sample was run in triplicate. For assay of serum FSH and LH, 25 µl of standard, control or treated serum samples were added to respective wells coated with anti-FSH and anti-LH antibodies and incubated with 100 µl of substrate was added to each well. After incubation for 10 minutes at room temperature, reactions were stopped using 50 µl of stop solution and the O>D measured at 450 nm. The intra-assay and inter-assay variations were found to be less than 9% and 10% respectively. Limit of detection for estradiol was 3.6 pg/ml, for LH was 0.45mlU/ml and for FSH was 0.28mlU/ml.

Signature of the Principal Investigator **Principal**