Annexure III

University Grants Commission

Bahadur Shah Zafar Marg

New Delhi- 110 002

Annual/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)-08/KABA023/UGC-SWRO
Dated 28/03/2008

3 Period of report from **:** July 2008 to June 2010

4.Title of the research project **:** "Functional Activity of Selenium on Arsenic Induced Tumours in Mice Liver."

5. a). Name of the Principal Investigator **:**Dr. Smt. S.N. Dharwadkar

b).Dept of University/College **:** Dept of Zoology, KLE's S. Nijalingappa College II Block Rajajinagar, Bengaluru-10

6. Effective date of starting the project **:** July 2008

7. Grants approved and expenditure incurred during the period of the project

a). Total amount approved **Rs.62,000/- (Rs.57,800**/- released

b). Total Expenditure **: Rs.63,130/-**

c). Report of the work done:

I). Brief objectives of the project

- To evaluate and **assess** the chronic effect of tri oxide on laboratory animal model (mice), their behavioural, morphological, physiological and anatomical changes for a period of One year.

- To evaluate and assess the effect of Sodium Selenite in induced tumours in the hepatic cells (Histopathology of Hepatic cells)

- To compare the toxicity of Arsenic (III) on the experimental animals and compare the data to that of the control.

- To evaluate and compare the concentration of protein in Aresenic pre-treated and compare it to that of Sodium Selenite treated, animal model.

II). Work done so far and results achieves and publications if any, resulting from the work. (Give details).

For the first few months our foremost task was to up-date the animal house and to train a person to take care of lab animals, and also to create hygienic conditions for the animals and complete sterilisation and fumigation of the animal house. Six to Eight months were required and needed for a scientific and hygienic animal house. Next step involved in setting up a lab exclusively for our project.

Materials and Methods: Chemicals Glassware, Reagents and other equipments.

The materials purchased are examination gloves, cotton absorbent, Nirlif sterile syringes, double distilled water BD 1ml syringes. The chemicals are sodium potassium tartarate, Benzoic acid, copper sulphate pentahydrate, Bovine Albumin fraction, sulphuric acid, sodium carbonate, anhydrous ammonium molibdate, trichloro acetic acid, animal feed, sodium selenite, arsenic trioxide(Merck GmbH,Germany), periodic acid schif,PB sodium salt, DPX, Glutaraldehide, aluminium foils, micropippetes, roter for the microtome, blades, repair and service of existing microtome, alcohol grades, watman filter paper, animal racks, plastic cages for animals, feeding bottles etc. Sodium hydroxide, female albino swiss mice, special tags, humidifier, weighing machine, thermometer etc. The chemicals purchased were of highest purity. A.R grade.

Animals and treatment: We have followed all the norms and the procedures for the use of Lab animals .We have an animal ethical committee and a registered animal house: No.626/02/a/CPCSEA.

- Animals and treatment: Sixty four(n=64) adult fertile female Albino Swiss Mice which were acclimatised for a period of one month (Dec 2009)(weight between 20-25 gm) were divided into nine groups were set for chronic exposure.

- Arsenic trioxide was prepared in 0.1N sodium The standard solution of ArsenHydroxide. Much before setting the experiment for toxicity testing the animals were brought from an authorised animal distributor Sri Venkateshwara traders suppliers of animals, animal cages and animal feeds.
- The animals were maintained in on a 12hr light/dark cycle starting from at 7.00 a. m with a temperature of 22± and 50% relative humidity. Mice were given access to feed with each one of them with a feeding bottle. Various concentrations of As_2O_3 and sodium selenite were prepared in duplicate.
- The experimental regimes were designed as **Set I, Set II & Set III**
- **Set I.** Received various concentrations ranging from 25 µmol (Group I), 50 µmol (Group II), 75 µmol(Group III) and 100 µmol (Group IV). (10^{-15}) dilutions respectively.
- **Set II.** Received various concentrations ranging from 20 µmol (Group V), 40 µmol (Group VI), 60 µmol (Group VII) and 80 µmol (Group VIII), (10^{-15}) I.P injections thrice a week at 10:00 am. The experimental regime was for a period of six months.
- **Set III.** Control mice,acted as IX group. Total No (n= 64).
- 0.2ml of standard solution of sodium selenite was prepared in double distilled water and was diluted up to 10^{-3}), was administered orally. Sodium Selenite treatment was started from March 2010 to June 2010.
- When Set I and Set II received IP Injections of chronic dose of arsenic trioxide, animals started behaving aggressively and violently, there was hair loss and weight loss (from 22gm to 21, 20, 19 and 18.5gm).
- Subsequently lesions were erupted on the drug administration site.
- In Set III (control) there was no hair loss and lesions did not erupt. They were healthy. At the end of the treatment both control and experimental mice were euthanized .
- 1 gm sample tissue was collected from each group, fixed in 10% formalin in saline, dehyderated in ascending grades of ethyl alcohol, cleaned in xylol and mounted in molten paraffin at 58^{o}-62^{o}C. 5µm sections were taken, stained with Eosin and evaluated for any structural changes under a bright field microscope.
- Subsequently Set IV & Set V were set for Sodium Selenite treatment (0.2 ml orally).

- Improvement and encouraging results like hair growth in hair loss area decrease in tumour size and healing in the area of legions.
- At the end of the treatment both control and experimental mice were euthanized.
- 1gm sample tissue was collected from few group, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraffin at 58^{o}-62^{o}C.
- 5µm sections were obtained, stained with Eosin and evaluated for any structural changes under a bright field microscope.

Histopathological Analysis:

- Light microscopic observation revealed that control hepatic tissue showed normal morphology.
- Large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged of kupffer cells.
- In contrast groups receiving trioxide (Set I & Set II), showed massive hepatotoxicity and the most pronounced histopathological abnormalities observed includes dissolution of hepatic cords, presence of dense focal inflammatory cells or necrotic tissues and vacuole accumulation.
- Changes in dense E.R and some mitochondria degeneration. The group receiving sodium selenite (Set IV & Set V) resulted in common histopathological observations showing much more or less like normal cell with normal morphology.

Biochemical Assay: Estimation of protein by Lawry's Method .The reagents such as 2% Sodium Carbonate, copper sulphate Folin'sCiocalteau, Bovine Serum Albumin(BSA), Trichloro acetic acid were standardised and stock solutions were prepared,1 gm of the tissue was homogenised then it was centrifuged ,the supernatant was taken the graph was plotted for protein .The optical Density at 640 nm (Blue filter) was calculated. for control, Arsenic trioxide treated and Arsenic + Selenium treated.

It was found that the arsenic treated mice have low concentrations of protein when compared to the Arsenic +selenium treated mice, when exposed for a chronic period. It was even more than the control values.

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.

Initially there was difficulty in setting up the lab and in maintaining the animal house .

V. If the project is not being completed please indicate the approximate time by which it is likely to be completed.

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 commission.

Yes. **Enclosed Annexure – VI P.No: 13-14**

VII. Any other information which will help in evaluation of the work done on the project. At the completion of the project, the first report should indicate the output, such as

- Man power trained
- Ph.D awarded
- Any other impact
- Publication of the results

Signature of the Principal Investigator **Principal**

Annexure V

Statement of Expenditure in respect of Minor Research Project

1. Name of the Principal Investigator : Dr.Smt S. N. Dharwadkar

2. Dept of University/ College : Dept of Zoology K.L.E's S.Nijalingappa College II Block Rajajinagar Bangalore-10

3. UGC approval No and date : F.No MRP(S) 08/KABA023/UGC-SWRO Dated 28/03/2008

4. Title of the research project : "Functional Activity of Selenium on Arsenic Induced Tumours in Mice Liver".

5. Effective date of the starting of project : July 2008

6. a). Period of expenditure : Aug 2009 to June 2010

b) Details of expenditure : Rs. 63,130/-

Sl No	Item	Amount Allocated	Amount Received	Expenditure Incurred
I	**NON RECURRING GRANTS**			
A	Equipments	20,000/-		18744/-
II	**RECURRING GRANTS**			
B	Contingency	12,000/-	**57,800/-**	24,000.-
C	Chemicals	20,000		14,980/-
D	Field work and Travel	10,000/-		3,000/-
E	Any other (Internet computer etc			2,400/-
	Total	**62,000/-**	**57,800/-**	**63,130/-**

This is to certify that the total grant of **Rs.57, 800/-**(Fifty Seven Thousand Eight Hundred) received from the University Grants Commission under the scheme of support for Minor Research Project Entitled "Functional Activity of Selenium on Arsenic Induced Tumors in Mice Liver". vide letters, **1). First Installment of Rs. 41000/-(**Forty One Thousand) vide Letter No . **F.No MRP (S) 08/KABA 023/UGC-SWRO Dated 28/03/2008** And**Second and Final Installment of Rs.16800/-(**Sixteen Thousand Eight Hundred) vide **Letter No. F.No MRP (S) – 005/07 08/KABA 023/UGC-SWRO Dated 13/11/2010** has been fully utilized for which purpose it was sanctioned and in accordance with terms and conditions laid down by University Grants Commission.

Principal Investigator Principal

Consolidated Statement of expenditure in Respect of Minor Research Project for the grant of Rs. 57,800/- released vide

1. Rs. 41,000/- I instalment sanction Order No. MRP(S). F.No. 08/KABA 023/UGC-SWRO dated 28/03/2008

2. Rs. 16,800/- Second and FinalInstalment Sanction order No.MRP(S) 005/07-08/KABA 023/UGC-SWRO dated 13/11/2010

Details of the Expenditure

Amount Sanctioned Rs.	**Amount released Rs.. I Instalment II&Final Instal**	**Amount utilised Rs.**
62,000/-	**41,000/- 16,800/-**	**63,130/-**

	Item	Particulars	Bill/V no	Amount
I	**NON RECURRING GRANTS**			
01	Equipments	Racks, Cages and Feeding bottles	873	17,859/-
02		Plastic Boxes	V-01	150/-
03		Boxes	1224	145/-
04		Surgical instruments	S/2298 7	426/-
05		Markers	6926	45/-
06		Plastic voils	765	119/-
II	**RECURRING GRANTS**			
07	Contingency including Hiring Charges	In handling animals assisting in lab for six months (Rs.4,000x6=24,000)	V-02	24,000/-
08	Chemicals	For histopathology and biochemical estimations	1467	4,985/-
09		Sodium Selenite	3499	526/-
10		Sodium Arsenite	860	485/-

11		Watman Filter paper	550	500/-
12		Syringes	30320	78/-
13		Hand wash	V-03	30/-
14		Watman filter paper	575	499/-
15		Animal and feed	873	7,650/-
16	Field work and Travel	Frequent visit to NIMHANS and IISC	V-04	3000/-
	Any other			
17		Film Roll	V-05	30/-
18		Marker	35	10/-
19		Markers	393	35/-
20		Soaps	557	50/-
21		K G Board	348	20/-
22		Voils	36	68/-
23		Internet, Photography	V-06	2,400/-
24		K G Road	40	20/-
	Total			**63,130/-**

Rs. Sixty Three Thousand One Hundred and Thirty only

Principal Investigator **Principal** **Statutory Authority**

Annexure-VI

Executive Summary: The Minor research project Entitled "Functional Activity of Selenium on Arsenic Induced Tumours in Mice Liver", was designed to investigation the effects of Selenium on arsenic induced tumours for chronic studies and its effects on behaviour histopathology, and biochemical assay.

Arsenic which has no known important functions is ubiquitous in nature. The presence of arsenic is in any form in amount greater than a trace, can be considered abnormal.

Regulation of genes is a vital process for the expression of a character. Cancer is Regular disease of genes in which the tumour suppressor gene p53 is damaged beyond repair, and hence results in the manifestation of cancer. The cancer may be of any kind, but the basic concept remains more or less the same. We propose to study the antineoplastic activities of selenium complex on arsenic which is a potential neoplastic promoter in mice hepatocyte with an objective to not only expand our knowledge but to know the molecular changes in mice neoplasm.

To evaluate andassess the chronic effect of arsenic trioxide on laboratory animal model (mice), their behavioural, morphological, physiological and anatomical changes for a period of One year. To evaluate and assess the effect of sodium selenite in arsenic induced tumours in the hepatic cells (Histopathology of Hepatic cells)

To compare the toxicity of Arsenic (III) on the experimental animals and compare the data to that of the control.

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Various concentrations of As_2O_3 and sodium selenite were prepared in duplicate. The experimental regimes were designed as Set I and Set IIfor chronic studies.

Set I. received various concentrations ranging from 25 µmol, 50 µmol, 75 µmol and 100 µmol (10^{-15}) dilution.

Set II.received various concentrations ranging from 20 µmol, 40 µmol, 60 µmol and 80 µmol (10^{-15}). I.P injections thrice a week at 10:00 am. The experimental regime was for a period of six months.

Set III acted as IX group, were the control animals (n= 64).

Sodium Selenite treatment was done from March 2010 to June 2010, We started treating the experimental animals(arsenic tri oxide pre-treated) with Sodium

Selenite, 0.2ml of standard solution of sodium selenite was prepared in double distilled water and was diluted up to 10^{-3}).

We observed abnormal pattern of animal behaviour including abnormalities in histopathology of hepatic cells and decreased concentrations in arsenic induced hepatic cell carcinoma when compared to a normal (control) tissue.

When Set I and Set II received IP Injections of chronic dose of arsenic trioxide, animals started behaving aggressively and violently, there was hair loss and weight loss (from 22gm to 21,20,19 and 18.5gm) Subsequently lesions were erupted on the drug administration site. In Set III (control) we neither noticed hair loss nor lesions, they were healthy. At the end of the treatment both control and experimental mice were euthanized. 1gm of liver tissue was collected from a few group, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleaned in xylol and mounted in molten paraffin at 58^{o}-62^{o}C. 5µm sections were taken, stained with Eosin and evaluated for any structural changes under a bright field microscope. Subsequently Set IV & Set V was set for Sodium Selenite. We administered 0.2 ml of Sodium Selenite solution orally. Subsequently Set IV & Set V were set for Sodium Selenite treatment (0.2 ml orally). Improvement and encouraging results like hair growth in hair loss area decrease in tumour size and healing in the area of legions. At the end of the treatment both control and experimental mice were euthanized. 1gm sample tissue was collected from few group, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraffin at 58^{0}-62^{o}C. 5µm sections were obtained, stained with Eosin and evaluated for any structural changes under a bright field microscope.

Histopathological Analysis: Light microscopic observation revealed that control hepatic tissue showed normal morphology, large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged of kupffer cells. In contrast groups receiving Arsenic trioxide (Set I & Set II)showed massive hepatotoxicity and the most pronounced histopathological abnormalities observed includes dissolution of hepatic cords, presence of dense focal inflammatory cells or necrotic tissues and vacuole accumulation. Changes in dense E.R and some mitochondria degeneration. The group receiving sodium selenite (Set IV & Set V) resulted in common histopathological observations showing much more or less like normal cell. with normal morphology.

Biochemical Assay: Estimation of protein by Lawry's Method .The reagents such as 2% Sodium Carbonate, copper sulphate Folin'sCiocalteau, bovine Serum Albumin (BSA), Trichloro acetic acid were standardised and stock solutions were prepared,1gm of the tissue was homogenised then it was centrifuged, the supernatant was taken the graph was plotted for protein .The optical Density at 640 nm (Blue filter) was calculated. For control, Arsenic trioxide treated and Arsenic + Selenium treated. It was found that the Arsenic + selenium treated for a period of six months have shown that increase in the protein level when compared to the normal.

The science of cancer chemotherapy has long been recognised a necessity to help a close interaction among chemists, biochemists, pharmacologists, molecular biologists and toxicologists .Such a concerned enterprise is sorely lacking in the cancer chemoprevention arena. Currently there are hundreds of chemicals that have been and are being evaluated for anticancer activity in both *in-vivo* and *in-vitro* models. Na-selenite acts as antagonistic agent to Arsenic trioxide there by reducing the toxicity to a greater extent. (Borne by experimental findings of the author).

The present investigation with the results in terms of chronic effects on various aspects studied in albino mice would serve as useful criteria to draw some conclusion with regards to Arsenic trioxide. The alteration in cancer manifestation in Arsenic pre-treated and the rate of protein levels in optical density and their behaviour would be taken as a pointer to show some metabolic strategies on mice may adapt to overcome the toxic stress provided that the stress falls within the zone of tolerance. The conclusion drawn at some places may perhaps be considered as useful signals for further probe into the toxicity of Arsenic health hazard.