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TARGETING GLYCOLYTIC STRESS TO STARVE CANCER CELLS BY SODIUM OXAMATE AND 5-BROMO ISATIN IN URETHANE INDUCED LUNG CARCINOGENESIS

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ABSTRACT

These studies have provided strong evidence that glycolysis up regulation represents a clear advantage for cancer cells and at the same time a target for new anticancer therapies. Balb/c mice of male sex were divided into 4 groups of 6 animals each. Sodium oxamate and 5-Bromo isatin was evaluated against urethane induced lung carcinogenesis and observed the body weight of animals daily for 26 week. At the end of the study lung tissue were subjected to evaluation for tumor incidence based on morphological and histological study and a portion of lung tissue was subjected to evaluation of parameters of glycolytic stress. Both drugs showed a significant anticancer activity in urethane induced lung carcinogenesis model. The anticancer activity of the sodium oxamate (250 mg/kg) and 5-bromo isatin (50 mg/kg) were found be extremely significant compared to cancer control animals. In treatment group, markers of glycolytic stress were significantly decreased; this might be due to treatment groups less uptake glucose through glycolytic pathway by glycolytic enzyme. Results obtained from the study indicates that sodium oxamate and 5-bromo isatin possess promising anticancer activity The activity might be drug treatment was inhibited glycolytic stress through blockage glycolytic enzymes.

KEYWORDS: Anticancer activity, Glycolytic enzyme inhibitors, Sodium oxamate, 5-bromo isatin, Urethane.

INTRODUCTON

Cancer can be defined as an unregulated growth of cells arising from one cell. The scientific or medical term for cancer is malignant neoplasm, which is defined as a relatively autonomous growth of tissue not subject to the rules and regulations of normal growing cells. Tumor is a general term indicating any abnormal mass or growth of tissue.

Glycolytic difference between normal cell and cancer cell

Cells require energy to absorb nutrients to react to changes in their environment, grow and replicate. Normal cells, under normal conditions, undergo aerobic respiration, which is a metabolic pathway that requires oxygen. Cells break down glucose into pyruvate, to eventually form ATP, while releasing CO_2 as a waste product. When there is not enough oxygen, cells switch to a different type of respiration called anaerobic respiration.

During glycolysis, glucose is subjected to a series of biochemical transformations devoted to demolishing its structure with the production of energy ATP, and each step is catalyzed by specific enzymes. In normal cells, the glycolytic process is mostly coupled to OXPHOS, so pyruvate enters into mitochondria and undergoes oxidative transformation into acetyl CoA, which then enters the tricarboxylic acid (TCA) cycle and eventually produces CO_2 together with a considerable amount of ATP. Under certain conditions, however, especially under oxygen deprivation, OXPHOS cannot take place, so pyruvate is instead converted into lactate by lactate dehydrogenase (LDH). This final step in fundamental because it allows regeneration of the oxidized cofactor NAD^+, which is required for the regular progress of glycolysis, even when there is not enough oxygen to promote NADH re-oxidation. In this case lactate is then ejected from the cell by monocarboxylate transporter-4 (MCT4) to maintain intracellular pH levels within an acceptable range. Extrusion of lactate from the cell is one of the main causes of extracellular acidosis occurring in these situations. This “anaerobic” glycolytic pathway is much less efficient than OXPHOS in producing energy, as only two molecules of ATP are produced by each glucose molecule, versus the -36 ATP units usually produced by the TCA cycle. However, Glycolysis generates ATP more rapidly than OXPHOS, and this offers a selective advantage for rapidly growing tumor cells.[1,2]

Increased glycolysis in cancer cells

Over 70 years ago, Warburg (Warburg 1930) observed that cancer cells frequently exhibit increased Glycolysis and depend largely on this metabolic pathway for generation of ATP to meet their energy needs. He attributed this metabolic alteration to mitochondrial respiration

injury and considered this as the most fundamental metabolic alteration in malignant and transformation or "the origin of cancer cells" (Warburg 1956). Although the underlying mechanisms responsible for the Warburg effect are rather complex and can be attributed to a variety of factors, such as mitochondrial defects and hypoxia, the metabolic consequences are similar. The compromised ability of cancer cells to generate ATP through oxidative phosphorylation forces the cell to increase Glycolysis to maintain their energy supply and thus renders cancer cells highly dependent on this metabolic pathway for survival. As such, it is conceivable that the metabolic alterations in malignant cells may be exploited to serve as a biochemical basis to develop therapeutic strategies to target this abnormality. One possibility is to inhibit Glycolysis and preferentially kill the cancer cells that are dependent on glycolytic pathway for ATP generation.[3,4,5]

Glycolytic enzyme inhibitors in cancer treatment

Glycolysis inhibitors can be used mostly in fighting cancer cells that have mitochondrial defect or in hypoxic condition. When there is glycolysis inhibition the normal body cells will be able to adapt to other metabolic pathway to generate the ATP energy through the TCA cycle and oxidative phosphorylation in the mitochondria. The cancer cells however cannot adapt to other source of ATP as they have defects hence will die as they will not be able to live without another source of ATP energy. The healthy body cells will be able to adapt to new source of energy like fatty acid and amino acid to produce metabolic intermediates channeled to the TCA cycle for ATP production through respiration. Glycolysis inhibition is therefore a clever way of killing the cancer cells without harm on the healthy body cells.[6,7,8]

Sodium oxamate (LDH inhibitor) has been studied as an inhibitor of carbohydrate metabolism in various models. Several invitro studies confirmed the anticancer property of sodium oxamate.[9,10] Isatin and its derivatives were found to inhibit this cytosolic enzymes such as, glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase in glycolytic pathway.[11,12]

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) [Proposed no: DAMCOP/IAEC/029] of Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India. Male Balb/c mice weighing 25-35 g were

used in this study. The animals were procured from Central lab animal facility, Amrita Institute of Medical Science and Research Centre, Ponekkara, Kochi.

Chemicals

Sodium oxamate and 5-bromo isatin procured from sigma Aldrich louis, mo, USA. Other chemicals was purchased from Chemdyescorp, Gujarat, India.

1. Experimental design

On the day of the experiment, the animals were divided randomly into control and experimental groups (n=6).

Group I serve as control and receive 0.5% w/v carboxy methyl cellulose suspension 1 ml/100 g once daily orally for a period of 26 weeks.

Group II serve as negative control and receive urethane 600 mg/kg dissolved in sterile 0.9% w/v of Nacl intraperitoneally once weekly for 10 weeks. Following the first injection of urethane the mice received the 0.5% w/v carboxy methyl cellulose 1 ml/100g once daily orally for a period of 26 weeks.

Group III serve as treatment group and receive urethane 600 mg/kg dissolved in sterile 0.9%w/v of Nacl intraperitoneally once weekly for 10 weeks. Following the first injection of urethane the mice received the sodium oxamate 250 mg/kg body weight in 0.5% w/v carboxy methyl cellulose once daily orally for a period of 26 weeks.

Group IV serve as treatment group and receive urethane 600 mg/kg dissolved in sterile 0.9% w/v of Nacl intraperitoneally once weekly for 10 weeks. Following the first injection of urethane the mice received the 5-bromo isatin 50 mg/kg body weight suspended in 0.5% w/v carboxy methyl cellulose once daily orally for a period of 26 weeks.

During the period of study the body weight of animals were recorded once weekly till the end of the study. At the end of the study blood samples was collected by retero orbital puncture under anaesthesia (Ketamine 100 mg/kg i.p) and evaluated for tumor marker (CEA-carcino embryonic antigen).

After blood collection the animals was euthanized and the lung immediately excised, washed with ice cold saline, dried using a whatman's filter paper and weighed to calculated the

relative lung weight. Gross morphological examination was carried out to observe lung tumor incidence, multiplicity of tumors and tumor load (sum of the tumor per lung in average). The lung tissue was subjected to evaluation of inflammatory markers (CRP- reactive protein) and subjected to evaluation of glycolytic stress. A portion of lung tissue was subjected to histopathological examination to evaluated the microscopical changes in the tissue.[13]

a) Preparation of blood sample

To 0.5 ml of blood added 2 ml of perchloric acid in an eppendrof tube. Stand for 10 minutes (room temperature) and micro centrifuged for 15 minutes at 3000 rpm. After centrifugation supernatant collected using syringe and kept in freezer (-200C).

b) Preparation of tissue sample

100 mg lung tissue was homogenized in 2 ml of 6% v/v perchloric acid. The homogenate centrifuged at 5000 rpm for 10 minutes. The supernatant was withdrawn and neutralized with 2 ml 5M potassium hydroxide. Again centrifuged and the supernatant taken for analysis which is kept in freezer (-20◦C).

2. Determination of blood glucose

Procedure

Took clean dry test tubes labelled as Blank (B), Standard (S) and Test (T). Pipette out 1ml of glucose reagent to all the test tube and added 0.01 ml of distilled water, glucose standard and sample to the Blank (B), Standard(S) and Test (T) respectively. Mix well and incubate at 37°c for 10 min or at room temperature (25°c) for 30 minutes. Measure the absorbance of standard and test sample against the blank within 60 minutes. The result was calculated by using the following formula.[14]

$$\text{Total Glucose in mg/dl} \quad \frac{\text{Abs. T}}{\text{Abs. S}} = \text{X } 100$$

3. Determination of Lactate in blood and tissue

Procedure

1. Prepare Reaction Mixture-Reconstitute the appropriate number of NAD vials (Catalog Number N8285) required by pipetting the following reagents into each vial: 2.0 ml Glycine buffer (Catalog Number G5418), 4.0 ml water, 0.1 ml L-Lactate dehydrogenase (Catalog Number L3916).

2. Cap and invert the vials several times to dissolve the NAD. The Reaction Mixture remains active for 4 hours at room temperature or 24 hours at 2–8°C.
3. Pipetted 2.9 ml of reaction mixture in to cuvettes labelled as BLANK and TEST.
4. To the cuvette labelled BLANK add appropriate blank solution both(water, 0.1ml 8% v/v Perchloro acetic acid)in to Blank. Mix gently by inversion.
5. Added 0.1 ml of biological sample in to Test and mix both gently by inversion. Incubate test tubes for 15 minutes at 37°C and measure the absorbance of Test Sample against the Blank as reference at 340nm.[15,16,17]

4. Determination of Pyruvate in blood and tissue

Procedure

Labelled the test tubes as Reagent Blank (B), Standard(S) and Test(T).1 ml of the biological sample was taken and to it 1 ml of 0.001 M 2,4- Dinitro phenyl hydrazine and 3 ml of 0.4 N sodium hydroxide to test sample. After 10 minutes, the optical density of the colour was measured in a spectrophotometer at 540 nm against the reagent blank. Pyruvate standards were prepared for comparison.[18,19]

5. Determination of SDH in tissue

Procedure

The reaction mixture containing 1 ml of 0.3 M of phosphate buffer pH(7.4), 0.1 ml of EDTA (0.03 M), 0.1 ml of 3% w/v BSA, 0.3 ml of 0.4 M sodium succinate pH(7.6), 0.2 ml 0.075 M potassium ferricyanide and made up to 2.8 ml with distilled water. The reaction was started by addition of 0.2 ml of mitochondrial suspension. The change in absorbance was recorded at 30 second interval for 3 minutes at 420 nm. The activity was expressed as nano moles of succinate oxidised per minute per mg of protein.[20,21]

6. Determinaton of LDH in blood and tissue

Procedure

Dilute the serum five-fold with saline by mixing 0.2 ml of serum with 0.8 ml of normal saline.

Determination of absorbance of the Blank and Test

Label the test tubes as Reagent Blank (B), and Test (T). Pipette 1 ml of buffered substrate and 0.2 ml of NAD solution in both tubes and placed them in a water bath (37◦C) for five minutes in order to equilibrate the temperature. Added 0.1 ml of serum in the tube marked “T and

added 1 ml of serum in the tube marked B (serum blank). Exactly after 15 minutes of incubation at 37◦C added 1 ml of hydrazine solution to both the tubes. Mixed the contents of each tube and continued incubation for another 15 minutes (exact timing is not necessary). Added 10 ml of NaOH solution (0.4 M) to both the tubes and mixed. Measured absorbance of Test Sample (Abs.T) and Blank (Abs.B) against water after 10 mins at 505 to 510 nm (green filter). The result was calculated by using the following formula.

▲A = Abs.T–Abs.B

Determine the absorbance of standard following way, Label the test tubes as Reagent Blank (B), and Standard (S) and mixed 1 ml of substrate and 0.3 ml of water in a blank test tube. In standard test tube was mixed 1 ml of substrate, 0.1 ml of pyruvate standard and 0.2 ml of water. Added 1 ml of hydrazine solution in both tubes, left for 15 minutes at 37◦C and then added 10 ml of NaOH (0.4N). The absorbance was determined the standard against the blank.[22]

7. Determination of CEA in blood

The amount of CEA present in the blood is determined by electrochemical luminescence.[23]

8. Determination of CRP in blood

The amount of CRP present in the blood is determined by high-sensitivity Immunoturbidimetry method.[24]

9. Determination of relative lung weight

After sacrificing the animals, lung was removed and the washed free of extraneous material and weighed. The relative lung weight was calculated as per the formula given below:

$$\text{Relative lung weight} = \frac{\text{Lung weight}}{\text{Animal body weight}} \times 100$$

Multiplicity of tumor

It is defined as average number of tumors per mouse. And it is obtained by dividing the total number of tumor by the total number of mice per group including non tumor bearing mice.

Tumor volume

$$\text{Tumor volume} = \frac{\Pi}{6} \times \text{length} \times \text{width} \times \text{height}$$

Tumor load

Tumor load = Mean number of tumors× mean tumor volume

Tumor volume

$$\text{Tumor volume} = \frac{\text{Number of effected mice}}{\text{Total no of mice available For examination}} \times 100$$

10. Histopathological examination of lung

BALB/c mice were sacrificed after 26 weeks of drug administration. Lung were perfuse with saline via the pulmonary artery and infused with 10% buffered formalin for 24 hrs. Fixed lung were paraffin embedded cut into 4µm sections, A fixed to glass slides and stained with haematoxylin/ eosin to visualize lung structure using an Olympus BX-41 Microscope with digital camera.

11. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM) by instat softwere. Different groups were assessed by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey test (Graph pad software Inc, La Jolla, CA. Trial version 7.03).

RESULTS AND DISCUSSION

1. Body weight of animals

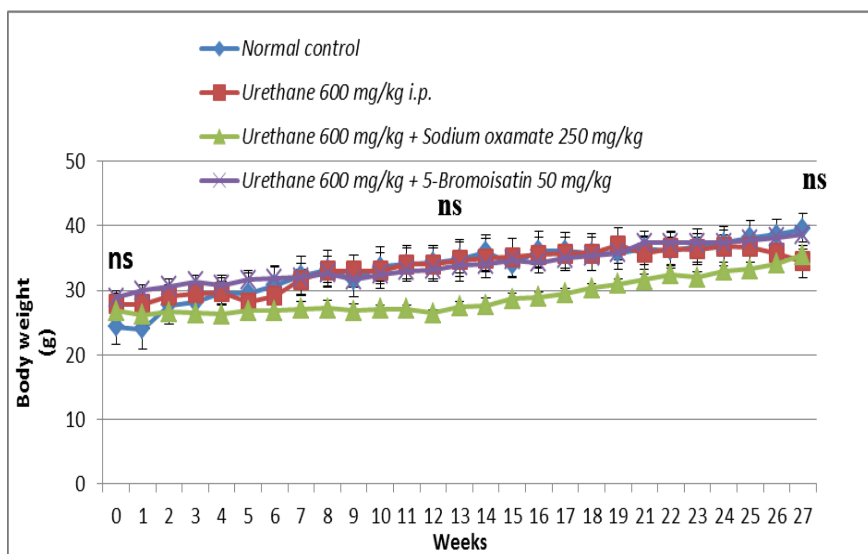

Fig.1: Effect of sodium oxamate and 5-bromo isatin on body weight of urethane induced lung carcinogenesis in Balb/c mice.

There was no significant difference between urethane treated animals and normal control. Animal weight of treated group was no significant difference compared with cancer control group.

2. Determination of CEA and CRP in blood

Table 1: Effect of sodium oxamate and 5-bromo isatin on serum CEA &CRP of Urethane induced lung carcinogenesis in Balb/c mice.

Group	Treatment	CEA(Mg/ml)	CRP(mg/L)
Group I	0.5 % CMC 1ml/100 g oral	2.00 ± 0.05774	1.00 ± 0.09121
Group II	Urethane 600 mg/kg i.p	7.58 ± 0.2136***a	7.60 ± 0.2164***a
Group III	Urethane 600 mg/kg/ i.p.+ Sodium oxamate 250 mg/kg oral	2.9±0.1211***b	2.725±0.133***b
Group IV	Urethane 600 mg/kg/ i.p.+ 5-bromo isatin 50 mg/kg oral	3.066±0.0881***b	2.52±0.133***b

Data expressed as Mean ± S.E.M. n=6 a –comparison between normal and negative control. b- Comparison between negative control and other treatment group excluding normal *p<0.05, **p<0.01, ***p<0.001.

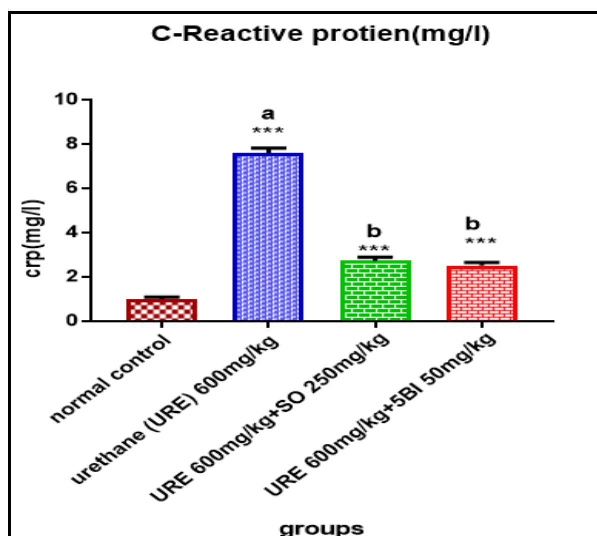

Fig. 2: Variation in CRP levels in different groups.

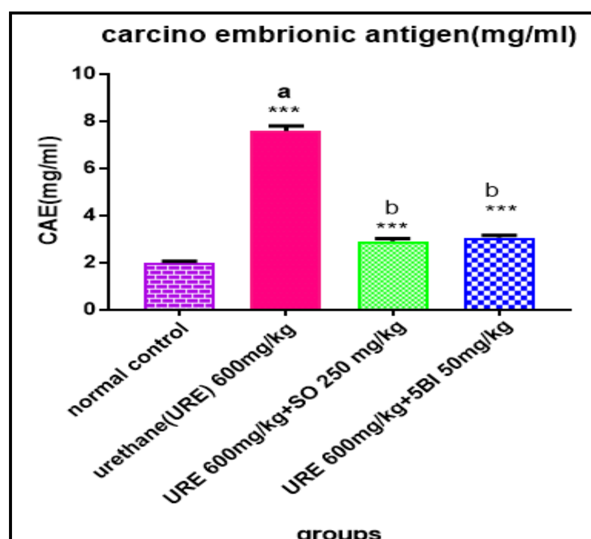

Fig. 3: Variation in CEA levels in different groups.

3. Determination of Lactate, pyruvate, LDH and glucose in blood

Table 2: Effect of sodium oxamate and 5-bromo isatin on blood parameters of urethane induced lung carcinogenesis in Balb/c mice.

S.	Group	Treatment	Lactate(mmol/L)	Pyruvate(mmol/L)	LDH (U/L)	glucose(mg/dl)
1.	Group I	0.5%CMC1ml/100g oral	2.386±0.05795	0.0426±0.00181	266.84±3.051	74.166±1.376
2.	Group II	Urethane 600 mg/kg i.p.	5.21±0.1930[***a]	0.123±0.0074[***a]	671.04±5.324[***a]	128.5±44.745[***a]
3.	Group III	Urethane 600 mg/kg/ i.p.+ Sodium oxamate 250 mg/kg oral	2.983±0.0424[***b]	0.073±0.00230[***b]	343.93 ±3.510[***b]	81.5±1.478[***b]

4.	Group IV	Urethane 600 mg/kg/ i.p.+ 5-bromo isatin 50 mg/kg oral	3.26±0.06947[***b]	0.0750±0.00371[***b]	371.32±1.6787[***b]	83.33±1.453[***b]

Data expressed as Mean ± S.E.M. n=6 a – comparison between normal and negative control. b- Comparison between negative control and other treatment group excluding normal *p<0.05, **p<0.01, ***p<0.001.

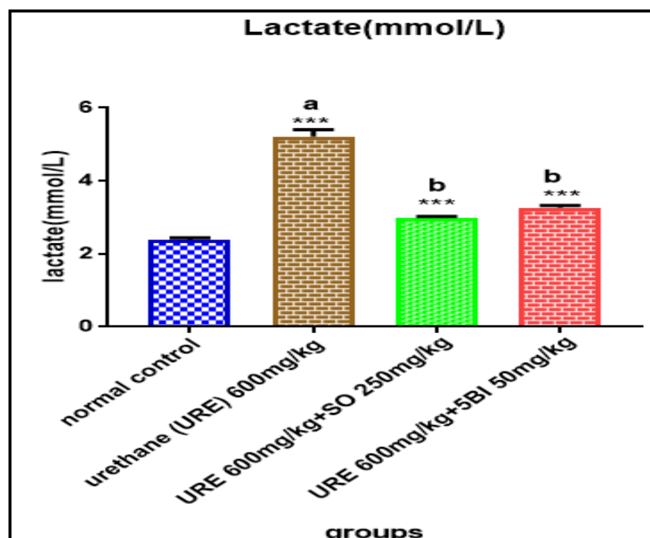

Fig. 4: Variation in blood Lactate levels in different groups.

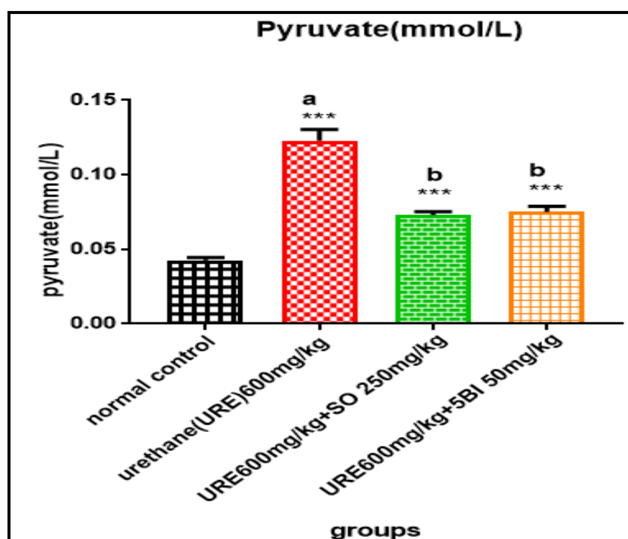

Fig. 5: Variation in blood pyruvate levels in different groups.

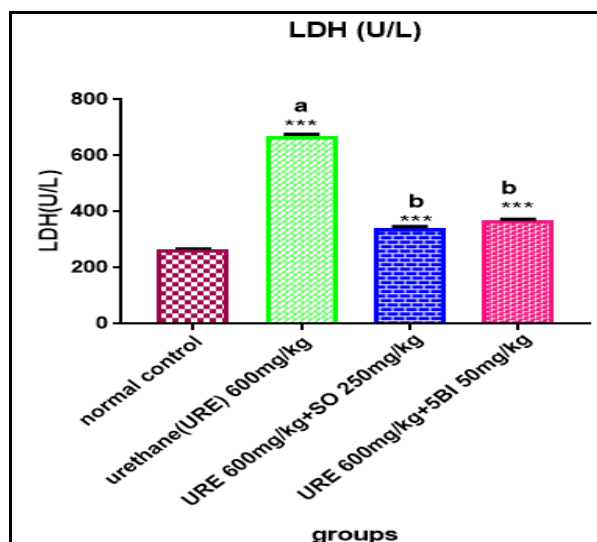

Fig. 6: Variation in blood LDH levels in different groups.

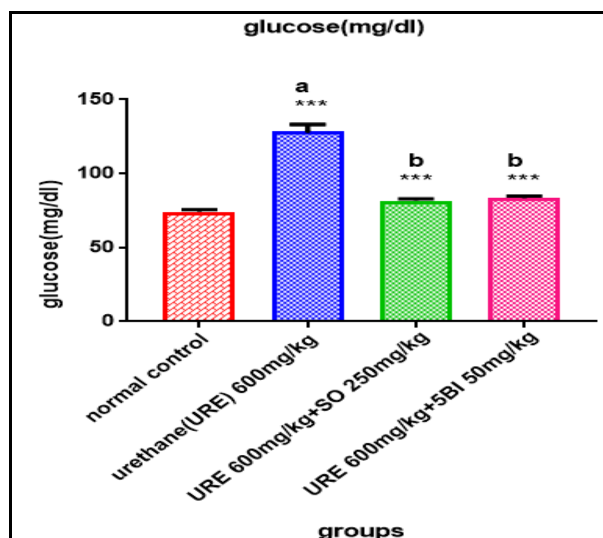

Fig. 7: Variation in blood glucose levels in different groups.

The blood parameters shows there was a significant increase in (lactate, pyruvate, glucose, LDH) treatment with urethane (Table No.2, Fig.4-7). After treatment with sodium oxamate and 5-bromo isatin along with urethane significantly reduced the levels of blood parameters.

4. Determination of Lactate, pyruvate, LDH and SDH in tissue

Table 3: Effect of sodium oxamate and 5-bromo isatin on tissue parameters of urethane induced lung carcinogenesis in Balb/c mice.

No	group	Treatment	Lactate (µmol/gm)	Pyruvate (µmol/gm)	LDH (nmole)	SDH (nmole)
1.	Group I	0.5 % CMC 1ml/100 g oral	1.5233±0.05071	0.04766±0.00270	266.47±5.985	114±2.582
2.	Group II	Urethane600 Mg/kg/i.p	7.92±0.09465***a	0.159±0.0037***a	630.89±6.638***a	61.833±2.535***a
3.	Group III	Urethane600 mg/kg/ i.p.+ Sodiumoxamate 250 mg/kg oral	2.961±0.0987***b	0.06583±0.00164***b	330.6 ±12.05***b	82±1.238***b
4.	Group IV	Urethane 600 mg/kg/ i.p.+ 5-bromo isatin 50 mg/kg oral	3.02±0.145***b	0.07080±0.002124***b	389.9±10.994***b	79.5±0.7638***b

Data expressed as Mean ± S.E.M. n=6 a – comparison between normal and negative control. b- Comparison between negative control and other treatment group excluding normal *p<0.05, **p<0.01, ***p<0.001

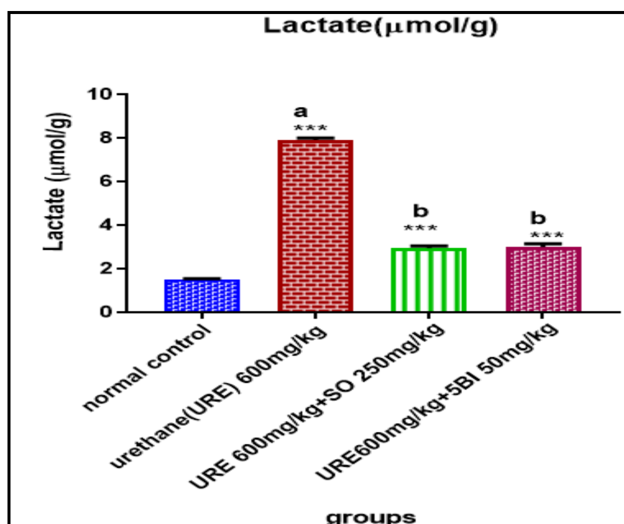

Fig. 8: Variation in tissue Lactate levels in different groups.

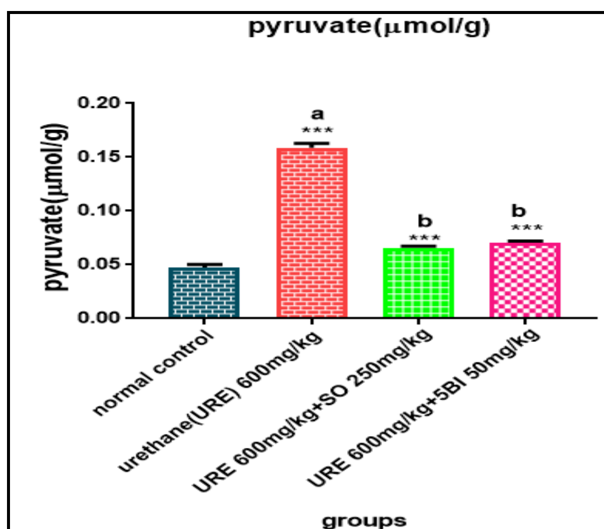

Fig. 9: Variation in tissue pyruvate levels in different groups.

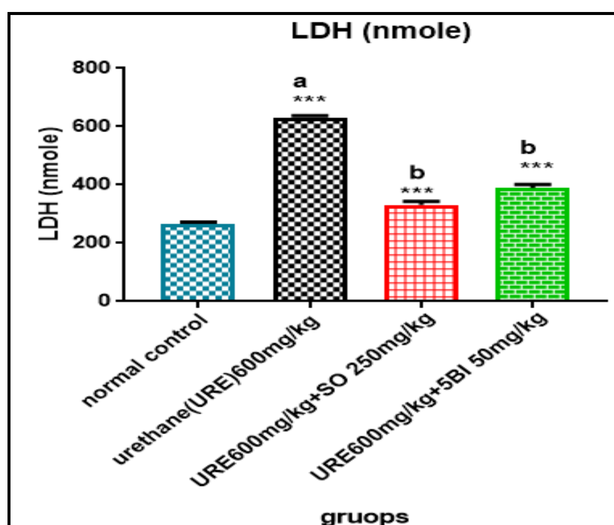

Fig. 10: Variation in tissue LDH levels in different groups.

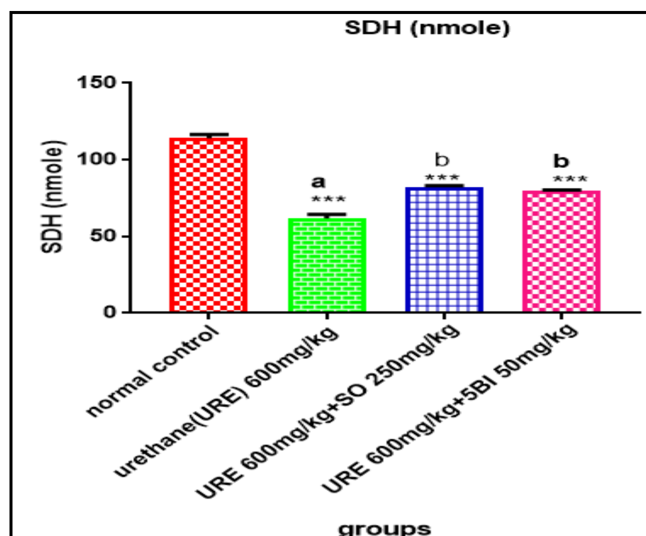

Fig. 11: Variation in tissue SDH levels in different groups.

The tissue parameter shows there was a significant increase in (lactate, pyruvate, LDH) after treatment with urethane except SDH value (Table 3, Figure 8-11). After treatment with sodium oxamate and 5-bromo isatin along with urethane significantly reduced the levels tissue parameters. It shows that urethane caused significant decrease in SDH and increase in level of LDH, lactate, pyruvate (Table 3). Treatment with drugs alters the levels, near to normal level.

5. Morphological examination of lungs

Table 4: Effect of sodium oxamate and 5-bromo isatin on tumor parameters of urethane induced lung carcinogenesis in Balb/c mice.

S.No.	Group	Treatment	Tumor incidence (%)	Tumor volume (mm^3)	Tumor multiplicity	Tumor load (mm^3)
1.	Group I	0.5 % CMC 1ml/100 goral	-	-	-	-
2.	Group II	Urethane 600 mg/kg i.p.	100	0.495±0.02320	67.33±7.94	33.3
3.	Group III	Urethane 600 mg/kg/ i.p. + Sodiumoxamate 250 mg/kg oral	50	0.205±0.01910	24.5 ±1.408	5.22
4.	Group IV	Urethane 600 mg/kg/ i.p. + 5-bromo isatin 50 mg/kg oral	50	0.2433±0.1116	26.5±1.054	6.447

Data expressed as Mean ± S.E.M. n=6 values are comparison between negative control and other treatment group excluding normal *p<0.05, **p<0.01, ***p<0.001.

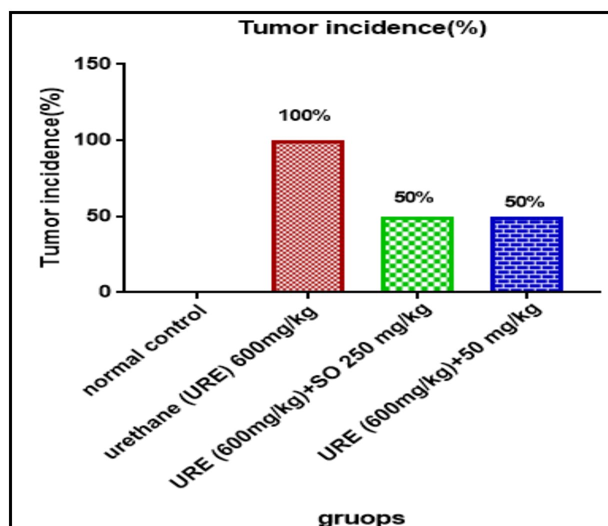

Fig. 12: Variation in Tumor incidence levels in different groups.

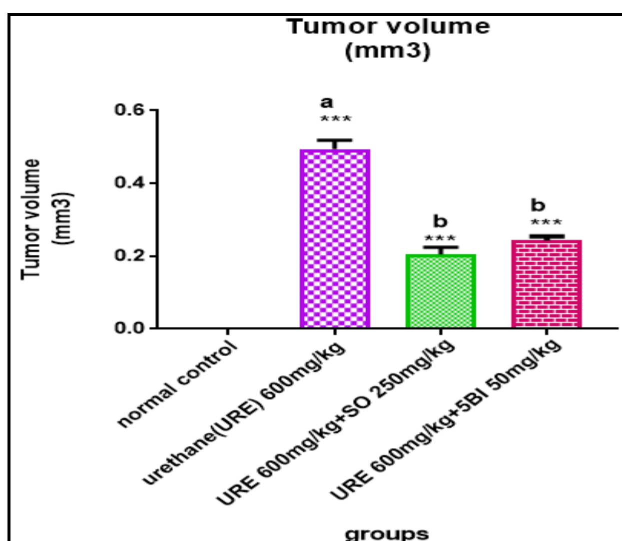

Fig. 13: Variation in Tumor volume levels in different groups.

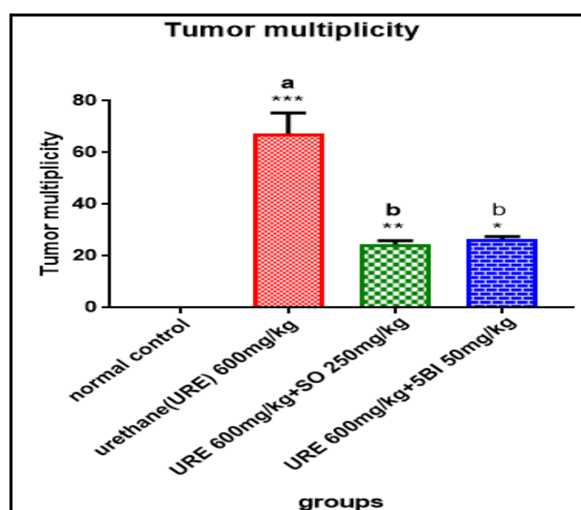

Fig. 14: Variation in Tumor Multiplicity levels in different groups.

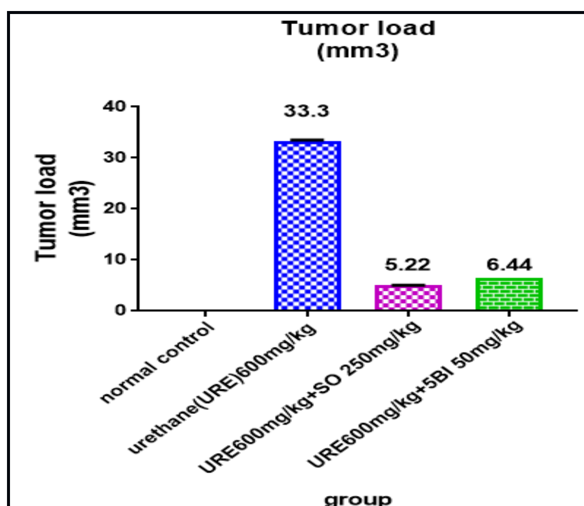

Fig. 15: Variation in Tumor load levels in different groups.

6. Determination of relative lung weight of Balb/c mice.

Table No. 7: Effect of sodium oxamate and 5-bromo isatin on relative lung weight of urethane induced lung carcinogenasis in Balb/c mice.

S.No	Groups	Treatment	Relative lung weight (g lung/100gm)
1.	Group I	0.5 % CMC 1ml/100 g oral	0.51±0.01862
2.	Group II	Urethane 600 mg/kg i.p.	0.85±0.07303[***a]
3.	Group III	Urethane 600 mg/kg/ i.p.+ Sodium oxamate 250 mg/kg oral	0.598±0.0160[***b]
4.	Group IV	Urethane 600 mg/kg/ i.p.+ 5-bromo isatin 50 mg/kg oral	0.563±0.00243[***b]

Data expressed as Mean ± S.E.M. n=6 a – comparison between normal and negative control. b- Comparison between negative control and other treatment group excluding normal *p<0.05, **p<0.01, ***p<0.001.

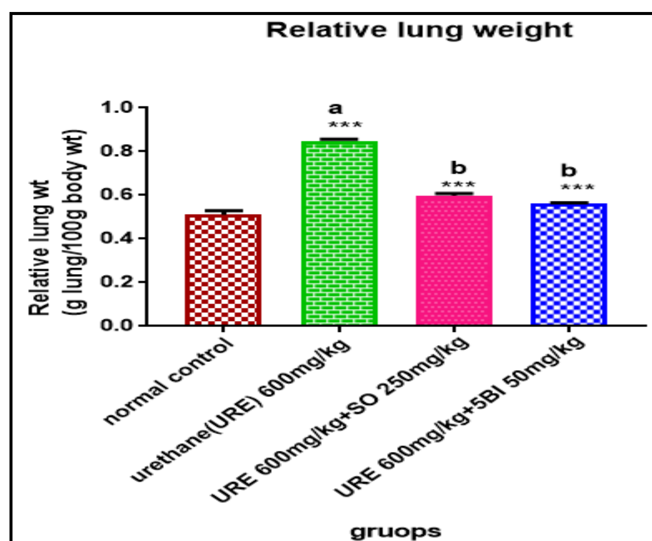

Fig. 16. Variation in relative lung weight levels in different groups.

There was a significant increase in the weight of lung treated with urethane (600mg/kg). Generally, a urethane treated lung increase in weight due to tumor formation. Treatment with sodium ooxamate and 5-bromo isatin significantly reduced the relative lung weight.

7. Structure of lungs

Fig. 17: Normal lung.

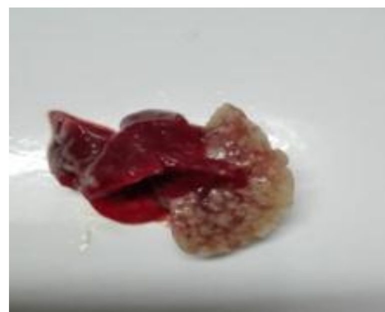

Fig. 18: urethane (600mg/kg).

Fig. 19: urethane(600mg/kg)+sodium oxamate (250 mg/kg).

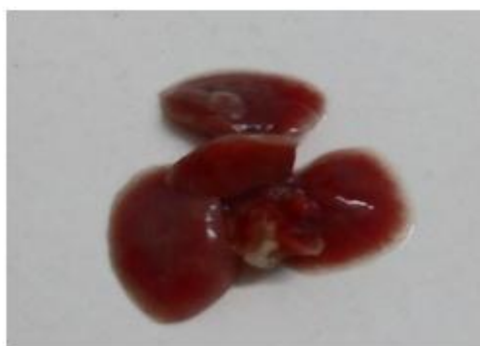

Fig. 20: Urethane (600mg/kg)+5-bromo isatin (50 mg/kg).

8. Histopathology of Lungs

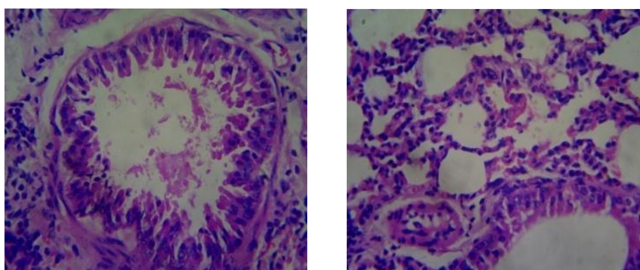

Fig. 21: Normal lung.

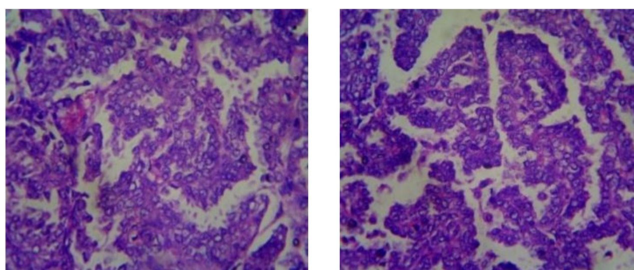

Fig. 22: urethane (600mg/kg).

Fig.21 showed the presence of normal bronchiole and alveoli and Fig.22 showed the presence of neoplasm cells.

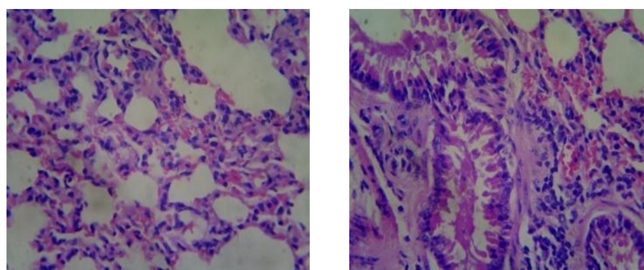

Fig. 23: urethane (600mg/kg)+sodium oxamate (250 mg/kg.

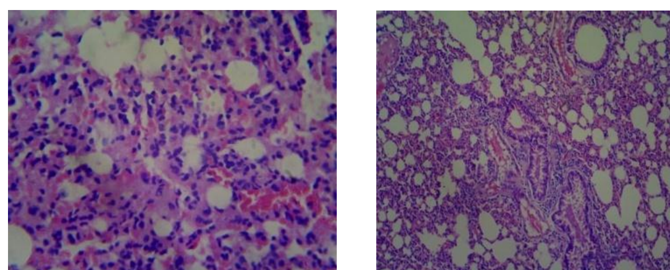

Fig. 24: urethane (600mg/kg)+5-bromo isatin (50 mg/kg).

Fig.23 showed the presence of Interstitial infiltration and Peribronchial inflammation and Fig.24 showed the presence of Inflammatory infiltrates and Blood vessel congestion.

DISCUSSION

Carcinogenesis is a complex, multistep process that requires the elimination of several cell-imposed barriers such as antiproliferative responses, programmed cell death-inducing mechanisms, and senescence.

Sodium oxamate is a derivative of pyruvate found to inhibit the conversion of pyruvate to lactate via lactate dehydrogenase, thus disrupting glycolysis. Sodium oxamate has been studied as an inhibitor of carbohydrate metabolism in various models. Isatin (1H -indole 2,3

dione) and its derivatives demonstrate a diverse array of biological and pharmacological activity including anticonvulsant, anti bacterial, anti viral and anticancer activity.

In present investigation, urethane (600mg/kg) treated group increased carcinogenic incidence and stimulated the markers of glcolytic stress such as lactate, pyruvate, LDH (in blood and tissue). This might be lung tumor cells that actively uptake glucose through glycolytic pathway by glycolytic enzyme for their energy source that increase the formation of lactate and pyruvate in lung tissue cells and blood. SDH level (mitochondrial enzyme) was found to be significantly decreased in urethane induced group. This might be due to disturbing mitochondrial function in cancer cells. Carcinogenic incidence, tumor multiplicity, tumor load and tumor volume increased significantly compared to treatment group due to increase numbers of tumor rate. Histopathological analysis of lung section was supported heamatologial and tissue parameters results.

In present investigation, animals treated with sodium oxamate and 5-bromo isatin showes significant decrease in glycolytic stress parameters (lactate, pyruvate LDH) in both blood and tissue comparing with cancer control (600mg/kg). This might be due to drug treatment cells that inhibited glycolytic stress through blockage glycolytic enzymes, comparatively higher than the tumor cells. So the drug treated groups decrease the formation of lactate and pyruvate in lung tissue cells and blood.

Sodium oxamate and 5-bromo isatin treated animal compared with cancer control showed significant decrease in tumor incidence rate, multiplicity, tumor volume and tumor load. Histopathological morphological observation was conformed the tumor progression compared to cancer control group. Unlike many differentiated cells, high rate of glycolysis in urethane induced lung carcinoma model, Based on these models was considered has better *in vivo* model investigation.

The present *in vivo* study on the effect of 5-Bromo isatin and sodium oxamate in urethane induced lung carcinogenesis might have a potential anti-cancer activity by blocking the glycolytic stress. This may provide a novel therapeutic target in the field of cancer chemotherapy.Thus the present work though of preliminary in nature suggests that the sodium oxamate and 5-bromo isatin have anticancer activity. Further elaborate research work involving molecular level mechanism of sodium oxamate and 5-bromo isatin for the enzyme inhibiting activity.

CONCLUSION

The present study was designed to evaluate the anticancer activity of sodium oxamate and 5-bromo isatin in urethane induced carcinogenasis model. The study indicated a significant protection by sodium oxamate and 5-bromo isatin against urethane induced lung carcinogenesis. The results suggest that the protection was showed by sodium oxamate and 5-bromo isatin might be due to inhibition of glycolytic stress through blockage of glycolytic enzymes in glycolytic payhway.

This study serves as supporting evidence and can be scientifically exploited for future research in *in vivo* cancer models focusing specifically on glycolytic enzyme in cancer cells, so that sodium oxamate and 5-bromo isatin can be used as a single compound or in combination with chemotherapeutic agents for control the malignant potential. Further elaborate research work involving molecular level mechanistic understanding of sodium oxamate and 5-bromo isatin for the enzyme inhibiting activity.

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REFFERENCE

1. Granchi C and Minutolo F. Anti cancer agents that counteract tumor glycolysis. Chem med.2012; 000(00): 1-34.
2. Pelicano H, Martin DS, Xu RH, Huang P.Glycolysis inhibition for anticancer treatment. Oncogene, 2006; 25: 4633-4646.
3. Warberg O. The metabolism of tumors. London: Constable co. Ltd., 1930.
4. Warberg O. On the origin of cancer cells.Science. 1965; 123: 309-314.
5. Zu XL, Guppy M. Cancer metabolism: fact, fantasy and fiction. Biochem Bioph Ys Rescommun. 2004; 313: 459-465.
6. Deberardinis RJ, Lam JJ, Hatzivassiliou G, Thompson CB. The biology of cancer metabolic reprogramming fuels cell growth and proliferation. Cell metab. 2008; 7: 11-20.
7. Li X, Gu J, ZQ. Review of aerobic glycolysis and its key enzymes-New targets for cancer therapy. Thoracic cancer. 2015; 6: 17-24.

8. Scatena R, Bottoni P, Pontoglio A, Masfrototaroc L, Giardina B. Glycolytic enzyme inhibitors in cancer treatment. Research gate. 2008; 17(10): 1533-1545.
9. Zhao Z, Han F, Yang S, Wu J, Zhan W. Oxamte –mediated inhibition of lactate dehydrogenase induces protective autophagy in gastric cancer cells: involvement of the Akt-mTOR signaling pathway. Cancer letter., 2015; 358: 17-26.
10. Chaube B, Malvi P, Sing SV, Mohammad N, Meena AS, Bhat MK. Targeting metabolic flexilbility by simultaneously inhibiting respiratory complex I and lactate generation retards melanoma progression. Oncotarget., 2015; 6(35): 37281-37299.
11. Win KL, Locke JM,Ranson M,Pyne SG In vitro cytotoxicity evaluation of some substituted isatin derivatives. Bioorganic and Medicinal Chemistry, 2011; 15: 931–938.
12. 18.PakravanP, Kashanian S, Khodaei MM, Harding FJ. Biochemical and pharmacological characterization of isatin and its derivative: from structure toactivity. Pharmacological Reports, 2013; 65: 313 -335.
13. Ma X, Deng. J, Cao N, Guo Z, Zheng Y, Geng S, Meng M, Lin H, Duan Y, Du G. Lasting glycolytic stress govern susceptibility to urethane- induced lung carcinogenesis in-vivo and in-vitro. Toxicology letters., 2016; 240: 130-139.
14. Julius A, Allen R, K Pramila, A Venkatesan and Swamikannu B. Comparitive study on the effects of serum and plasma on glucose and cholesterol estimation.Biotech Res Asia., 2014; 11(1): 359-363.
15. Marbach EP, And Weil M.H.Rapid enzymatic measurement of blood lactate and pyruvate.Clin Chem. 1967; 13-314.
16. Fiesher W.R. Enzymaticmethodsfor lactic and pyruvic acids in standard methods of clinical chemistry.Macdonald R.P ed. Acadamic pores. 1970; 6: 345-259.
17. Loomis M.E. An enzymatic fluorometric method for the determination of lactic acid in serum. Lab clin Med., 1961; 57: 966.
18. Segal. S, Blair A. E. Spectrophotometric method for the determination of pyruvate in blood sample., 1956; 48: 137.
19. JA Gloster, P Harris.Observations an enzymatic method for estimation of pyruvate in blood. Clin chem. Acta. 1962; 7: 206-211.
20. Slater EC And Bonner WD. Effects fluoride on succinate oxidase system.Biochem J., 1952; 52: 185-196.
21. King TE, Ohnish T, Winter DB, Wu JJ. Biochemical and EPR probes for structure function studies of iron sulfer conters of sccinate dehydrogenase. Adv. Exp Me. 1976; 74: 182-227.

22. Ramnic Sood. Text book of medical laboratoty technology. Jaypee. 2009; 5: 600-610.
23. Chen Zong, Jie Wu, Chen wang, Huangxian JU and Feng Yan. Chemiluminescence imaging immunoassay of multiple tumor markers for cancer screening. Anal chem., 2012; 84: 2410-2415.
24. Shine B, Debeer FC, Pepys MB. Solid phase radio immunoassay for c reaction. Clinchem Ata., 1981; 177: 13-23.