5. Result

5.1.Components in *Bellamya bengalensis* tissue extract

The level of nutrients and micronutrients are presented in the table 1. The protein content in the flesh of *Bellamya* sp. was found to be 54.59mg/ gm tissue. Flesh of *Bellamya* sp. contains moderate amount of carbohydrate, 25.75mg/gm and lipid, 242µg/gm. Similarly, the micronutrient and non-protein soluble thiol (free -SH) levels are also described in the table 1.

Table 1 Composition of Nutrients and micro-nutrients in the *Bellamya Bengalensis flesh extract.*

Parameters	**Concentration in BBE**
Phosphorous (µgm/gm)	52.24 ± 3.89
Vitamin C (µgm/gm)	71.85 ± 4.74
Calcium (mg/gm)	0.36 ± 0.023
Protein (mg/gm)	54.59 ± 4.75
Total lipid (µg/gm)	242 ± 23.54
Total carbohydrate (mg/gm)	25.75 ± 2.94
Total amino acid (mg/gm)	0.96 ± 0.056
NPSH (µgm/gm)	50.8 ± 3.7

5.2.Arsenic toxicity in *B.bengalensis* tissue

A dose and time dependent increase in malondialdehyde (MDA) production is noticed in *Bellamya bengalensis* tissue which has been ceased and decreased after a longer period of time (figure 1). This result is found to be related by the antioxidant component i.e. non protein soluble thiol (NPSH). No significant DNA fragmentation is noticed during the present treatment ($NaAsO_2$) schedule (figure 1).

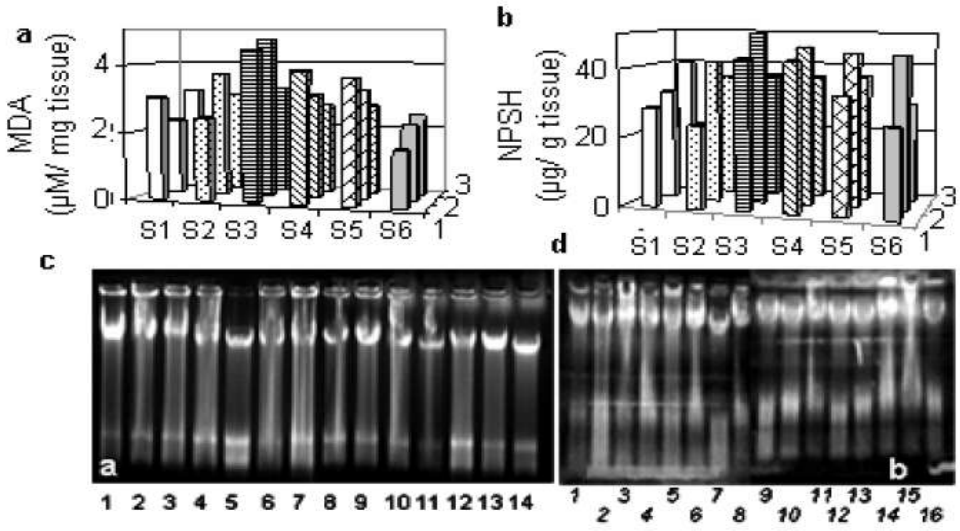

Fig 1. Malondialdehyde (MDA, fig 1a) and non-protein soluble thiol (NPSH, fig 1b) level in *Bellamya bengalensis* tissue after arsenic (NaAsO2) exposure in live organism. Bars in the figure represent as follows; S1 control, S2 10 ppm, S3 12.5 ppm, S4 15 ppm, S5 17.5 ppm, S6 20 ppm NaAsO2 exposed group and 1 denotes 48 hours, 2 is 72 hours and 3 is 96 hours. Fig 1c and 1d show the quality and stability of tissue DNA of *Bellamya bengalensis* after arsenic exposure. Lane distribution; panel a- Lane 1- 48 hrs control, 2- 48 hrs control, 3- 48 hrs 10 ppm, 4- 48 hrs 12.5 ppm, 5- 48 hrs 15 ppm, 6- 48 hrs 17.5 ppm, 7- 48 hrs 20 ppm, 8- 96 hrs control, 9- 96 hrs control, 10- 96 hrs 10 ppm, 11- 96 hrs 12.5 ppm, 12- 96 hrs 15 ppm, 13- 96 hrs 17.5 ppm, 14- 96 hrs 20 ppm of NaAsO2 in the water where the animals were kept. Fifteen ppm of NaAsO2 was treated to the *B. bengalensis* in a time dependant manner (1d). Lane distribution; Lane 1- 24 hrs control, 2- 24 hrs of NaAsO2 exposed, 3- 48 hrs control, 4- 48 hrs of NaAsO2 exposed 5- 72 hrs control, 6- 72 hrs of NaAsO2 exposed 7- 120 hrs control, 8- 120 hrs of NaAsO2 exposed, 9- 144 hrs control, 10- 144 hrs of NaAsO2 exposed, 11- 168 hrs control, 12- 168 hrs of NaAsO2 exposed, 13-192 hrs control, 14- 192 hrs of NaAsO2 exposed, 15- 216 hrs control, 16- 216 hrs of NaAsO2 exposed. At every 48 hrs, the animal incubation water was refreshed maintaining the stipulated concentration of NaAsO2.

5.3.In-vivo

5.3.1. Arsenic toxicity in rat liver

The malondialdehyde (MDA) content in liver homogenates significantly increased in the sodium arsenite-exposed rats (P<0.05) which is restored in the BBE supplemented group and this result is significantly substantiated by the tissue NPSH level (Fig. 2). The strong antioxidant enzymes i.e. lactoperoxidase (P<0.05) and xanthine oxidase (P<0.01) and the oxidant-neutralizing molecule uric acid (P<0.05) have been found to be decreased significantly in arsenic treated group and restrained in the BBE supplemented group (Fig. 3).

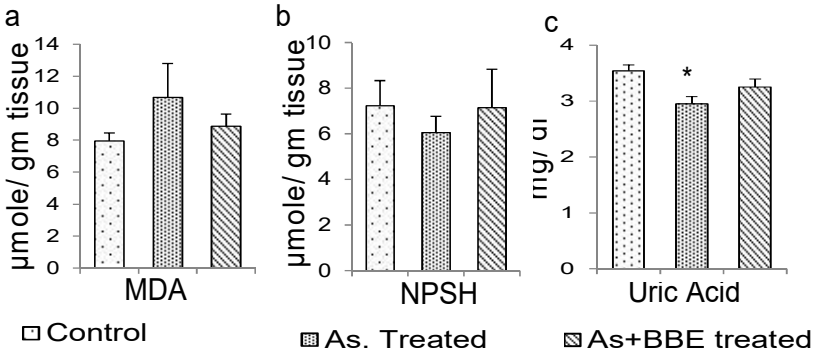

Fig: 2. The malondialdehyde (MDA) is regarded as the oxidative stress marker representing the deleterious effects of Reactive Oxygen Species (ROS) on cellular lipid components. All the parameters were measured in the hepatic tissues except the antioxidant uric acid, which was measured in serum sample. Results are mean ±SE (n=6 in each group). Data of treated group or supplemented group is compared to the corresponding vehicle treated group (Student 't' test). Levels of significances are denoted as; *=*P<0.05; **=P<0.01*

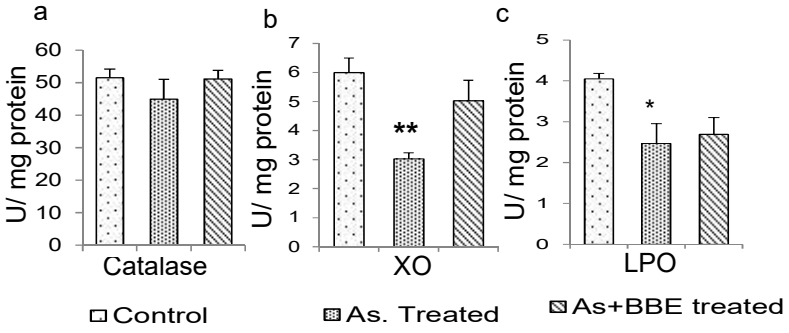

Fig: 3. Effects of BBE; BBE on arsenic-induced hepatic antioxidant parameters in female rats (data in the graph represent mean ± SE, N=6). Arsenic or arsenic + CS treated groups are compared with control and the level of significances are denoted as *; p<0.05, **; p<0.01 (ANOVA followed by multiple comparison two tailed t test).

5.3.2. Liver and kidney function test in rat blood serum

Liver and Kidney Function Test Serum glutamic pyruvic transaminase (SGPT) ($p<0.01$) and alkaline phosphatase (ALP) activities (86%) are significantly/notably increased in arsenic intoxicated rat suggesting necrotic tissue damage which has been ceased and restored by the BBE supplementation (figure 4 and table 2). Similarly, the increase in the kidney function marker, urea and general tissue degeneration marker like Lactate dehydrogenase (LDH) are also nullified by the BBE supplemented group in arsenic exposed rats (figure 5). In several of the instances, changes among groups were not found significant due to the higher inter individual variability and lower sample size

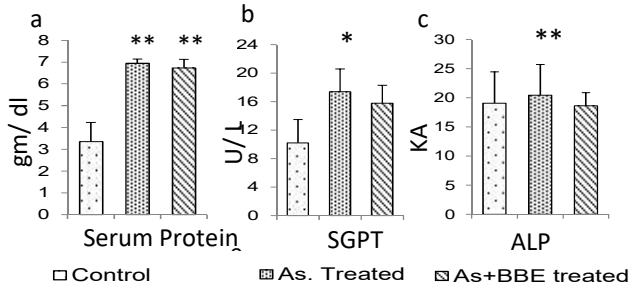

Fig: 4. Result shows that arsenic induced impairment of liver function tests are significantly restored by the BBE supplementation. Results are mean ± (n= 6 in each group).Data of treated group or supplemented group is compared to the corresponding vehicle treated group. (student 't' test). Levels of significance are detonated as; *=P <0.05; **=P < 0.01.

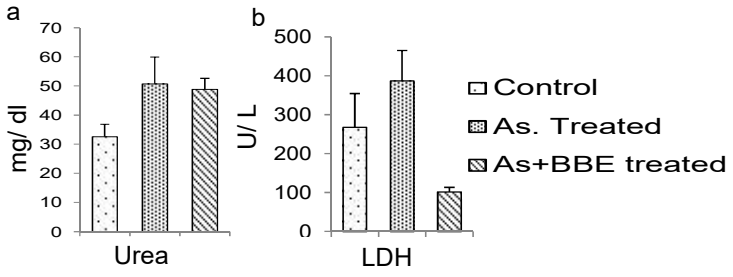

Fig: 5. Result shows that arsenic induced impairment of kidney function marker like urea and tissue degeneration marker LDH are significantly restored by the BBE supplementation. Results are mean ± (n= 6 in each group).Data of treated group or supplemented group is compared to the corresponding vehicle treated group. (student 't' test). Levels of significance are detonated as; *=P <0.05.

Table 2 Different antioxidant and cellular toxicity parameters are shown in liver tissues (uric acid, protein, SGPT, ALP, urea and LDH were measured in the serum sample) of rat after arsenic exposure. For details procedure please refer to the 'Materials and method' section. Results are mean ± SE (n = 6 in each group). Data of treated group or supplemented group is compared to the corresponding vehicle treated (Student 't' test). Levels of significances are denoted as; *, p<0.05; **, p<0.01 and ***p<0.001.

Parameters	Control	As treated	As + BEE treated
ALP	15.19±5.4	28.19±3.62	18.64±2.24
Catalase	51.45±2.73	44.91±6.13	51.12±2.63
LDH	267.14±86.66	386.86±77.92	101.19±12.18
LPO	4.05±0.13	2.47±0.48**	2.69±0.41*
MDA	7.95±0.50	10.66±2.13	8.87±0.76
NO	1.59±0.14	0.71±0.22**	0.92±0.19*
NPSH	8.18±0.65	5.78±0.51*	7.83±2.02
Serum protein	3.35±0.88	6.94±0.2**	6.73±0.39**
SGPT	15.01±1.49	23.36±1.35**	19.11±1.3
TNF-α	55.76±2.35	86.19±13.1*	70.35±3.75**
Urea	32.63±4.24	50.74±9.21	48.88±3.73*
Uric acid	3.54±0.11	2.95±0.13**	3.25±0.14
XO	5.99±0.50	3.02±0.21***	5.02±0.71

5.3.3. In vivo and in-vitro SOD regulation in rat liver tissue

Present result suggests that in-vivo SOD activity did not alter significantly in arsenic treated group (lane 4-7) with comparison to the control (figure 6a). But in the BBE supplemented group this activity augmented significantly suggesting its protective role. In the in vitro study, a significant decrease in arsenic + H_2O_2 treated group (lane 3, figure 6b) is noticed which is markedly reversed BBE and *Bellamya bengalensis* venom (BBV) treated group (lane 4 and 5 respectively, figure 6b). Lane 6 and 7 served the control to test the possible SOD activity in the only BBE and BBV respectively. Similarly, only H_2O_2 was used in oxidant-stress associated SOD inactivation and its reversal and even activation by the BBE and BBV (figure 6c).

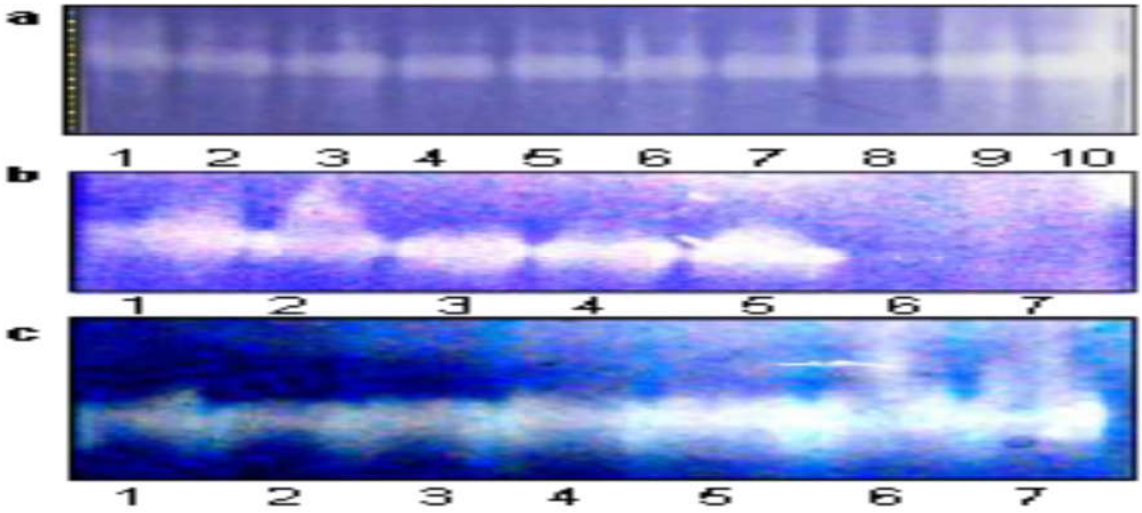

Fig: 6. Cytosolic SOD (Cu-Zn SOD or SOD1) activity of hepatic cells is shown on a poly acrylamide gel. The NBT soaked acrylamide gel is shaken in SOD solution (TEMED, riboflavin, and potassium phosphate at pH 7.8). The soaked gel is placed on a clean acetate sheet and illuminated with UV, and the transparent SOD-activity band is visualized. The gel is scanned when the maximum contrast between the band and background has been achieved. Upper panel a (in vivo experiment) - Lane distribution; 1, 2, 3 control; 4, 5, 6, 7 arsenic treated, and 8, 9, 10 arsenic + BBE treated. Middle panel b- In vitro H2O2 inactivation of rat cytosolic SOD1 and its protection by BBE and BBV. Lane distribution: Lane 1 control, 2-Control (2h), 3- H2O2 (100mM) (2h), 4- H2O2 (100 mM) + BBE 15µl (25%) (2h), 5- H2O2 (100 mM) + BBV 15µl (2h), 6-BBE 15µl (25%) (2h), 7- BBV 15µl (25%) (2h). Lower panel c- In vitro H_2O_2 inactivation of rat cytosolic SOD1 and its protection by BBE and BBV. Lane 1-control., 2 - con incubated 2hrs., 3- H2O2(1M) 2hrs., 4- BBE 18 µl(25%) + H2O2(1M))2hrs., 5- BBE 18µl(25%) + H2O2 (1M) 2hrs., 6- BBV 18 µl + H2O2(1M) 2hrs., 7- BBV 18 µl + H2O2(1M) 2hrs.

5.3.4. DNA Fragmentation in rat liver from in vivo assay

Arsenic ingestion with the present dose and duration decisively resulted in appreciable amount of "ladder" of DNA fragments (lane 4, 5) in liver with comparison to the control rats (lane 1, 2, 3), whereas, the DNA is found to be partially but significantly protected from fragmentation in BBE co-administered group (Fig. 7). The DNA/ladder density (normalized value) is calculated for the different migrated locations on the lane in the gel and the mean values are plotted against the relative migrated location which clearly reveals the outcome noticed in the ladder image.

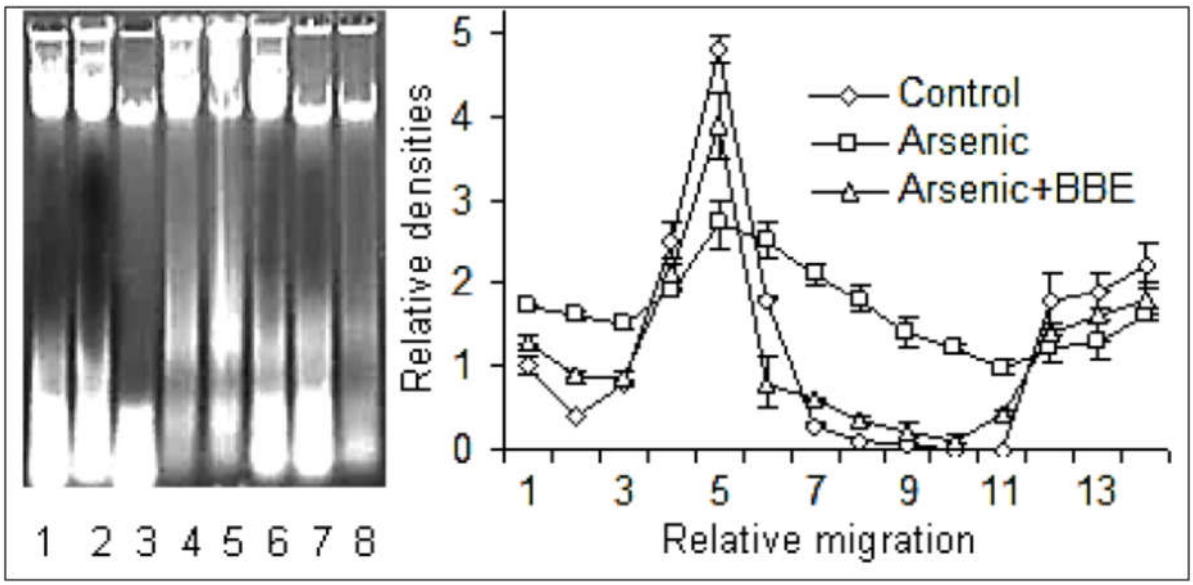

Fig: 7. DNA fragmentation result is shown in liver of female rats treated with arsenic. Lane distribution; Lanes 1, 2, 3- control; lanes 4, 5- NaAsO2 exposed and lanes 6, 7, 8- NaAsO2 + BBE (left panel). Densitometry analysis of the different bands was done in ImageJ software and the mean normalized values are plotted at the position of their relative migration.

5.3.5. Single- cell DNA damage in rat liver by COMET assay

The single cell DNA status (comet assay) basically supports the DNA fragmentation results. The numbers of comet forming cells are found to be higher in arsenic exposed rat liver whereas, in BBE supplemented group the genetic materials of the cells are noticed to be highly protected (Fig. 8).

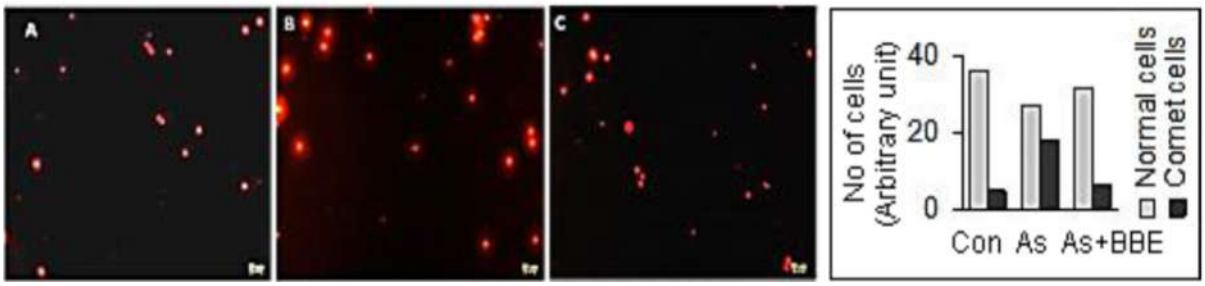

Fig: 8. Arsenic induced hepatic DNA breakage in single cell apoptotic damage which is markedly prevented by the BBE administration. Panels are represented as control rat (a) NaAsO2 exposed (b) NaAsO2+BBE (c). The alkaline comet assay is done following the lyses of cells then electrophoresis and staining with ethidium bromide.

5.3.6. Hepatic tissue architecture in rat liver

The results on the DNA stability in the Figures 4 and 5 are concordant with the histo-architecture picture (Fig. 9). We noticed a significant alteration in the liver functions of the experimental animal. This is supported by our present hepatic histological study. Arsenic caused hepatic injury due to the necrotic tissue lesions and disruption of the central canal and associated convergent lobular structure of the hepatic cells.

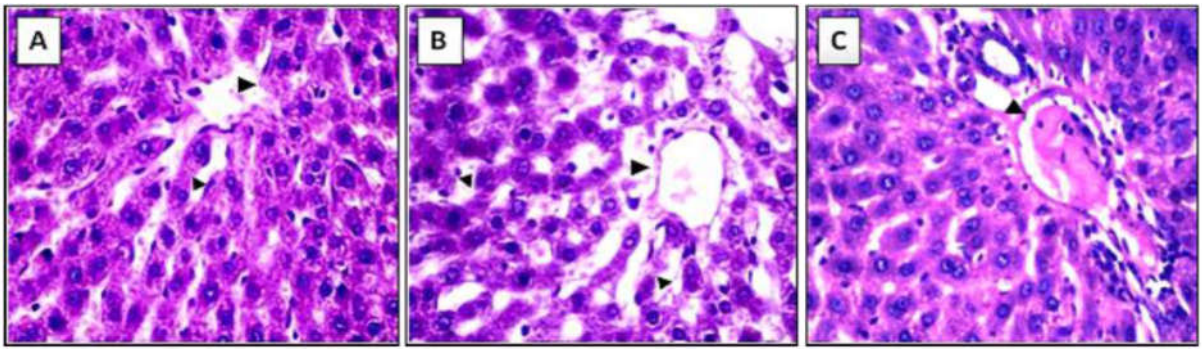

Fig: 9. The hepatic histoarchitecture is shown by H&E staining (10X) of female rat treated with arsenic. Control rat (a) or treated with sodium arsenite (b) or sodium arsenite + BBE. The lumen lining of the central vein of liver is present. The hepatic lobules are comprised mostly of plates of hepatocytes. Tissue degeneration is clearly visible in slide b as the formation of a mesh like structure losing the eccentric feature of hepatocyte organization. The present histoarchitectural results are justified by the serum markers of liver and kidney functions and tissue degenerative marker i.e. LDH activity.

5.3.7. Qualitative and Quantitative measurement of mitochondrial membrane potential in rat liver

Mitochondria inner membrane bound free redox carrier molecule like cytochrome C, ubiquinone and others molecule are important marker molecule for the membrane stability and charge caring capacity of the mitochondria. A large amount of signal rodamin 123 bound redox active substance suggest that mitochondria is in active condition, The low level of signal suggest the lower amount of redox substance . In the current study the pic of the rodamin 123 signal proposed that high amount of signal is present in control in rat liver. Mitochondria after 28 days of arsenite treatment we noticed a significant a significant lost membrane redox value, so less amount of signal is noticed after arsenite treatment. This impairment was prevented after BBE supplementation. The signal was significantly restored in this group of animals. It is observed in the lower panel in fig 10 a. Thus florescence spectrometry data also support the above findings. The fluorescence density which was noticed in the control animals impaired in arsenic treated group and that was restored in the BBE supplemented animals and the result is presented in the line diagram.

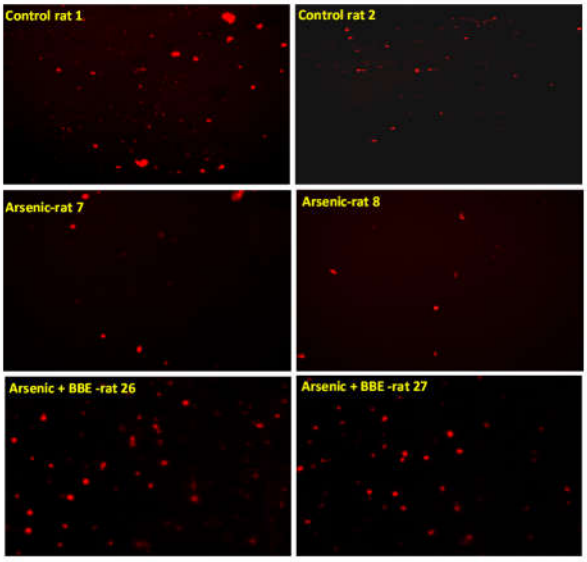

Fig. 10.a

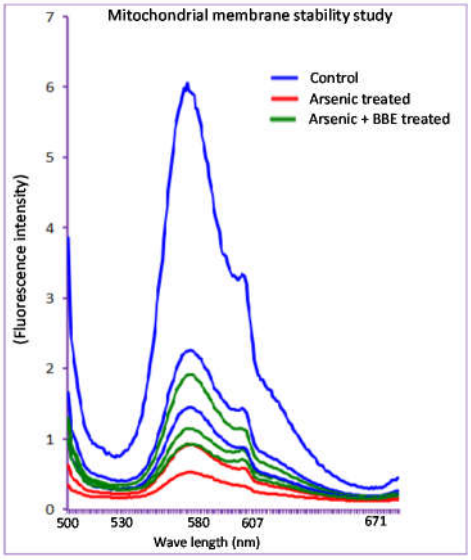

Fig 10.b

Fig-10. Qualitative and quantitative measurement of mitochondrial membrane potential (MMP) of experimental rat liver. MMP was determined by measuring rodamine 123 fluorescence intensity using both fluorescence spectrophotometer (10, b) fluorescence microscopy (10, a) as described in the materials and methods section 4.7.15

In-vivo experiment in arsenic exposed intestinal epithelial cells of rat

5.3.8. Status of Oxidative Stress and necrotic Markers in intestinal epithelial cells on rat model

The malon di-aldehyde (MDA) and conjugated di-ene (CD) content increased significantly ($P<0.01$, respectively) in the sodium arsenite-exposed rats. However, administration with BBE combined with arsenic prevented MDA and CD elevation when compared to the arsenic only treated group ($P/0.001$). (fig.12.b, 11.b) There was a significant decrease in intestinal SOD and catalase activities in arsenic-treated rats when compared to control group ($P<0.001$) Restoration of SOD (fig.11) and catalase (fig.12.a) activities were observed in the BBE supplementation group. However, administration of BBE in addition to arsenic significantly prevented their elevations ($P<0.001$) and also regained NPSH level than the arsenite-exposed rats (fig.13. a). Arsenic caused a significant increase ($p<0.05$) in the level of proinflammatory cytokines TNF-α and significant decrease ($p<0.01$) in NO level in the arsenic treated groups. Though not significant, appreciable reversal was noticed in both parameters of BBE supplemented group (fig.14).

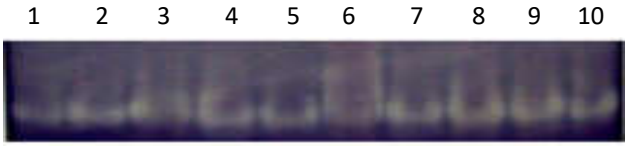

Fig 11. Cytosolic SOD (Cu-Zn SOD or SOD1) activity of intestinal epithelial cells are shown on a polyacrylamide gel. Lane distribution: 1, 2, 3

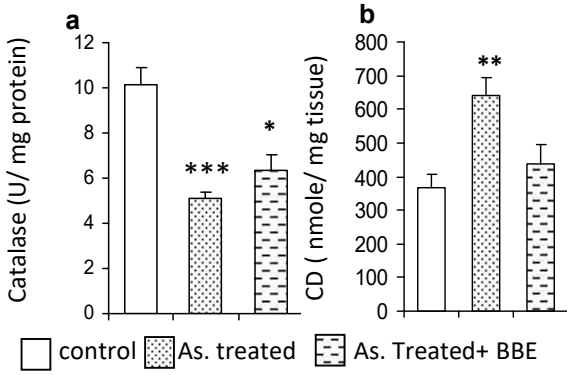

Fig: 12. The catalase assay (a), CDs are determined by a standard method (b). Data in the graph represent mean+SE, N=6. The levels of significances compared to the control are denoted as: a; P<0.05, b; P<0.01, and c; P<0.001 (ANOVA followed by multiple comparison two tailed t test).

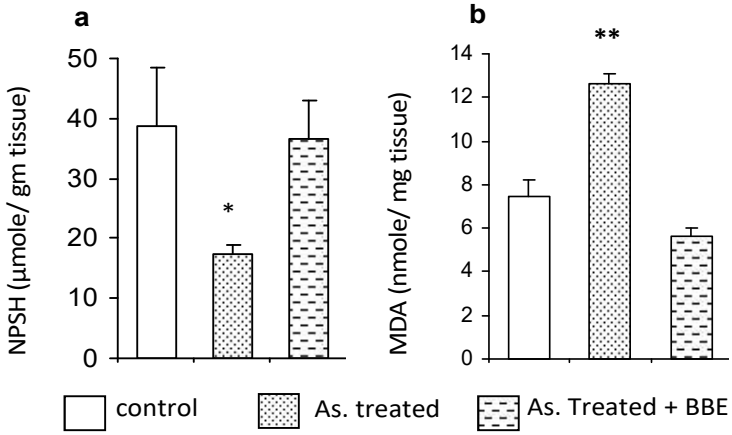

Fig: 13. The NPSH in intestinal cells homogenate is determined by standard DTNB method (a). The MDA is measured with its molar extinction coefficient of MDA (1.56 x 10^5 cm^2/ mmol) (b). Data in the graph represent mean+SE, N=6. The levels of significances compared to the control are denoted as: *; P<0.05, **; P<0.01 (ANOVA followed by multiple comparison two tailed t test).

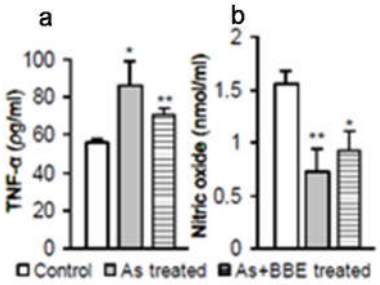

Fig 14. The serum level of TNF-α (a) and nitric Oxide (b) are shown. Levels of significances (Student 't' test) are denoted as; * ,P< 0.05; ** , P< 0.01.

5.3.9. Liver and Kidney Function Test

The result suggests that the serum Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) and Alkaline Phosphatase (ALP) enzymatic activities are significantly increased ($p<0.05$) in arsenic intoxicated rat suggesting necrotic tissue damage which has been ceased and restored by the BBE supplementation (fig.15. d,e,f). Similarly, the increase in the kidney function marker urea and creatinine, restored by the BBE supplemented group in arsenic exposed rats (fig.-15.b, c). A significant depletion of serum uric acid by arsenic ($P<0.05$) and its restoration by BBE are noticed (fig.15. a).

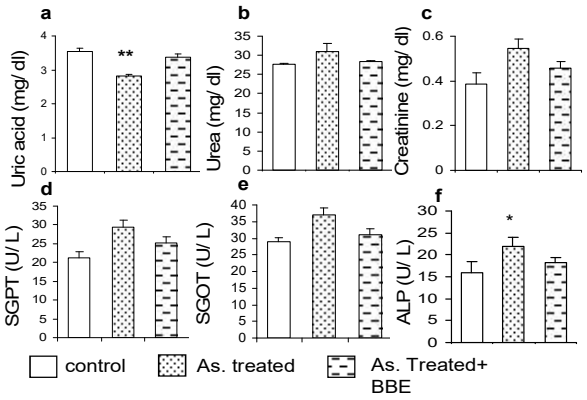

Fig 15. Results on liver and kidney function marker, metabolic inflammation, and tissue necrotic markers are presented. SGPT (d), SGOT (e), and ALP (f), urea (b), creatinine (c), and uric acid (a) are measured by standard protocol with the assay kits. Result shows that arsenic induced impairment of liver and kidney functions are significantly restored by the BBE supplementation. (Data in the graph represent mean + SE, N=6). The levels of significances compared to the control are denoted as: *; $P<0.05$. **; $p<0.01$, ANOVA followed by multiple comparisons two tailed t test).

5.3.10. Hematopoietic Profile

Their hematopoietic profiles (total count (TC) and differential count (DC) of WBC) are presented table 3, these data suggest that dramatically increased total count of WBC in arsenic intoxicated rat blood compared with control group and that was restrained by BBE supplemented group. Results of DC indicate that neutrophil increased by 9% and lymphocyte decreased 8% in arsenic-treated rats when compared to control group and its restoration by BBE are noticed table 3 and 4.

Table 3: TC and DC arsenic intoxicated rat blood compared with control group

Parameters	**Control**		**Arsenic treated**	
	N	**Mean±SE**	**N**	**Mean±SE**
TC	3	7533.33±384.42	5	8360.00±366.88
Neutrophil	3	55.67±7.13	5	64.80±2.27
Lymphocyte	3	39.67±6.69	5	31.80±1.62
Monocyte	3	2.00±0.58	5	2.00±0.55
Eosinophil	3	2.67±0.67	5	1.80±0.37

Table 4: TC and DC, arsenic + BBE rat blood compared with control group

Parameters	**Control**		**Arsenic treated +BBE**	
	N	**Mean±SE**	**N**	**Mean±SE**
TC	3	7533.33±384.42	5	7920.00±201.00
Neutrophil	3	55.67±7.13	5	60.40±2.66
Lymphocyte	3	39.67±6.69	5	35.80±2.89
Monocyte	3	2.00±0.58	5	1.20±0.20
Eosinophil	3	2.67±0.67	5	2.60±0.51

5.3.11. DNA fragmentation in of intestinal epithelial cells in rat

Results of agarose gel electrophoresis of intestinal epithelial cells DNA from different experimental groups showed (Fig.16) lane 1, 2 control, 3, 4, 5 in only arsenic-treated group, which is significantly restrained in BBE supplemented arsenic-exposed group in preceding lanes 6, 7. Graph of Fig. 16, b describes the band intensity of intestinal DNA at its different relative positions on the agarose gel, which suggest the highly damaging effect arsenic is significantly protected by As+ BBE treated group.

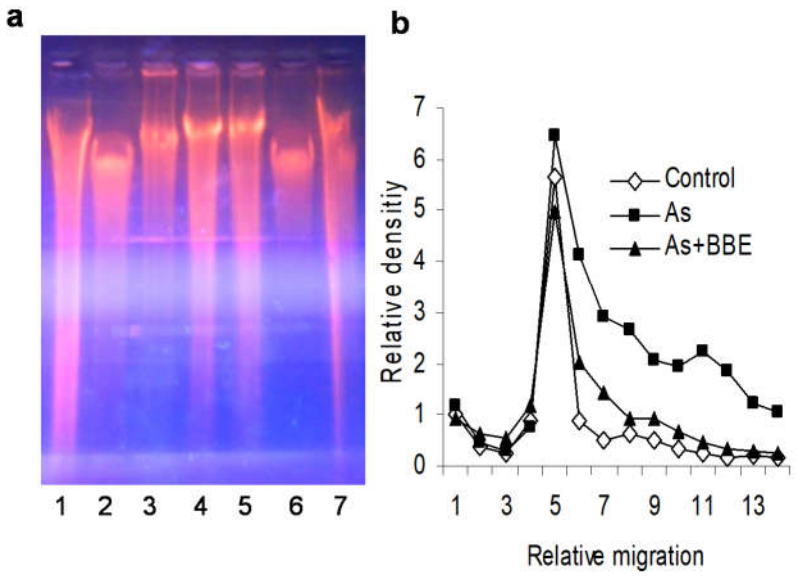

Fig-16. DNA fragmentation result is shown in intestinal tissue of female rats treated with arsenic. Lane distribution; Lanes 1, 2 - control; lanes 3, 4, 5- $NaAsO_2$ exposed and lanes 6, 7 - $NaAsO_2$ + BBE. Densitometry analysis of the different bands was done in ImageJ software and the mean normalized values are plotted at the position of their relative migration.

5.3.12. Comet assay result in rat intestinal epithelial cell

The single cell DNA status (comet assay) basically supports the DNA fragmentation results. The number of comet forming cells is found to be higher in arsenic exposed rat intestinal epithelial cell whereas, in BBE supplemented group the genetic materials of the cells are noticed to be highly protected (fig.17.a,b,c).

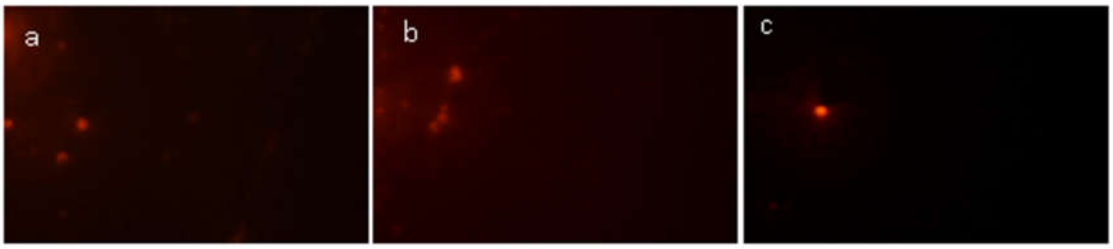

Fig- 17. Intestinal DNA – breakage is shown in single cell apoptosis (comet assay; a= control, b= arsenic treated and c= supplemented).

5.3.13. Intestinal tissue architecture

Arsenic ingestion resulted in intestinal tissue disarrangement with lobular/epithelial degeneration. The histo-pathological evaluations suggest the event of hemorrhages and infiltration of mononuclear cells in intestine. The layers of muscularis mucosae (inner and outer layer) of intestinal wall are found to be impaired in arsenic-exposed rat. But the co administration of BBE shows partial but significant protection which is evident from the picture (Fig. 18.a, b, c). PAS staining suggest the micronecrotic lesions in the tissues and impairment in the mucin producing abilities. It has been significantly restored by the BBE supplementation (Fig. 18.d, e, f).

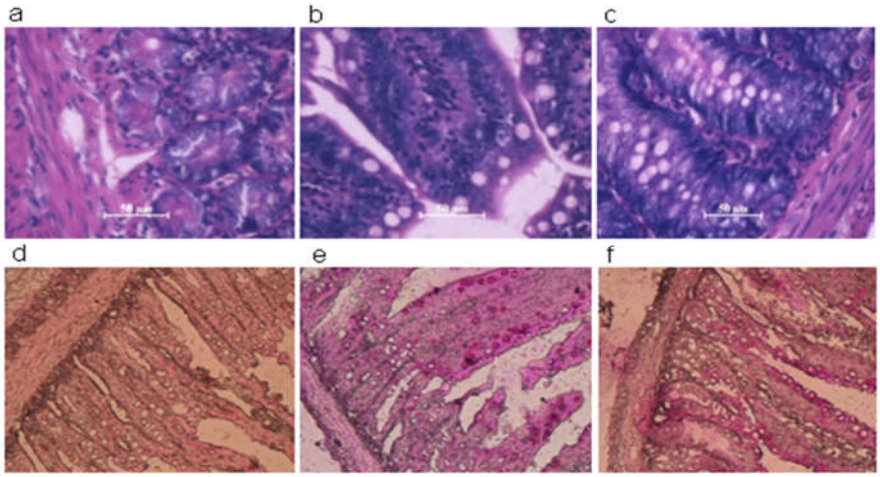

Fig-18. The intestinal histoarchitecture is shown by HE staining (magnification X 50) of female rat treated with arsenic. Control rat(a) or treated with sodium arsenite (b) or sodium arsenite + BBE (c). Pictures of upper panel are of crypts lieberkeuhn (intestinal gland). Degeneration of glands, smooth muscle, and lamina propria in arsenic – treated group (b) . A certain degree restoration is noticed in BBE supplemented group. PAS staining results in the lower panel show a significant micro necrosis in the cells (e) compared with control (d) which is markedly restored by BBE exposure (f)

5.4.In vitro study

5.4.1. MDA (malondialdehyde) and NPSH (Non protein soluble thiol) Assay result in liver slices

In the lipid peroxidation study, we notice a moderate increase of lipid peroxidation in liver slices. In this condition, the BBE treated seems to be more protective in long duration. Where as arsenic treatment shows it toxicity after long duration but the increase in lipid peroxidation is possible circumvented by the thiol increase in NPSH assay. So in can be said the lipid peroxidation might have some limited scope for the tissue toxicity manifestation (fig.19,a). Non Protein soluble thiol assay shows that NPSH in liver cells increase in all As treated groups time dependently. But it is significantly increased only in arsenic treated group and all BBE treated group. This suggests that in case of As toxicity thiol plays an adaptive role and in BBE treated this increase showed an adaptive and protective role against As induce hepatic toxicity. NPSH is the thiol precursor for all other thiol containing biomolecule. Which plays a direct and indirect antioxidant against oxidative stressed induce threatening apart from that and disulphide maintain intercellular redox balance and thiol is the active component of several enzyme receptor or signalling molecule .So thiol induce protection was notice in BBE treated group.(fig.19, b).

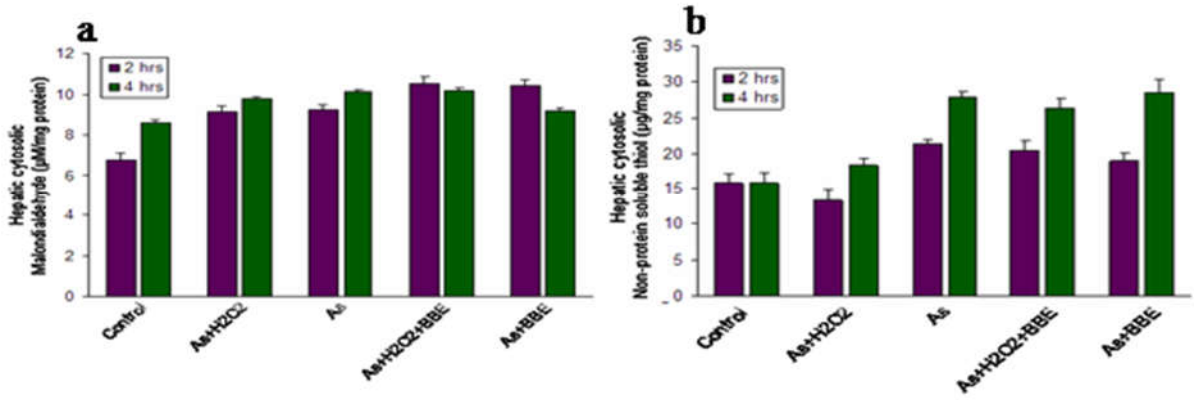

Fig 19. The graphical representation of MDA (a) and NPSH (b) level in liver slices in different group's incubation treated with sodium arsenite and its protection by BBE.

5.4.2. Catalase activity in Gel-zymography in rat liver slices

Catalase assay in the gel shows that the catalase activity is decrease by the As + H_2O_2 & As in the lane (3, 4) (5, 6) respectively than the control (lane1&2). The activity of the catalase was regained when the *Bellamya* extract was treated as the therapeutic agent against As & H_2O_2 in lane (7, 8) & (9, 10) (Fig.20, a).

5.4.3. Superoxide Dismutase (SOD) activity in Gel-zymography in rat liver slices

Superoxide dismutase (SOD) assay in gel shows that the SOD activity was decrease by the As & H_2O_2 and As in the lane (3,4) & (5,6) respectively than the control(lane 1&2).Whereas the SOD activity was regain when the *Bellameya* extract was use as remediation against the As+H_2O_2 and As in the lane (7&8) and (9&10) (Fig.20, b).

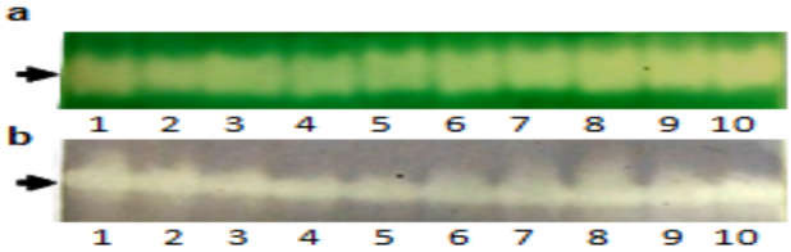

Fig. 20. The liver slice catalase (a) and cytosolic SOD (Cu-Zn SOD or SOD1) (b) activity by gel-zymography in different group's incubation treated with sodium arsenite and its protection by BBE. Lane distribution: Lane 1- control (2 h), 2-Control (4h), 3- NaAsO2 (1mM)+ H_2O_2 (100mM) (2h), 4- NaAsO2 (1mM)+ H_2O_2 (100mM) (4h), 5-NaAsO2 (1mM) (2h), 6-NaAsO2 (1mM) (4h), 7 - NaAsO2 (1mM) +H_2O_2(100 mM) +100ul BBE(40%) (2h), 8- NaAsO2 (1mM) +H_2O_2(100 mM) +100ul BBE(40%) (4h), 9 - NaAsO2 (1mM) + 100ul BBE (40%) (2h), 10 - NaAsO2 (1mM) + 100ul BBE (40%) (4 h).

5.4.4. Results of In vitro study of Catalase and SOD (Cu-Zn SOD or SOD1) activity by sodium arsenite with free phosphorus and cysteine in rat liver slice

Present liver-slice experiment suggests that $NaAsO_2$ (As3+) alone or in combination with H_2O_2 inactivates the SOD1 activity time dependently fig 20.a, lane 2, 3. Fig 21. a, lane 4,5,6,7, shows that free phosphorus and cysteine are found to be definitely SOD1 protecting agent. Catalase assay in the gel shows that the catalase activity is decrease by the As + H_2O_2 & As in the lane (2, 3) respectively than the control (lane1). The activity of the catalase was regained when the free phosphorus and cysteine were treated as the therapeutic agent against As & H_2O_2 in lane (lane 4, 5, 6, 7) (Fig.21, b).

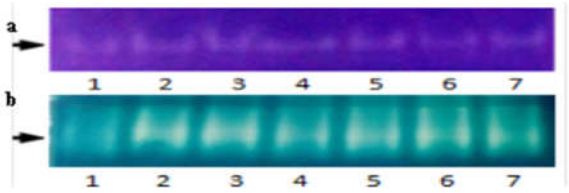

Fig. 21. The invitro liver slice catalase (e) and SOD (Cu-Zn SOD or SOD1) (d) activity by gel-zymography in different group's incubation treated with sodium arsenite and its protection by Na_2HPO4 and L-cysteine. Lane distribution: 1-control, 2- $NaAsO_2$ (250µM), 3 -$NaAsO_2$ (250µM) +H_2O_2 (100mM), 4- $NaAsO_2$ (250µM) + Na_2HPO_4, 5- $NaAsO_2$ (250µM) + L- Cysteine (100mM), 6- $NaAsO_2$ (250µM) +H_2O_2 (100mM) + Na_2HPO_4, 7- $NaAsO_2$ (250µM) +H_2O_2 (100mM) + L-Cysteine (100mM).

5.4.5. In- vitro comet assay studies in liver slice

The comet assay showed that the intestinal DNA in the arsenic treated cells have damaged as per the (fig.-22.b,c) where cells seems to be larger due to dispersion of DNA as compared to the control (fig.- 22.a), whereas remission was observed in the As + BBE (Fig.22.d,e)

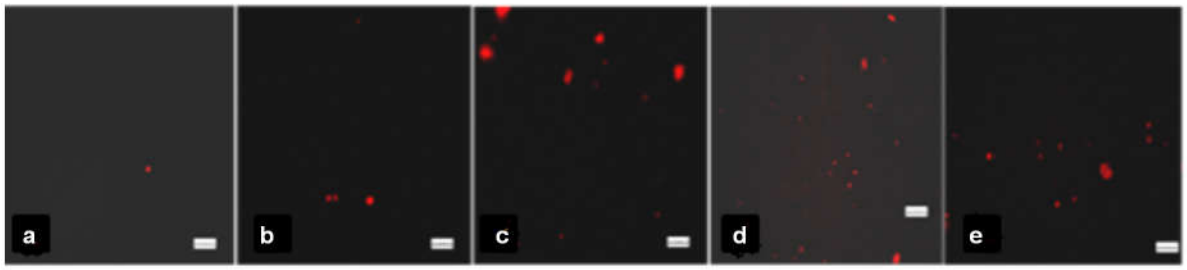

Fig 22. The in vitro intestinal tissue DNA-breakage is shown in single cell apoptosis a- control (2 h), b- $NaAsO_2$ (1mM)+ H_2O_2 (100mM) (2h), c -$NaAsO_2$ (1mM) (2h), d- NaAsO2 (1mM) +H_2O_2(100 mM) +100ul BBE(40%) (2h), e - $NaAsO_2$ (1mM) + 100ul BBE (40%) (2h). Picture shows that arsenic induced severe DNA breakage which is noticed in single cell apoptotic damage is markedly prevented by the BBE exposure.