ABSTRACT
Swiss Albino mice *Mus musculus* was exposed to endosulfan@2 mg/kg. body wt., rogor @20 mg / Kg. body wt. separately & a combination of endosulfan(@1 mg /kg. body wt) and rogor (@10 mg/Kg .body wt.) for 7, 4 & 21 days respectively. At the termination of each exposure, blood sample were collected & serum was extracted to analyze the quantitative estimation of total protein and amino acid composition by standard method of Moore & Stein using thin layer chromatography. The hepatic tissues were processed for light microscopic studies. The results showed an increasing trend of serum protein content at all the exposure level of rogor while a decreasing trend was reported against endosulfan exposure. An abrupt trend of fluctuation in serum protein was reported in combined exposure. Amino acid profile of control group showed a very high percentage of valine, serine, aspartic acid, tyrosine & glutamic acid while a very diminished concentration of these amino acids were found in the serum of treated groups. The hepatic tissues showed corresponding anomalies in different treated groups. Hence abrupt fluctuation in serum protein, diminished amino acid contents can be considered as sensitive indices of toxicity generated by agrochemicals in mice.

KEYWORDS: *Mus musculus*, Endosulfan, Rogor, Hepatic tissues, Light microscopy, TLC, Total protein, Amino acid.

INTRODUCTION
The widespread use of pesticides in public health and agriculture has caused severe environmental pollution and health hazards. Several cases of severe, sub- chronic and chronic
human poisoning due to non judicious application of agrochemicals have been reported (Repetto and Balija, 1996; Mars, 1997; USDA, 1994 and Yamashita et al, 1997). Rogor is an oreganophosphate with acetylcholine esterase inhibiting activity (Dutta et al, 1995). It bears acute and chronic toxicity apart from possessing reproductive, teratogenic, mutagenic and carcinogenic effects (Hayes, 1982 & Hellenbeck, 1985). Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of cyclodiene subgroup. Endosulfan and acrylamide induced significant decline in body weight, decrease in number of RBC and hemoglobin content in experimental Swiss Albino mice Mus musculus have been reported by Sharma et al (2008) who studied the neoplastic and hematological effect of endosulfan, bleomycin and acrylamide in the Swiss albino mice. Endosulfan induced neurotoxicity in rats and mice have been reported by Gupta(1976,2004) and Gupta & Gupta (1979). Total protein is useful for monitoring gross changes in protein levels caused by various disease states. It is usually performed in conjunction with other tests such as serum albumin, liver function tests or protein electrophoresis. An albumin/ globulin ratio is often calculated to obtain additional information. Total protein level may increase in several pathological conditions viz: - cholelithiasis, leishmaniasis, liver cirrhosis macroglobulinemia,, multiple myeloma, pheochromocytoma, rheumatic fever. total protein level may decrease in several diseases like- amyloidosis, analbuminemia, chronic lymphocytic leukemia, chronic renal failure diabetes mellitus, eclampsia Hodgkin’s disease, malaria nephrotic syndrome, Non-Hodgkin’s lymphoma, pre-eclampsia, acute post streptococcal glomerulonephritis, epidemic typhus (severe cases), gastritis gastrointestinal carcinoma (stomach, colon, rectum, etc.), glomerulonephritis, hemolytic uremic syndrome (HUS), hepatolenticular degeneration, toxic hepatitis, ulcerative colitis & Whipple’s disease.

Amino acids are the building blocks of protein containing 16% nitrogen. The proteins used in making of the body are not directly derived from diet, but the dietary proteins are firstly broken down into respective amino acids and then body reconstitutes these amino acids into specific proteins, needed as per requirement. Enzymes and hormones regulating body functions are also protein. Amino acids are used in almost every body processes ranging from regulating the way the body works to the sophisticated functioning of brain. The liver manufactures about 80% of these amino acids but the remaining 20% of such amino acids must be supplied directly by diet, referred as essential amino acids. The xenobiotics exposures have serious implications on amino acid profile of mice muscle as well as serum.
Liver and kidney are the primary organs of degradation, detoxification & elimination in vertebrates. These are the most affected organs by the toxic assault. Changes occurring to any part of the body first disrupt the liver function. Several works have reported the harmful effects of this dreaded pesticide on liver (Kurutas, 2006; Chevallier et al, 1994; Myara, 1987and Safari &Friedman,2002).Thus present study is an attempt to observe the comparative toxicity of these two pesticides on serum total protein content, free amino acids pool and associated hepatotoxicity in Mus musculus.

MATERIALS AND METHODS

Test animal

For the present investigation adult Swiss albino mice Mus musculus were selected. Thirty female albino mice of same age group and average weight of 23 gm were procured from Animal Research Laboratory; Mahavir Cancer Sansthan, Phulwarisharif, Patna. The albino mice were housed in poly propylene cages and maintained in controlled temperature (27°C), humidity (0.5-10%) and and a photo period of 12 hours. They were fed with cereal made bread and gold mohar brand animal feed manufactured by Lipton India limited company, Delhi and water ad libitum. All the experimental mice were categorized into following groups:-Group C – Normal, Group R7 (7days rogor treated mice@20 mg/kg body wt.), R14 (14days rogor treated mice@20 mg/kg body wt.), R21 (21days rogor treated mice@20 mg/kg body wt), E7 (7days endosulfan treated mice @ 2 mg/kg body wt.), E14 (.14days endosulfan treated mice @ 2 mg/kg body wt.).E 2I (21days endosulfan treated mice @ 2 mg/kg body wt.), RE7 (7 days concomitant treated mice with rogor @10 mg/kg body wt and endosulfan@1 mg/kg body wt), RE14 (14 days concomitant treated mice with rogor @10 mg/kg body wt and endosulfan@1 mg/kg body wt) & RE21 (21 days concomitant treated mice with rogor @10 mg/kg body wt and endosulfan@1 mg/kg body wt).

Pesticides used

The two pesticides of analytical grade selected were “Endocel (EC 35%) manufactured by Excel Industries Ltd., Ruvapari Road, Bhawanagar (Gujrat)” , and “Rogor (EC 30%)” manufactured by ANU products, old Faridabad (Haryana).The LD₅₀ of endosulfan and rogor for mice were calculated as per standard method(APHA,2005) and confirmed by pilot test. Accordingly a separate dose of endosulfan (@2mg/Kg/body wt.) & rogor (@20 mg/Kg/body wt.) and a combined dose mixture of rogor (10mg/Kg/ body wt.) and endosulfan (@1 mg/Kg/body wt.) were considered for its administration to mice for 7, 14 and 21 days. The stock
solutions were prepared in distilled water and administrated orally by gavages method for the requisite period. Group-I mice were given only normal saline.

At the termination of each exposure of 7th, 14th, and 21th day the test animals were anesthetized, blood sample were collected in different vials, by puncturing ocular vein with the help of sterile hypodermic syringe. Blood were extracted and refrigerated at -20°C in sterilized paraffin covered tubes for amino acid and biochemical analysis. Serum was analyzed for quantitative estimation of Total protein respectively. After each schedule exposure, liver tissues were dissected out, ringed in NaCl (85%) to remove any adhering unwanted tissues, cut into small pieces with sharp surgical blades, and were preserved in neutral formalin and further processed for Light microscopy as per routine method of our laboratory for histopathological studies.

Methods for amino acid estimation
Amino acid composition of blood serum were determined by the method of Moore and Stein (1954) using Thin Layer Chromatography( TLC aluminium sheets No-1.05554.0007 purchased from Merck KGa A 64271 Darmstadt, Germany) and quantified on Systronic UV-Spectrophotometer (UV-U75-Spectrophotometer) at 570 nm Using lysine (1.13mg./ml) for comparison.

Methods for estimation of total protein
Total protein level were estimated according to the method of Lowery et al (1951) using bovine serum albumin as standard .Blood was centrifuged to separate serum at 3000 rpm and analysis was done on BT-260 plus Semi-Automatic Chemistry Analyzer, manufactured by Nanchang Biotech A&C Biotechnical Industry Co. China.

Statistical analysis
Six observations at random were taken in each case, then arithmetic mean and Standard deviation is calculated and then subjected to unpaired “t” test. The value obtained were referred to fisher’s table to see level of significance at (P<0.05) and (P<0.01). All the biochemical data were shown in text graph. All the statistical parameters were done on Sigma plot 8.0 version.
RESULTS AND DISCUSSION

Histo-pathological observations
The control (group C) liver of *Mus musculus* showed polygonal hepatocytes with normal arrangement of sinusoids and well defined central vein. (Plate I, fig.1). The spherical nucleus was almost centrally placed. The bile canaliculi were detected as a small canal formed by grooves in the two opposing cells (plate I, fig.1).

Numerous histopathological changes in the hepatic tissues were observed in different test group viz. rogor, endosulfan and simultaneously treated mice. The liver of test group R7 showed degeneration in hepatocytes in terms of intense vacuolization, widening of sinusoids, and extensive eosinophilic inclusion (plate I fig.2). The test group R14 showed further increase in sinusoid space and eosinophilic inclusion. Besides constriction of hepatocytes and glycogen depots were also prominently marked (Plate I, fig.3). The test group R21 showed increased necrotic area and sinusoid space while decreased cytoplasmic content were marked in constricted hepatocytes (plate I, fig.4).

The liver of test group E7 showed massive degeneration in hepatocytes as evidenced by the presence of numerous vacuoles, widening of sinusoids, eosinophilic inclusion and few prominently marked basophilic area (Plate II, fig.1). The test group E14 showed further widening in sinusoid space and constriction in central vein, eosinophilic inclusion, mild necrosis, and pycnotic nuclei (plate II, fig.2). The test group E21 showed degenerated murulia of hepatocyte with very wide sinusoid space, increased number of vacuoles (Plate II, fig.3&4). The liver of test group RE7 showed massive degeneration of hepatocytes in terms of increased sinusoid space, degenerated central vein with in filtered eosinophils (Plate III, fig.1). The liver of test group RE14 showed peripheral shifting of nucleus with the formation of numerous vacuoles in the hepatocytes. (Plate III, fig.2). The liver of test group RE21 showed extensive widening of sinusoid space, increased vacuoles within cells, nuclear destruction, eosinophilic inclusion and accumulation of fibrous tissue. (Plate III, fig.3&4).

Total protein related observation
All the rogor treated groups showed characteristics increasing trend in serum total protein content where as decreasing trend was marked in endosulfan treated groups. A similar trend in serum total protein was marked in concomitant groups. (Text graph-1).
Amino acid profile related observations

The study of amino acid profile showed aberrant fluctuation. Amongst rogor treated groups valine increased more than normal in R7 and then disappeared in other groups. Similar was the fate of glutamic acid. Tyrosine was detected in control (C), R7 and R21 except R14. Aspartic acid appeared only in control and R7 while methionine, alanine and serine appeared only in R21. (Text graph II). Valine, serine and Glutamic acid were present in very high quantity in control but found to be nearly absent in all the treated groups of endosulfan treated mice. The data clearly shows the loss of such amino acids in the serum of treated groups. Aspartic acid was detected in high concentration in control but it showed decreasing trend from E7 to E21. (Text graph III). In the concomitant treated groups, glutamic acid appeared in high quantity in control, comparatively lowered in RE7 while it was almost absent in RE14 and RE21.Tyrosine was detected only in control. Serine and methionine appeared only in RE21. Aspartic acid appeared in very high concentration in control, decreased variably in RE7 and RE21 and was absent in RE14. (Text graph IV).

![Text graph-I.](image)
Fluctuation of serum amino acid in control, Rogor, endosulfan and simultaneous (Rogor & Endosulfan) treated *Mus musculus* for 7 days

Text graph-II.

Fluctuation of serum amino acid in control, Rogor, endosulfan and simultaneous (Rogor & Endosulfan) treated *Mus musculus* for 14 days

Text graph-III.
The present study indicates that oral administration of sublethal doses of rogor (@20 mg/Kg body wt.), endosulfan (@2 mg/Kg body wt.) and their combinations (rogor @10 mg/Kg body wt.+ endosulfan@1 mg/Kg body wt.) brings about significant histopathological changes pointing towards severe damaging impact on liver cells. It is further confirmed by depletion in some of the important amino acids as well as abrupt alterations in the serum protein contents of various test groups of Mus musculus. Various histopathological anomalies observed in different test groups were irregular shape of hepatocytes, their inflammation and fusion at many places. Besides increased cellular edema, reduction in nucleo-cytoplasmic ratio, necrosis of hepatocytes and congregation of pycnotic nuclei were prominent. Other major anomalies incurred were shrinkage of central vein, deposition of fibrous tissue around the endothelial wall of central vein, collapsed sinusoidal space with their indistinct opening into the central vein, infiltration of few eosinophils and kupffer cells into sinusoids, etc. All these histological alterations points towards hepatic failure in mice. Similar impact of endomethacin, an anti-inflammatory drug on the liver of mice have been documented by Hameeda et al. (1998), who observed dilation of blood sinusoids, eroded endothelial blood vessels containing haemolyzed blood, neutrophils infiltrations and appearance of pycnotic nuclei etc in the liver of treated mice. The biochemical and histopathological impact of dimethoate and navacron were evaluated on the pregnant rats and their progenies by Abdel and Abdeen (1997), who also observed increased formation of vacuoles, lymphocytic
infiltration, nuclear pleomorphism, haemorrhage and increase in the number of kupffer cells in the liver. Ergul Belge Kurutas et al (2006) studied the effect of endosulfan on histology of liver in *Mus musculus*. They reported lobular inflammation as a typical sign of liver cell necrosis and chronic toxic hepatitis in liver with infiltration of polymorphonuclear cells, eosinophils & leucocytes. Qing-Wei Hu et al (2006) studied dimethylnitrosamine induced liver fibrosis in mice and reported centric-nuclear necrosis, distortion of hepatic sinusoids and fibrosis. The side effect of ketoprofen, non-steroidal anti inflammatory drugs (NSAID) @ 1.34 mg/kg body wt. (therapeutic dose) 2.65 mg/kg body weight (overdose) on the liver of female albino rats were elucidated by Abdel et al. (1998). Shrinkage of central vein is primarily due to the deposition of fibrous tissue around the endothelial wall and widening of sinusoids can be correlated with simultaneous shrinkage of hepatocytes at a particular region due to endosulfan and rogor toxicity. Increase in eosinophilic inclusion in the lobule of liver after endosulfan and rogor treatment marks the stressful condition of mice. Here in the present investigation, endosulfan and rogor initiate a wave of biochemical imbalance, which is evidenced by characteristic fluctuation in amino acids and serum total protein respectively. Four amino acids (Val, Tyr, Asp, Glu) were detected in good concentration in control group of mice but found consistently decreasing in 7th day and 14th day while increase in concentration of these amino acids were marked in all the test groups of 21 day exposure. There is no rise of additional amino acid. The decrease in Free Amino Acid (FAA) is probably due to their utilization for new protein synthesis or for production of energy to cope up with prevailing toxic condition due to intoxicant induced stress while increase is probably attributed to proteolysis or increased synthesis of FAA by transaminase reaction (Wilson and Poe, 1974). Total protein starts shooting up gradually onwards from 7th day of treatment and reaches its peak at 12 days of endosulfan treatment. However, even in7 days rogor treated group it shows slight elevation but much less in contrast to endosulfan treated group at similar duration. At combined exposure total protein shows a consistent decreasing trend at all the exposure level but in all the cases, it is still higher than the control, signifying toxic status of mice. Similar haematological findings were also reported by Padmaja et al (2000) in Wister rat due to selenium toxicity. The decreasing trend of protein content may be due to metabolic utilization of ketoacids to gluconeogenic pathway for synthesis of glucose or due to directing FAA for synthesis of necessary protein for maintenance of osmotic and ionic regulation in stressed condition (Schmidt 1975) while increase in protein content could stimulate protein synthesis or detoxification enzymes at the expense of glycogen to meet additional requirement in synthesis activity to retrieve the body from toxic stress.
PLATE-I

Fig. 1. The transverse section of control liver showing normally arranged hepatocytes (H) and sinusoids (S) with well defined central vein (CV) X100.

Fig. 2. Rogor treated (7 Days) liver of mice showing degenerated hepatocytes with increased vacuoles, widening of sinusoids (S) and some deposition in central vein (CV). X 200.
Fig. 3. Rogor treated (14 days) liver of mice showing increased sinusoidal space(s) and condensed hepatocytes(CH). X100.

Fig. 4. Rogor treated (21 days) liver of mice showing constriction in hepatocytes (CH), increased sinusoidal space(S) and degenerated nucleus (N) X200.
Fig 1:- The transverse section of liver of endosulfan treated (7 days) mice, showing eosinophilic inclusion (Ei) in hepatocytes, widening of sinusoid (s) and decreased cytoplasmic content. X 200.

Fig 2:- Section of mice liver after 14 days of endosulfan treatment, showing vacuole, RBC, eosinophilic inclusion (Ei) and pycnotic nuclei (PI) X 200.
Fig 3: Section of mice liver after 21 days of endosulfan treatment, showing massive degeneration of hepatocyte with widened sinusoidal space(s) and increased vacuoles (V) X 400.

PLATE-III

Fig 1: The transverse section of simultaneous rogor and endosulfan treated (7 days) liver of mice showing degenerated hepatocytes with eosinophilic inclusion(Ei) and few vacuoles, widened sinusoid space(s) and degenerated central vein(CV), X 200.
Fig 2: Loss of cyto architecture of hepatocytes with degenerated nuclei altered nucleo-cytoplasmic ratio and increased sinusoidal space. X200.

Fig 3: Section of liver of mice after 21 days of simultaneous treatment showing immensely increased sinusoidal space, vacuoles in hepatocytes, nuclear degeneration, eosinophilic inclusion (Ei) and development of fibrous tissue. X200.
CONCLUSION
In conclusion, the result of present study suggests that rogor and endosulfan set in a wave of biochemical imbalance leading to abnormalities on concerned tissues. The synergistic impact of these two pesticides is even more intense and damaging.

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