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Eucaryotic cell intoxication by Gram-negative pathogens: A novel bacterial outermembrane-bound nanovesicular exocytosis model for Type-III secretion system*

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Abstract

Gram-negative pathogens can intoxicate their target or host eucaryotic cells by translocating bacterial pathogenicity proteins and associated virulence determinants from interactive pathogens to directly inside the cytosol of the recipient cells. Classified as Type-III secretion system of gram-negative organisms this process has been proposed to operate through microinjection-like 'injectisome' multiprotein 'needle complexes' (Cornelis, GR, 1998, J. Bacteriol. 21, 5495-5504). Such a model for translocation, therefore, pre-supposes a direct physical contact between the organisms and the host cells which, on the contrary, has not been found to be necessary for the functioning of the Type-III secretion system (Daefler,S, 1999. Mol. Microbiol. 31, 45-57). Therefore, an alternate model is proposed here, on the basis of a revolutionary discovery of a previously un-envisaged process of vesicular exocytosis in prokaryotes, reported for gram-negative organisms (YashRoy, R C,1993, Indian J. Anim. Sci., 63, 99-102). In this process, secretory materials are translocated as bacterial outermembrane-bound nanovesicles (nOMVs), which are liberated by pinching off extended periplasmic pockets of interactive pathogens. The nOMVs, may in turn, fuse with the plasma membrane of eucaryotic host cells (YashRoy, RC, 1998, Current Sci. 75, 1062-1066) or else, may be engulfed by phagocytic cells (YashRoy, RC, 2000, Indian J. Poult. Sci. 35, 276-281). According to the model presented here: (1) accumulation of exo-proteins in the pathogen-periplasm, secreted by general secretory pathway of host-interactive gram-negative organisms, results in appearance of numerous outer-membrane bound protrusions designated, periplasmic organellae. (2) Large-sized periplasmic organellae pinch off as 50-90 nm diameter nOMVs. (3) From detailed analysis of ultra-structural observations, the above referred 'needle complexes' are ascribed here, a new function of 'riveting' or buttoning together, the bacterial outer and inner membranes, thus allowing only small pockets of periplasm to inflate out with 'stimulated' transport of excessive amounts of exo-enzymes across the cell membrane. Hence these supramolecular complexes have been given here a more appropriate name of 'rivet complexes'. These complexes span through both the bacterial cell and outer membrane and help release nOMVs in a mode analogous to soap-bubble formation from a bubble-tube. A single protein, synthesized at the cytoplasmic-end of rivet complex may, however, be transported through the narrow passage inside this complex along its length, as that may become necessary for elongation of the extendable 'needle' component. A tube-like assembly may be constituted by three or more rivet complexes, aligned closely parallel to one another, with their thin needles protruding out of the outer-membrane of the actively secreting organisms. Rivet complexes may be visualised to draw closer to one another by lateral diffusion of these supra-molecular protein complexes in a fluid membrane-matrix. Such a movement is proposed to be directed by a 'stretch-force' that may develop on the portion of the outer-membrane which is covering the inflating periplasmic organellae. (4) The nOMVs containing pathogenicity exoproteins inside and lipopolysaccharide (LPS)-lining at their exterior, then dock on the eucaryotic host-cell surface via chelation through bivalent Ca^{2+}/Mg^{2+} ions. (5) Focal fusion of vesicular membrane with host cell-membrane is likely to be effected through specialized coiled-coil proteins like Sip B in *Salmonella,* that have been shown to be present in/on nOMVs (Hayward, R.D., 2002, Cambridge Univ., England,

personal communication). Translocation of Type-III secretion materials from nOMVs to recipient host/target cell-cytosol may take place through a fusion-pore. Flow of materials is proposed to be effected uni-directionally, by a self-driven contraction process of the stretched LPS membrane of the fused nOMVs. (6) Docking of nOMVs on phagocytic cells may also result in the engulfment of nanovesicles, as such, thus translocating both LPS and pathogenicity proteins into the cytoplasm of the host cells. (7) Translocation of these biochemical signals from the interacting pathogens into eucaryotic host cells may, in turn, result in a variety of host cell responses like cytoskeletal reorganization, altered phagocytosis, change in ion fluxes, apoptosis, invasion of pathogens, cytokine release, differentiation of phagocytes, etc.

Key words: Type-III secretion model, Gram-negative pathogens, nanovesicular translocation mechanism, eucaryotic cell intoxication, *Salmonella* infection.

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Estimation of acute toxicity of zinc chloride by histopathological analysis of the epidermal linings of operculum of the catfish *Heteropneustes fossilis* (Bloch)

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Abstract

Acute toxicity (75 ppm; 96h LC50 value) of the trace metal salt zinc chloride on the outer and inner opercular epidermal linings of *Heteropneustes fossilis* has been analysed histopathologically. The mucous cells of both the epithelial linings show periodic fluctuations in their density and staining properties at different exposure time. The outer opercular lining shows more extensive damage than the inner one which is having better mucogenic potentiality. The other histopathological alterations include periodic sloughing of damaged epithelial cells and their regeneration leading to hyperplasia alongwith extensive vacuolisation. Club cells also show severe necrotic changes including vacuolisations and rupture leading to release of their contents over the damaged skin surface. Major histopathological alterations in the inner opercular epidermis include periodic hyperplasia and mild sloughing of epithelial cells. The histopathological analysis of the damages induced to the outer as well as inner opercular epidermis may be used as a potential bio-indicator of the disturbed aquatic environment.

Key words : Acute toxicity, *Heteropneustes fossilis*, histopathology, skin, and zinc chloride.

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Biochemical and histoarchitectural alteration in mouse testis on exposure to infrared radiation

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Abstract

Infrared radiation has been recognised by the Technology Assessment Task Force for Reproductive Health Hazards as one of the potent environmental agents causing reproductive injury. The present study was aimed, therefore, at determining the effect of whole-body exposure to infrared radiation on the testicular function of adult male albino mice (Mus musculus). The animals were exposed for a period of 7 days, 1 hour daily, to a source of infrared radiation (IR-R), having wavelength range between 900-1200nm. The study revealed no alteration in weight of the testis, however a reduction was observed in the testis cholesterol and ascorbic acid concentrations, activities of 3β and 17β hydroxysteroid dehydrogenases and serum testosterone levels, suggesting impaired steroidogenesis. Infrared radiation exposure also led to elevated testis DNA, RNA and protein concentrations. Significant histoarchitectural alterations were observed in the testis of IR-R exposed animals, with evidence of arrest of spermatogenesis, which correlated with a reduction in the number of implantation sites on fertility testing. The data suggest that exposure to infrared radiation leads to marked testicular injury and impaired fertility. These findings hold significance in view of the rampant use of infared radiation.

Key words : Histoarchitectural alteration, testis, infrared radiation

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Studies on the haematological profile of salinomycin toxicity and efficacy of certain antioxidants in broilers

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Abstract

Salinomycin toxictiy was evaluated by assessing haematological profile at therapeutic (60 ppm) and toxic (120 ppm) dose levels in day old male broiler chicks and the efficacy of antioxidants such as vitamin E @ 300 mg/kg+Se@ 0.3 mg/kg, zinc @ 80 mg/kg and Endox Dry @ 125 mg/kg of feed was studied for prophylactic and therapeutic management of salinomycin-induced toxicity.The toxicity was assessed in terms of haematological parameters. The results revealed a significatnt (P<0.01) decrease in Hb, TEC, TLC, PCV, MCV and MCH as compared to control and remaining groups, whereas MCHC and DLC values were found to be non-significant. In groups X to XV, the haematological parameters were revived to normal at 7th week, following antioxidant therapy. Thus, it was concluded that ionophore toxicity was due to oxidative damage by free radicals, and hence it is recommended to use Vit.E+Se, Zinc and Endox Dry as antioxidants in feed to avoid any accidental ionophore toxicity.

Key words: Salinomycin, oxidative damage, Vit.E, Selenium, zinc, Endox Dry, haematology, broilers.

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Blood lead levels during pregnancy and their impact on birthweight - An Indian study

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Abstract

A cross-sectional study on blood lead levels during pregnancy was carried out on 100 women from general population reporting at local Hospitals in Lucknow(U.P) India. The age of women ranged from 15 to 32 Years. None of the subjects had any occupational exposure to lead (Pb). All the subjects screened for blood lead levels were apparently healthy, non-alcoholic, non smoker and non consumer of illicit drugs . Blood lead levels at full term were signigficantly higher ($p<0.001$) than during first trimester. Significant negative correlation ($r = -0.75$, $p< 0.001$) was observed between blood lead levels at full term and reduction in birthweight of neonates. Thus a possible eausal relationship between the two can not be ruled out.

Key words: Blood, Lead Levels, Pregnancy, Birthweight, Neonates

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Hypoglycaemic effect of petroleum ether extracts of neem (*Azadirachta indica*) seed husk and kernel in streptozotocin- induced diabetic rats

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Abstract

Effect of neem seed husk (NSH) and neem seed kernel (NSK) was studied on body and organ weight in normal and diabetic rats. NSK (2.00 mg/kg b. wt, orally daily for 28 days) significantly increased (+10.73 %) body weight as compared to diabetic control (-13.95%,$P<0.05$). The percent gain was comparable to control. The absolute weight of spleen, heart, liver and testes were significantly decreased in streptozotocin-induced(STZ@ 55mg/kg b.wt iv, tail vein) diabetic rats, whereas NSK (2.0 mg/kg, b wt orally daily for 28 days) significantly normalized the organ weights. The hypoglycaemic effect of single oral doses of NSK (0.5,1..0 and 2.0 mg/kg b wt) and NSH (0.09,0.18 and 0.36 mg/kg, b wt) was studied after oral administration of glucose (4ml/kg b wt of 25% solution).The higher dose of NSK (2.0 g/kg, b wt) and lower dose of NSH (0.09 g/kg, b wt) were found to be effective in lowering the blood glucose levels. In another experiment, insulin-dependent hypoglycaemia was studied using streptozotocin (STZ@ 55 mg/kg iv, tail vein) induced diabetes in rats. The animals were given petroleum ether extracts of neem seed kernel (NSK, 2.0 g/kg, b wt) and husk (NSH, 0.09 g/kg, b wt) orally daily for 28 days. Both NSK and NSH lowered the glucose levels. The decrease in blood glucose levels after NSK was significant at day 7, whereas after NSH, it was significant after day 14. The efficacy of NSK and NSH in lowering blood glucose levels was comparable to that of insulin (positive control). The study suggests that both NSK and NSH produce significant hypoglycaemia in rats. However, hypoglycaemia produced by NSH is time-dependent.

Key words : Blood glucose, Glucose tolerance, Hypoglycaemia, Streptozotocin, neem.

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Pesticide residues in formulators and their relevance to certain biological indices

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Abstract

The study on the biological monitoring of the residues of persistent pesticides in formulators engaged in formulation of various pesticides in an industrial setting was conducted. Total hexachlorocyclohexane (HCH) and its residues were significantly higher ($p<0.005$) in formulators. β-HCH was the chief contaminant and it accounts for about 93% of the total HCH content. A total of four residues of DDT (pp'-DDE, pp'-DDT, op'-DDT and pp'-DDD) were also detected. However, significant elevation ($p<0.01$) was noticed in the level of pp'-DDE and total DDT in formulators. The pp'-DDE contributed about 73% of the total DDT content. On comparison with the levels of various organochlorine insecticides detected in this study, a significant positive correlation was found between α-HCH and lgM ($r=0.526$, $p<0.005$) and total HCH and lgM ($r= 0.401$, $p<0.05$). These findings merit surveillance insight reflective of industrial exposure and may be regarded as an aid towards understanding of the environmental problems resulting from pesticide formulation practices in industrial settings.

Key words : Formulators, pesticide residues DDT, HCH, lgM

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Subacute oral toxicity of amitraz in cross-bred cow calves - Effect on some haematological parameters

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Abstract

The effect of repeated oral administration of amitraz at doses of 0.25 and 1.0 mg/kg/day for 21 consecutive days was studied on some haematological parameters in cross-bred male cow calves. Amitraz at dose rate of 0.25 mg/kg/day produced a significant decrease (52.7%) in erythrocyte sedimentation rate, and an elevation in haemoglobin concentration (28.1%). Amitraz at both doses for 21 days did not cause any significant alteration in total leucocyte count and total erythrocyte count, but higher dose elevated (17.3%) values of packed cell volume. Amitraz at different doses produced significant elevation in the values of mean corpuscular volume (21.8%), mean corpuscular haemoglobin (57.6%) and mean corpuscular haemoglobin concentration (30.0-32.2%) in male cow calves.

Key words : Amitraz, cow calves, haematological parameters, subacute toxicity

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Hepatoprotective effect of silymarin in experimentally induced aflatoxicosis in broilers

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Abstract

Silymarin, an antihepatotoxic principle isolated from *Silibum marianum* was able to afford protection to liver as observed by favourable changes in serum total proteins, alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in broilers, subjected to aflatoxin toxicity. However, the protective effects of the drug were not complete.

Key words : Aflatoxin, silymarin, hepatoprotective.

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Gasoline, methanol and gasoline : methanol blend exposure-induced nephropathy in male rats

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Abstract

The present study was designed to evaluate the effect of repeated inhalation exposure of gasoline, methanol and gasoline: methanol (90:10) vapours (20 ml/h; 6 h/day for 28 days) on kidneys of rats. The effect of withdrawal on cessation of exposure for 14 days was also investigated. Results revealed significant ($p<0.05$) increase in absolute and relative kidney weight in male rats exposed to gasoline and gasoline: methanol vapours. Histopathological studies revealed degenerative changes in the tubular epithelium, presence of renal epithelial casts in the tubular lumen alongwith engorged blood vessels and dilation of intertubular capillaries and proximal renal tubule in gasoline and gasoline: methanol treated male rats. In addition, significant ($p<0.05$) rise in blood urea nitrogen, creatinine and total bilirubin in the serum of male rats, as compared with that of the control, was also observed in gasoline and gasoline: methanol exposed rats. The toxicity was comparatively higher in gasoline exposed rats than that in gasoline: methanol mixture. No significant changes in any of the above parameters were observed in methanol vapour alone exposed rats. Withdrawal of treatment caused marked recovery. The present study clearly indicates that the addition of 10% methanol to gasoline did not cause any rise in toxicity and the effect of the treatment are transient and reversible.

Key words: Gasoline, Methanol, Inhalation, Rat, Nephropathy

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Effect of *Awala* on carbon tetrachloride induced hepatotoxicity in rats

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Abstract

Effect of *Awala* (*Emblica officinalis*) was studied on hepatotoxicity in rats. The hepatotoxicity was induced by administration of carbon tetrachloride @0.15 ml/kg body weight (20% v/v solution in liquid paraffin) once on 3rd day of the experiment. *Awala* was administered orally @200 mg/kg body weight once a day orally for 7 and 15 days. The efficacy was assessed on the basis of histopathological studies and biochemical profile of transaminases (AST and ALT). From the results it was observed that *Awala* showed protective action against carbon tectrachloride hepatotoxicity in rats.

Key Words : Hepatotoxicity, Emblica officinalis, Liver, Alanine aminotransferase, Aspartate aminotransferase, Rat.

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Role of pollutants, environmental and social factors in causing shortage of drinking water : A global study with focus on India

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Abstract

The major environmental problems and factors that affect the quality and/or quantity of drinking water are acidic deposition, mercury deposition, global warming, population growth, water usage for agricultural production, and deforestation. Of these, acid and mercury depositions affect the quality of water whereas all others affect the quantity of fresh water available for drinking purposes. The quantity of fresh water available for drinking is finite. The need for fresh water has increased four-fold due to growth in human population and water usage in agricultural production. Global warming-related increases in temperature may be accompanied by changes in rainfall-patterns and potential for droughts. Forests play vital roles in the onset of rainfalls and absorption of water underground for storage in aquifers. The shortage of drinking water in India is mainly due to explosive population growth, global warming (temperature up to 120°F), deforestation, and depletion of water in underground storages (aquifers). There is a need to control and stabilize human population growth; introduce new techniques for irrigating farm lands and refilling underground aquifers; plant and grow millions of trees; regulate pollution to clean air and water; and conserve water. All water conservation techniques are briefly described.

Key words: Explosive population growth, global warming, deforestation, depletion of key aquifers,
conservation techniques

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Ameliorative effect of vitamin E on aflatoxin-induced changes in accessory sex organs of male mice

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Abstract

Ameliorative effect of vitamin E on aflatoxin-induced changes in male accessory sex organs, adult male albino mice were evaluated by orally administering vitamin E (2mg/animal/day) 1 hour before administration with 25 and 50 g of aflatoxin/animal/day (750 and 1500g/kg body weight) for 45 days. Seminal vesicle and ventral prostate with their secretory contents, were isolated, blotted free of blood and used for analysis. Aflatoxin treatment for 45 days caused significant, dose-dependent reductions in absolute and relative weights, as well as fructose content in the seminal vesicle as compared with vehicle control. Also absolute and relative weights, as well as protein content in the prostate were significantly reduced in aflatoxin-treated mice. However, acid phosphatase activity was significantly higher in aflatoxin-treated mice than that of vehicle control. Vitamin E (2 mg/animal/day) pretreatment significantly ameliorated aflatoxin-induced changes in low dose aflatoxin-treated group as data were almost comparable with the controls. However, amelioration was partial in high dose aflatoxin-treated group, as values were significantly different from the control. It is concluded that vitamin E pretreatment significantly ameliorates aflatoxin-induced changes in male accessory sex organs of mice.

Key words : aflatoxin, vitamin E, seminal vesicle, prostate

Vol. 10, No. 2, 2003 pp 81-87

Impact of antioxidants on N-nitrosodiethylamine-induced biochemical toxicity in rats

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Abstract

Present study reports the importance of exogenous antioxidants (vitamin C, vitamin E and their combination) in neutralizing the N-nitrosodiethylamine (NDEA)-induced oxidative stress. Vitamin C or vitamin E was incorporated @ 200 mg/kg and vitamin C+vitamin E @ 100mg/kg of each in the diets of rats. Rats of these groups alongwith control rats receiving normal diet without supplement, were injected NDEA @200 mg/kg body wt., and animals were sacrificed after 48 h of injection. Supplement of diets with antioxidant vitamins protected the animals, against the toxicity of NDEA through varying degrees. It improved feed intake and prevented loss of body weight to a reasonable extent. Combination of vitamins C+E appeared to be most effective in improving the oxidative stress of N-nitrosodiethylamine followed by vitamin C alone and vitamin E alone.

Key words: N-nitrosodiethylamine, vitamin C, vitamin E, oxidative stress

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Prediction of age from body weight of randomly trapped monkeys

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Abstract

Baseline variables such as age, body weight etc. are influencing factors for biological response during safety and toxicity testing. Normally the non-human primates e.g. *Macaca mulatta* provided for such experiments are wild caught or randomly trapped. The body weight of these monkeys can be measured easily, but their age remains unpredicted. A comparison of two mathematical models to predict the age of trapped rhesus, langoors and bonnet monkeys is discussed in the present communication.

Key words : Primatology, age prediction, age-body weight relationship, non-human primates

Vol. 10, No. 2, 2003 pp 95-103

Effects of acute and chronic toxicity of four household detergents on RBC counts and their morphology in a freshwater fish *Gambusia affinis*

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Abstract

Acute exposure (96 hours) of freshwater fish *Gambusia affinis* to 6 concentrations (5-30ppm) of four household detergents (2 washing powders and 2 cakes) and chronic exposure (15 & 30 days) to only washing powders (10ppm) resulted in morphological abnormalities in their RBCs, alongwith reduction in their counts (acute toxicity: 12-64%; chronic toxicity: 23-58%). The percentage of morphologically abnormal RBC (poikilocytosis) was higher among fish exposed to cakes (24-52%), in comparison to those exposed to washing powders (12-48%) for 96 hours; increasing further (65-70%) at chronic exposure to washing powders. The RBC size decreased (microcytic condition) after both acute (8-34%) and chronic (12-24%) exposures of fish to detergents. The aforesaid adverse effects were dose dependent during acute toxicity studies, whereas these were governed by exposure periods during the chronic toxicity.

Key Words : Acute toxicity, chronic toxicity, washing powder, cake, *Gambusia affinis*.

Vol. 10, No. 2, 2003 pp 105-109

Relative sensitivity of *Gambusia affinis* to different concentrations of textile effluent

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Abstract

Bioassay studies indicate that toxic ingredients of textile effluents induce behavioural changes such as loss of equilibrium, unusual lethargy and gasping for breath (asphyxia) in *Gambusia affinis* along with the high mortality at 25 to 40% concentrations. Relative toxicity of effluent in terms of lethal concentrations was also evaluated. Dissolved oxygen content was found lower at all concentrations during bioassay. Textile effluent was found highly acidic in nature. Higher levels of chemical oxygen demand, conductivity and total hardness were recorded. The effluent was also found to contain heavy metals (Cu, Pb, Fe, Mn, Cd, Zn) some of them in higher levels than the permitted limits.

Key words: Textile effluent, mortality, lethal concentrations.

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A study on the mechanism of toxicity of deltamethrin in poultry

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Abstract

A study was conducted to evaluate the mechanisms of toxicity due to deltamethrin in poultry. Broiler chicks were fed on ration containing deltamethrin (100 mg/Kg) for 6 weeks. Body weights and serobiochemical parameters were evaluated at every 2 weeks interval. The study revealed a significant ($P< 0.05$) reduction in the weight gains, glutathione and high density lipoproteins with treated birds. A significant ($P< 0.05$) increase in the activities of glutathione peroxide, glutathione reductase, catalase, aspartate aminotransferase and lactate dehydrogenase, and the concentrations of total cholesterol, triglycerides, low density lipoproteins, serum urea, serum creatinine and blood urea nitrogen was recorded in the group fed on deltamethrin-containing feed, as compared to the control. Results of the present study suggest that deltamethrin induces toxicity in birds by inducing oxidative stress due to over production of free radicals or reactive oxygen species.

Key words: Broilers, deltamethrin, oxidative stress

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Effect of *Awala* on carbon tetrachloride-induced hepatotoxicity in rats

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Abstract

Effect of *Awala* (*Emblica officinalis*) was studied on hepatotoxicity in rats. The hepatotoxicity was induced by administration of carbon tetrachloride @0.15 ml/kg body weight (20% v/v solution in liquid paraffin) once on 3rd day of the experiment. *Awala* was administered orally @200 mg/kg body weight once a day orally for 7 and 15 days. The efficacy was assessed on the basis of histopathological studies and biochemical profile of transaminases (AST and ALT). From the results it was observed that *Awala* showed protective action against carbon tectrachloride hepatotoxicity in rats.

Key Words : Hepatotoxicity, Emblica officinalis, Liver, Alanine aminotransferase, Aspartate aminotransferase, Rat.