

Research Article

Evaluating Systemic Toxicity in Rabbits after Acute Ocular Exposure to Irritant Chemicals

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Received 9 September 2014; Revised 15 October 2014; Accepted 20 October 2014; Published 19 November 2014

Academic Editor: Kanji Yamasaki

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Acute systemic toxicity via ocular exposure route is not a well understood aspect. Any material/drug/chemical that comes in contact with the eye can evade the first pass metabolism and enter the systemic circulation through the conjunctival blood vessels or via the nasolacrimal route. In this study, the effect of ocular irritant chemicals on the systemic toxicity was assessed in rabbit. Eyes of rabbits were exposed to known ocular irritant (cetyl pyridinium chloride, sodium salicylate, imidazole, acetaminophen, and nicotinamide) for 24 h and scored. After a period of 72 h, blood was collected from the animals for examining the hematological and biochemical parameters. The animals were then sacrificed and the eyes were collected for histopathology and cytokine analysis by ELISA. Splenocyte proliferation was assessed by tritiated thymidine incorporation assay. The liver and brain of the treated animals were retrieved for evaluating oxidative damage. The chemicals showed moderate to severe eye irritation. Inflammation was not evident in the histopathology but proinflammatory markers were significantly high. The splenocyte proliferation capacity was undeterred. And there was minimal oxidative stress in the brain and liver. In conclusion, acute exposure of ocular irritants was incapable of producing a prominent systemic side effect in the current scenario.

1. Introduction

Toxic effects seen when chemicals/drug/materials are exposed in single or multiple doses over a period of 24 h are referred to as acute toxicity. Acute toxic effect of a chemical will be different from the prolonged exposure (subacute) to a chemical. Toxicity of a substance is affected by multifaceted parameters and is complex. Routes of exposure are one of the parameters that affect the toxicity of a substance and can cause a systemic effect. Intravenous administration of a substance elicits the most profound toxic effect; however the substance is susceptible to first pass metabolism in the liver. Other prominent routes of exposure include inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral, and topical. Ocular exposure of drugs or chemical is a relatively unexplored territory. Any chemicals that come in contact with the eye accidentally or by deliberate administration (ophthalmologic drug or cosmetics) can enter the systemic circulation through the rich network

of conjunctival blood vessels or via the nasolacrimal route [1, 2]. Entry of drug through the above pathways ensures high plasma concentration of the substance due to evading of the first pass metabolism. The tight junctions in the cornea protect most the entry of the chemicals through paracellular route onto the systemic circulation. However, many harsh chemicals (such as ocular irritants) can compromise the integrity of the tight junction and aid in access to systemic circulation. Moreover, these chemicals can induce inflammation, making the vasculature leaky [3]. Once these chemicals enter the circulation, it can cause damage to the central nervous system, liver, spleen, kidney, and so on. So this study was carried out in order to elucidate whether acute exposure of an ocular irritant chemical can cause systemic side effects.

In this study, eyes of rabbits were exposed to 5 known ocular irritant chemicals: cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide (NIC) for a period of 24 h. The

capacity of these chemicals to induce ocular inflammation and their ability to alter the blood parameters, to affect the splenocyte proliferation, and to induce oxidative damage in liver and brain was assessed. It was found that most of the ocular irritant chemicals were capable of inducing proinflammatory marker. However, they did not elicit any systemic toxicity.

2. Materials and Methods

2.1. Chemicals and Reagents. Cetyl pyridinium chloride (Sigma), sodium salicylate (Sigma), imidazole (Merck), acetaminophen (Sigma), nicotinamide (Sigma), rabbit IL-1 β , IL-1 α , IL-8, and TNF- α ELISA kits (Cusabio, China) were used. Thiobarbituric acid (TBA), reduced glutathione (GSH), oxidized glutathione (GSSG), and dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Pyrogallol (PG), diethylene triamine pentaacetic acid (DTPA), trichloroacetic acid (TCA), and other chemicals and reagents used were of analytical grade.

2.2. Experimental Animals. Studies were carried out using New Zealand white rabbits, procured from the Division of Laboratory Animal Sciences (DLAS) of Sree Chitra Tirunal Institute for Medical Sciences and Technology. Animals of both sexes were used for the study. They were housed in controlled environments (temperature: $22 \pm 3^\circ\text{C}$; humidity: 50 to 70%) and fed with standard feed and free access to water and a 12 h light and dark cycle was maintained. This study conformed to the guiding principles of Institutional Animal Ethics Committee (IAEC), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and the guide for the care and use of laboratory animals.

2.3. Exposure to Ocular Irritant Chemicals. A total number of 15 animals of both sexes were chosen for the study. Three animals were assigned per chemicals. The chemicals were exposed at the following doses: CPC (100 mg/mL), SS (100 mg/mL), IMI (100 mg/mL), ACT (300 mg/mL), and NIC (300 mg/mL). The dose was determined based on in vitro tests (data not shown) and literature reviews. The chemicals were dissolved in physiological saline. Right eye of the rabbits was kept as test whereas the left eye was the control (for scoring and histopathology). Animals that were untreated with any chemicals were used as control for ELISA, hematological and biochemical, splenocyte proliferation, and antioxidant assays. The lower lid was pulled away from the eye ball and the chemical solution was added drop wise into the conjunctival sac. The eyelids were held together for a few seconds so as to ensure contact with the ocular tissue. After 24 h, the eyes were thoroughly washed with physiological saline. The animals were then kept for further observation for 72 h and scored periodically (0, 24, 48, and 72 h). At the end of 72 h blood was collected from the animal for hematological and biochemical parameters and then sacrificed.

2.4. Histopathology. The eyes from animals that were exposed to ocular irritant (test and control) were enucleated and preserved in Davidson's fluid. The ocular tissue was dehydrated and processed for histopathological analysis. Hematoxylin and Eosin (H&E) staining was carried out and corneal thickening, macrophage infiltration, inflammation, blood vessel congestion, and so on were observed under a microscope.

2.5. Cytokine Analysis

2.5.1. Preparation of Homogenate. The corneas were retrieved from the test as well as the control animals. 5 mL of phosphate buffered saline (pH: 7.4) was added and the tissue was homogenized under cold conditions using a polytron homogenizer. After this, the homogenate was spun at 12000 rpm for 30 min in a refrigerated centrifuge. The pellet was discarded and supernatant was stored in cold conditions for cytokine analysis using ELISA.

2.5.2. Analysis of Cytokines. IL-1 β , IL-1 α , IL-8, and TNF- α using ELISA assay were carried out as per the instructions provided by the manufacturer. In short, the sample (100 μL) was added to the wells and incubated for 2 h at 37°C . The liquid was aspirated out carefully after incubation and 100 μL of Biotin-Antibody (1X) was added to each well and was kept further for an hour at 37°C . Supernatant was removed and was washed thoroughly with wash buffer. Then, 100 μL HRP-Avidin (1X) was added to each well and incubated. After the incubation period (1 h), the wells were washed again (5 times). TMB substrate (90 μL) was added and incubated in the dark for 15–30 minutes. The reaction was stopped by adding 50 μL stop solution and read at 450 nm spectrophotometrically (Labtech LT-4000 microplate reader). Triplicates of each sample were run. Concentration was estimated using standard curve of each cytokine.

2.6. Hematological and Biochemical Parameters. Blood from the ear vein of rabbits (treated and control) was collected into collecting tubes. The blood was collected in EDTA coated tubes for analyzing hematological parameters such as hemoglobin concentration (Hb), total erythrocyte count (RBC), hematocrit (HCT), and total leukocyte counts. This was determined using hematology counter (vet animal blood counter).

Similarly, blood was collected in uncoated tubes for analyzing biochemical parameters. The tubes were allowed to stand for a while so that blood clot was formed. Serum was separated by centrifuging the tubes at 3000 rpm for biochemical estimation. Biochemical parameters such as ALP, SGOT, SGPT, GGT, albumin, total protein, glucose, total cholesterol, chloride, urea, creatinine, total bilirubin, phosphorous, and so forth were estimated using ERBA XL 300 biochemical fully automated analyzer.

2.7. Splenocyte Proliferation Assay. Incorporation of [^3H]thymidine is used to estimate the replicative capacity of cells. Spleens from the animals (test and control) were retrieved for this purpose. Splenic lymphocytes or

splenocytes were isolated using histopaque (Sigma, USA) and cultured in RPMI 1640 (Himedia, India) supplemented with 10% FBS (Invitrogen, USA). Cells were seeded at a cell density of 2×10^5 cells per well, onto 96-well plates, and kept in 37°C at 5% CO₂. At the 48th h, 0.5 μ Ci of [³H]thymidine was added per well. After 72 h in culture, the cells were harvested and the radioactivity was measured using scintillation counter (Hidex, Finland). Data is reported as mean CPM of triplicate samples.

2.8. Antioxidant Enzyme Assay of Liver and Brain

2.8.1. Preparation of Liver and Brain Homogenate. Liver and brain from the rabbits used for the acute ocular irritation study were collected. 10% liver and brain homogenate was prepared in phosphate buffered saline (PBS). The supernatant was collected after centrifugation at 3500 rpm for 10 mins. The resultant supernatants were maintained in an ice bath until used for the estimation of total protein, lipid peroxidation (LPO), glutathione reductase (GR), reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) using standard protocols with slight modifications. Liver and brain from untreated rabbits served as control.

2.8.2. Total Protein. Total proteins in the liver and brain homogenates of rabbits exposed to ocular irritant chemicals were estimated by the method of Lowry et al. [4] using bovine serum albumin as standard.

2.8.3. Lipid Peroxidation (LPO). The extent of oxidative damage to lipids was estimated by determining the concentration of malondialdehyde (MDA), which is a nonreactive end product of LPO. The protocol was followed as described by Okado-Matsumoto and Fridovich [5]. It uses thiobarbituric acid (TBA) which reacts with thiobarbituric acid reactive substrates (TBARS) like MDA to form a pink coloured product which is measured at 532 nm. The concentration is expressed in nmol/mg protein.

2.8.4. Reduced Glutathione (GSH). The level of GSH in the liver and brain of the animals was determined by the method of Moron et al. [6], with slight modifications, in which Ellman's reagent or DTNB (5, 5'-dithiobis-(2-nitrobenzoic acid) reacts with GSH to form a spectrophotometrically detectable, yellow coloured product (GSH-TNB) at 412 nm. The change in absorbance is a linear function of the GSH concentration in the reaction mixture. The amount of GSH was expressed as nmol/mg protein.

2.8.5. Glutathione Reductase (GR). GR activity in liver and brain homogenate was determined by measuring the reduction of GSSG in the presence of NADPH as described by Mize and Langdon [7]. Briefly, this assay measures the rate of NADPH oxidation to NADP⁺, which is accompanied by a decrease in absorbance at 340 nm. Thus, one GR unit is defined as the reduction of one μ M of GSSG per minute at

TABLE 1: *In vivo* scoring of rabbit eyes, after exposure to ocular irritant chemicals (acute) at the end of 72 h. CPC: cetyl pyridinium chloride, SS: sodium salicylate, IMI: imidazole, ACT: acetaminophen, and NIC: nicotinamide. All values are mean \pm SD, $n = 3$.

	Control	CPC	SS	IMI	ACT	NIC
Cornea						
Degree of opacity	0 \pm 0	0 \pm 0	1 \pm 0.57	0 \pm 0	0 \pm 0	0 \pm 0
Area of cornea	0 \pm 0	3 \pm 0.57	2 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0
Iris	0 \pm 0	2 \pm 0	2 \pm 0	1 \pm 0	2 \pm 0	2 \pm 0
Conjunctiva						
Redness	0 \pm 0	3 \pm 0	3 \pm 0	1 \pm 0	2 \pm 0	2 \pm 0
Chemosis	0 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0	2 \pm 0
Discharge	0 \pm 0	2 \pm 0.57	2 \pm 0	2 \pm 0.57	1 \pm 0	2 \pm 0

0: normal, 1: slight, 2: moderate, and 3: severe.

25°C and pH 7.6. Enzyme activity is measured in units/mg protein.

2.8.6. Glutathione Peroxidase (GPx). Activity of GPX in liver and brain homogenate was assayed by the method described by Rotruck et al. [8]. The remaining GSH after the enzyme catalyzed reaction complexes with DTNB, which absorbs at maximum wavelength of 412 nm. Enzyme activity was expressed as μ g of GSH consumed/min/mg protein.

2.8.7. Superoxide Dismutase Assay (SOD). Assay of superoxide dismutase was done in liver and brain tissue homogenate using modified pyrogallol autooxidation method [9] and is spectrophotometrically measured at 420 nm.

All measurements were carried out using Lambda 25, UV/Vis spectrophotometer, Perkin Elmer, USA.

2.9. Statistical Analysis. All experiments were repeated thrice and all data are presented as the mean with the standard deviation (mean \pm SD). Significance has been calculated using Student's *t*-test. * denotes a statistical significance (**P* value \leq 0.05) with respect to control.

3. Results

3.1. Scoring. The eyes (control and test) were scored based on their severity on a scale of 0–3, with 3 being the most severe irritant. Parameters like redness, swelling, opacity, discharge, and so forth were examined. All of the chemicals showed moderate to severe irritation potential. CPC and SS showed the highest degree of irritation when compared to IMI, ACT, and NIC. Table 1 indicates the scores assigned to the chemicals.

3.2. Histopathology. Histopathological analysis of eyes of CPC treated animals showed that the morphology of cornea remained normal (Figure 1(b)). However, there was mild

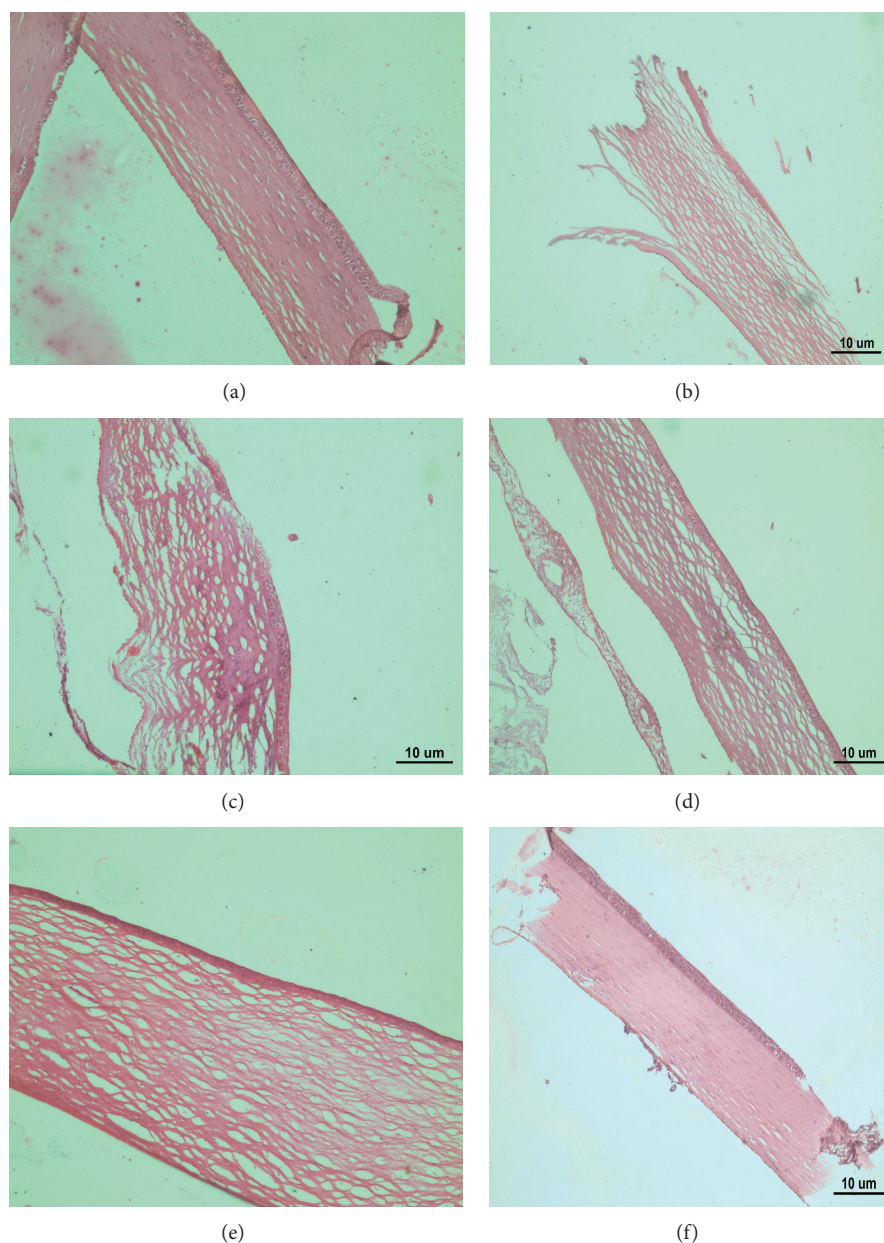


FIGURE 1: Histopathology of cornea treated with ocular irritant chemicals using H&E staining. (a) Control, (b) cetyl pyridinium chloride (CPC), (c) sodium salicylate (SS), (d) imidazole (IMI), (e) acetaminophen (ACT), and (f) nicotinamide.

acute inflammation in the ciliary body and congestion of blood vessel in the choroid of CPC treated animals.

Animals treated with SS showed corneal morphology similar to control, as evident from Figure 1(c). However, chronic inflammation was visible in the ciliary body and mild inflammation was evident in the choroid and retina of SS treated animals.

From Figure 1(d), it was observed that IMI treated animals, when compared to control, had normal corneal morphology. There were no evident signs of inflammation, except slightly congested blood vessels in the ciliary body.

In comparison to control, cornea was normal in ACT treated animals (Figure 1(e)). Sign of mild inflammation was

detected in the ciliary body due to macrophage infiltration. It was observed that the blood vessels in the choroids region were also congested.

In cornea of ACT treated animals, it was seen that there was cornea which appeared normal when compared with control (Figure 1(f)). Ciliary body showed mild inflammation in ACT treated animals.

3.3. Cytokine Analysis. Inflammation was further assessed by analyzing the concentration of proinflammatory cytokines (IL-1 α , IL-1 β , IL-8, and TNF- α) after treatment with CPC, SS, IMI, ACT, and NIC. It can be seen from Figure 2(a) that IL-1 α shows a statistically significant increase in CPC

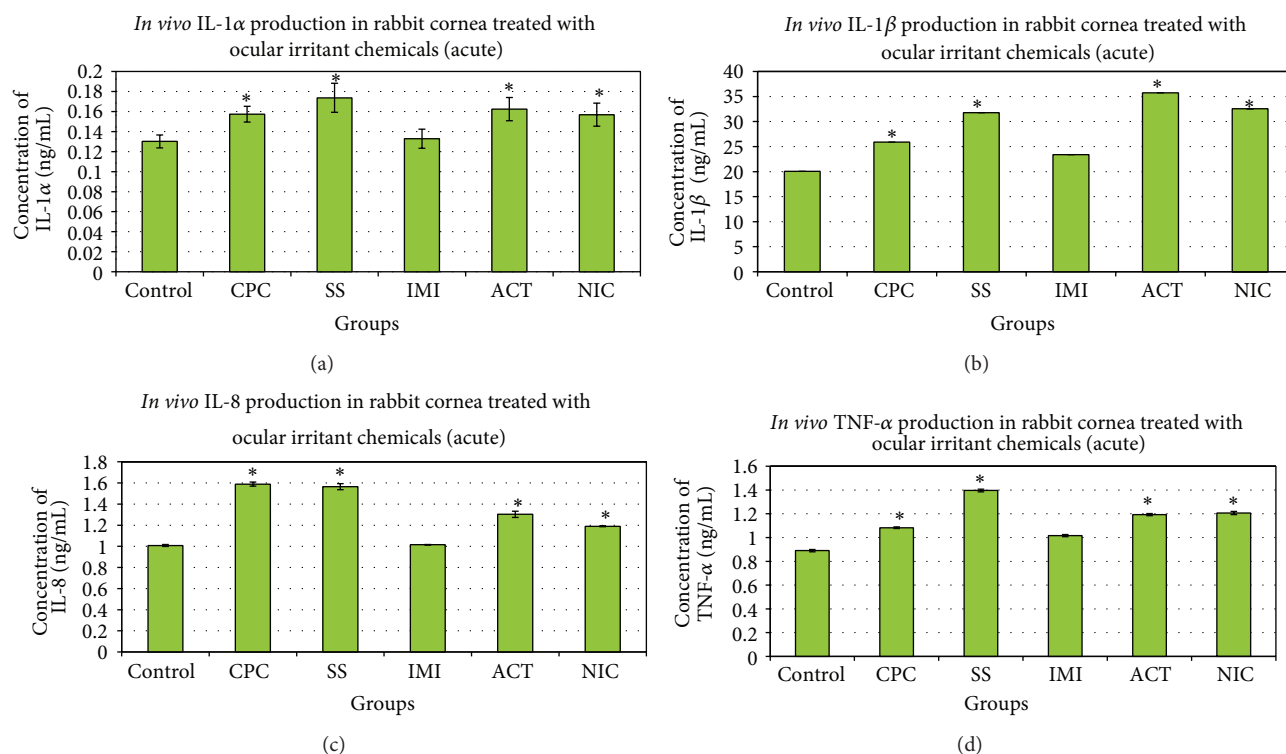


FIGURE 2: Cytokine production in cornea of rabbits treated with ocular irritant chemicals (Acute). CPC: cetyl pyridinium chloride, SS: sodium salicylate, IMI: imidazole, ACT: acetaminophen, and NIC: nicotinamide. (a) IL-1 α production, (b) IL-1 β production, (c) IL-8 production, and (d) TNF- α production. * $P \leq 0.05$.

(0.157 ± 0.007 ng/mL), SS (0.173 ± 0.014 ng/mL), ACT (0.162 ± 0.011 ng/mL), and NIC (0.156 ± 0.011 ng/mL) treated eyes when compared to control (0.13 ± 0.006 ng/mL). IMI (0.132 ± 0.009) treated rabbit cornea was almost similar to that of control. IL-1 β production also showed a similar trend as IL-1 α , where all the chemicals (CPC: 25.9 ± 0.002 ; SS: 31.775 ± 0.014 ; ACT: 35.733 ± 0.005 ; NIC: 32.566 ± 0.007 ng/mL) except IMI (23.4 ± 0.002 ng/mL) showed an increased concentration in comparison with control (20.066 ± 0.003 ng/mL). IL-1 β production is depicted in Figure 2(b). Chemotactic factor IL-8 production is illustrated in Figure 2(c). It can be seen that IL-8 increases in CPC (1.007 ± 0.009 ng/mL), SS (1.563 ± 0.028), ACT (1.302 ± 0.029 ng/mL), and NIC (1.188 ± 0.003 ng/mL) when compared to the control (1.007 ± 0.009 ng/mL) and this was statistically significant. However, IL-8 production in IMI (1.014 ± 0.004 ng/mL) treated eyes remains similar to control. From Figure 2(d), TNF- α is shown to increase significantly with respect to control in all treated animals except IMI treated animals. The values which are expressed in ng/mL are as follows: control: 0.89 ± 0.009 , CPC: 1.083 ± 0.006 , SS: 1.396 ± 0.011 , IMI: 1.016 ± 0.009 , ACT: 1.193 ± 0.009 , and NIC: 1.206 ± 0.013 .

3.4. Hematological and Biochemical Parameters. After acute exposure to the chemicals, blood was collected from the rabbits and the hematological parameters (HB, WBC, RBC, platelet, MCV, MCH, and MCHC) were analyzed. There were

slight alterations in the parameters; however, they were well within the normal range as given in Table 2.

Biochemical parameters such as GPT, GOT, ALP, GGT, uric acid, calcium, phosphorous, chlorides, and creatinine were also assessed. It was seen that there was slight variation with respect to control. However, they were well within the normal range. This is shown in Table 3.

3.5. Splenocyte Proliferation Assay. As depicted in Figure 3, the splenocyte proliferation in the treated animals showed a slight decrease when compared with that of the control. However they were insignificant statistically. The values which were expressed in CPM were as follows: control: 923.833 ± 108.875 , CPC: 797.333 ± 3.511 , SS: 823 ± 29.051 , IMI: 753 ± 96.814 , ACT: 772.333 ± 213.509 , and NIC: 791 ± 138.264 .

3.6. Antioxidant Enzyme Assay

3.6.1. Lipid Peroxidation (LPO). Livers of rabbits exposed to ocular irritant chemicals (Figure 4(a)) showed a slight increase (CPC: 16.785 ± 2.484 , SS: 15.014 ± 1.272 , IMI: 15.563 ± 2.014 , ACT: 13.465 ± 0.372 , and NIC: 13.652 ± 0.912) when compared to control (12.984 ± 0.845), though it was not statistically significant. The concentration of malondialdehyde, which is the end product of LPO, was expressed in nmol/mg protein.

TABLE 2: Hematological parameter of rabbits treated with ocular irritant chemicals (acute). CPC: cetyl pyridinium chloride, SS: sodium salicylate, IMI: imidazole, ACT: acetaminophen, and NIC: nicotinamide. All values are mean \pm SD, $n = 3$.

Parameters	Control	CPC	SS	IMI	ACT	NIC
HB (g/dL)	15.05 \pm 2.62	12.07 \pm 1.26	13.77 \pm 1.1	13.5 \pm 0.4	15.85 \pm 3.18	13.7 \pm 0.28
WBC ($\times 10^3$ /mm ³)	5.7 \pm 1.41	6.8 \pm 3.54	4.63 \pm 2.66	7.3 \pm 2.35	5.55 \pm 1.91	3.4 \pm 1.13
RBC ($\times 10^6$ /mm ³)	7.08 \pm 1.12	5.5 \pm 0.76	6.42 \pm 0.46	5.34 \pm 1.63	7.18 \pm 1.74	6.38 \pm 0.17
PLT	229 \pm 26.87	665.33 \pm 55.43	642.33 \pm 220.29	402 \pm 49.51	533 \pm 200.82	330 \pm 90.51
MCV (μ m ³)	69.5 \pm 0.71	69.37 \pm 2.78	67.13 \pm 2.92	68.2 \pm 1.9	69.45 \pm 1.34	67.25 \pm 0.92
MCH (pg)	21.7 \pm 0.28	22.03 \pm 0.74	21.43 \pm 1.1	21.63 \pm 0.85	22.25 \pm 0.92	21.4 \pm 0.14
MCHC (g/dL)	31.1 \pm 0.14	31.7 \pm 0.72	31.93 \pm 0.25	31.73 \pm 0.38	31.95 \pm 0.63	31.85 \pm 0.21

TABLE 3: Biochemical parameter of rabbits treated with ocular irritant chemicals (acute). CPC: cetyl pyridinium chloride, SS: sodium salicylate, IMI: imidazole, ACT: acetaminophen, and NIC: nicotinamide. All values are mean \pm SD, $n = 3$.

Parameters	Control	CPC	SS	IMI	ACT	NIC
SGPT (IU/L)	44 \pm 3.53	118.65 \pm 5.16	79.16 \pm 6.81	85.06 \pm 23.42	84.9 \pm 2.88	84.9 \pm 18.80
SGOT (IU/L)	21.3 \pm 1.41	19.35 \pm 6.15	22.86 \pm 7.47	25.06 \pm 4.77	20.33 \pm 4.12	168.15 \pm 19.70
ALP (IU/L)	61 \pm 2.82	40 \pm 5.65	35.66 \pm 26.38	84.33 \pm 11.06	54.33 \pm 19.50	62.5 \pm 19.09
GGT (IU/L)	10.85 \pm 0.77	7.5 \pm 0.84	5.33 \pm 4.16	3.2 \pm 2.36	12.26 \pm 5.02	1.5 \pm 0.56
Uric acid (mg/dL)	2.52 \pm 3.06	0.23 \pm 0.01	1.03 \pm 0.49	2.41 \pm 1.91	0.29 \pm 0.07	2.21 \pm 0.65
Calcium (mg/dL)	13.4 \pm 0.14	13.75 \pm 0.07	13.8 \pm 0.79	14.83 \pm 0.41	13.93 \pm 1.10	14.2 \pm 0.28
Phosphorous (mg/dL)	3.43 \pm 0.17	4.32 \pm 0.03	4.64 \pm 0.80	5.33 \pm 0.63	4.65 \pm 0.16	5.26 \pm 0.12
Chlorides (mmol/L)	104.3 \pm 9.33	115.35 \pm 1.34	110.7 \pm 3.08	116.5 \pm 1.47	109.23 \pm 2.51	110.45 \pm 1.62
Creatinine (mg/dL)	1.22 \pm 0.24	1.05 \pm 0.01	1.28 \pm 0.42	1.09 \pm 0.05	1.61 \pm 0.82	0.92 \pm 0.04

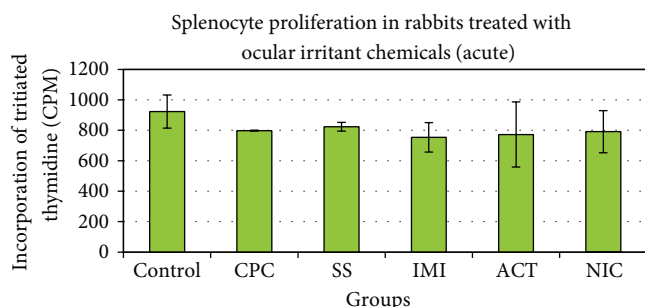


FIGURE 3: Splenocyte proliferation in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide.

LPO in brain of animals exposed to CPC (0.809 ± 1.613 nmol/mg protein), SS (14.007 ± 0.067 nmol/mg protein), IMI (11.277 ± 1 nmol/mg protein), ACT (11.156 ± 1.829 nmol/mg protein), and NIC (10.633 ± 1.39 nmol/mg protein) showed slight variations with respect to control (8.898 ± 0.45 nmol/mg protein). Nonetheless it was statistically insignificant. This is illustrated in Figure 4(b).

3.6.2. Reduced Glutathione (GSH). Concentration of GSH in the liver of rabbits exposed to ocular irritant chemicals (CPC: 1.277 ± 0.325 , SS: 1.280 ± 0.164 , IMI: 1.548 ± 0.055 , ACT: 1.037 ± 0.105 , and NIC: 1.035 ± 0.209 nmol/mg protein) showed a

slight decreasing trend but was insignificant in comparison with control (1.615 ± 0.153 nmol/mg protein). This is seen in Figure 5(a).

GSH in the brain showed that CPC (0.524 ± 0.071 nmol/mg protein), SS (0.471 ± 0.133 nmol/mg protein), IMI (0.572 ± 0.115 nmol/mg protein), ACT (0.58 ± 0.189 nmol/mg protein), and NIC (0.691 ± 0.225 nmol/mg protein) were slightly decreased when compared to control (0.653 ± 0.008 nmol/mg protein). However, it remained insignificant and is shown in Figure 5(b).

3.6.3. Glutathione Reductase (GR). Activity of GR in the liver of rabbits which were exposed to ocular irritant chemicals is shown in Figure 6(a). It can be seen that there was no statistically significant change in treated group (CPC: 0.404 ± 0.07 , SS: 0.372 ± 0.037 , IMI: 0.334 ± 0.151 , ACT: 0.27 ± 0.004 , and NIC: 0.325 ± 0.089 units/mg protein) with respect to control (0.268 ± 0.109 units/mg protein).

Brain of liver treated with ocular irritant chemicals (CPC: 0.437 ± 0.052 , SS: 0.425 ± 0.076 , IMI: 0.419 ± 0.009 , ACT: 0.387 ± 0.055 , and NIC: 0.337 ± 0.005 units/mg protein) showed that the GR activity had slight variations when compared with control (0.325 ± 0.029 units/mg protein). However, it was found to be statistically insignificant (Figure 6(b)).

3.6.4. Glutathione Peroxidase (GPx). Liver of rabbits treated with ocular irritant chemicals CPC (0.152 ± 0.031 units/mg

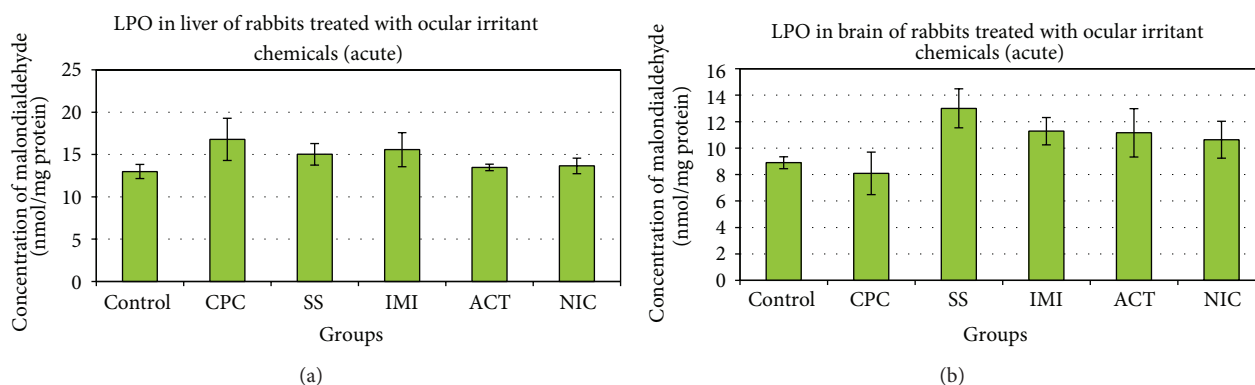


FIGURE 4: Lipid peroxidation (LPO) in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide. (a) LPO in liver and (b) LPO in brain.

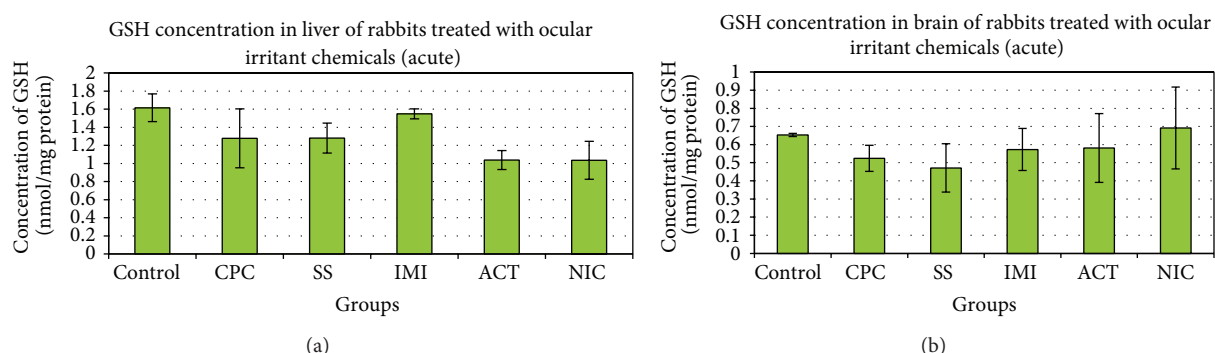


FIGURE 5: Concentration of reduced glutathione (GSH) in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide. (a) GSH in liver and (b) GSH in brain.

protein), SS (0.167 ± 0.042 units/mg protein), IMI (0.152 ± 0.005 units/mg protein), ACT (0.119 ± 0.014 units/mg protein), and NIC (0.124 ± 0.055 units/mg protein) shows slight alterations when compared with control (0.118 ± 0.018 units/mg protein) yet it is not significant. This can be seen in Figure 7(a).

In the brain of rabbits treated with ocular irritant chemicals (CPC: 0.04 ± 0.004 , SS: 0.043 ± 0.007 , IMI: 0.046 ± 0.016 , ACT: 0.056 ± 0.002 , and NIC: 0.124 ± 0.055 units/mg protein) the GPx activity was almost similar to that of the control (0.039 ± 0.012 units/mg protein). This is shown in Figure 7(b).

3.6.5. Superoxide Dismutase (SOD). Activity of SOD in liver of animals treated with CPC (0.134 ± 0.02 units/mg protein), SS (0.135 ± 0.026 units/mg protein), IMI (0.11 ± 0.021 units/mg protein), ACT (0.092 ± 0.004 units/mg protein), and NIC (0.106 ± 0.014 units/mg protein) showed slight changes when compared to control (0.107 ± 0.008 units/mg protein). But they were not significant. This is depicted in Figure 8(a).

SOD in the brain of rabbits treated with ocular irritant (CPC: 0.166 ± 0.001 , SS: 0.161 ± 0.006 , IMI: 0.134 ± 0.001 , ACT: 0.149 ± 0.002 , and NIC: 0.129 ± 0.019 units/mg protein) show similar results as in liver (Figure 8(b)). Though there were slight alterations when compared with control (0.127 ± 0.019 units/mg protein), it was insignificant statistically.

4. Discussion

The eye consists of a variety of barriers, like the conjunctiva and tear, which are meant to keep the systemic circulation separate from the ocular tissue. However, conjunctiva has wide intercellular spaces and is supplied with rich network of blood and lymphatic vessels, which carries any large molecules away from the eye to the systemic circulation [1]. Apart from that, rapid absorption of drugs is reported via the nasolacrimal duct through the highly vascularized nasal mucosa into the systemic circulation. Both these passages bypass the first pass metabolism. About 2–10% of the drug is retained in the eye and exerts its local effect and the rest can enter the systemic circulation by the above-mentioned routes [2]. The cornea consists of tight junctions that make it relatively impenetrable to macromolecules via the paracellular pathway. However, if the corneal epithelium integrity is breached as a result of trauma or inflammation, it can compromise the barrier [10]. This in turn can cause the entry of the substance to systemic circulation and have adverse effects. Hence unforeseen systemic reactions may be observed on ocular exposure of a substance and this has to be addressed.

In this study, five known ocular irritants, cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide (NIC), were

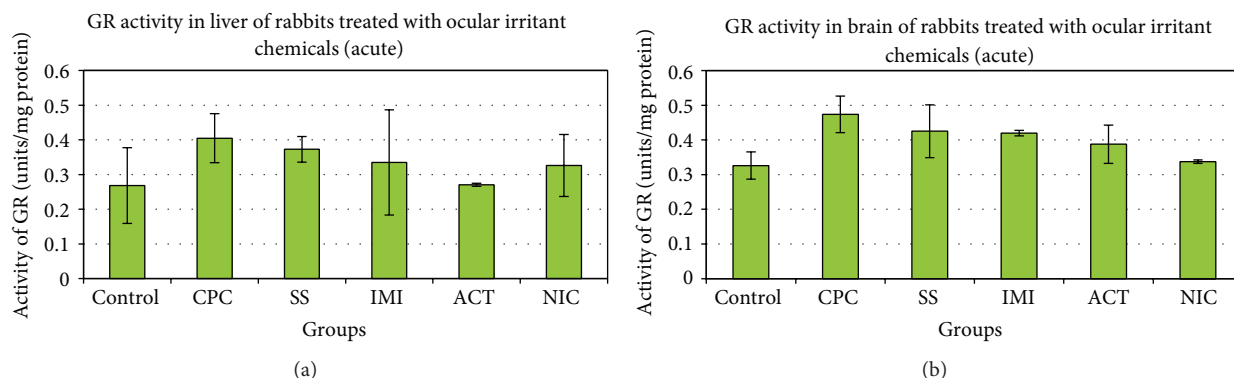


FIGURE 6: Activity of glutathione reductase (GR) in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide. (a) GR in liver and (b) GR in brain.

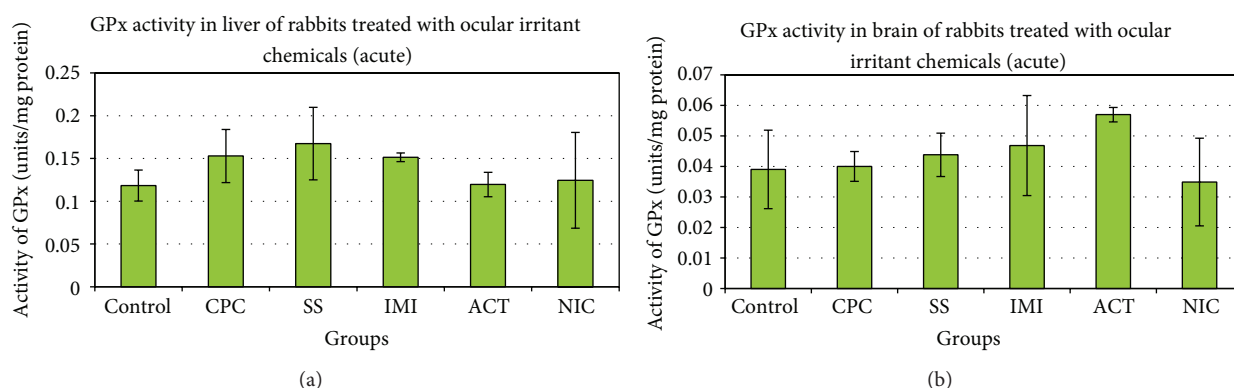


FIGURE 7: Activity of glutathione peroxidase (GPx) in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide. (a) GPx in liver and (b) GPx in brain.

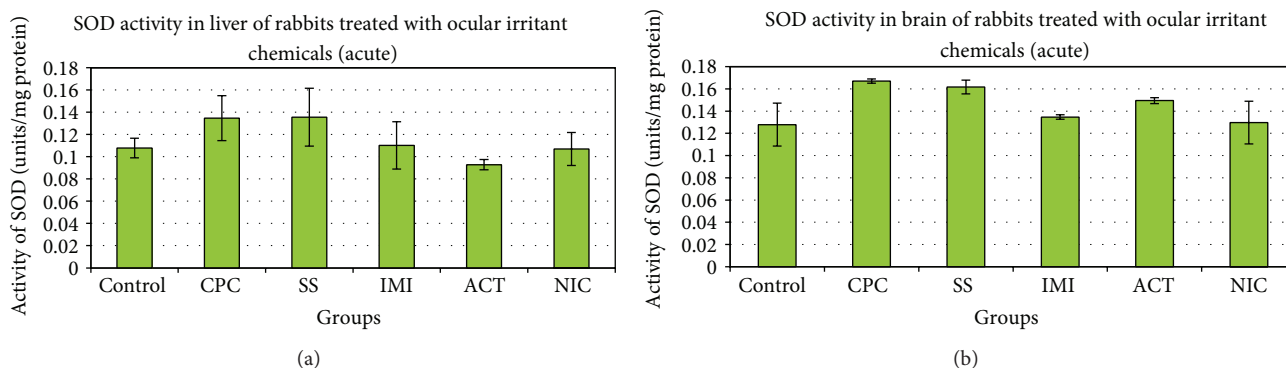


FIGURE 8: Activity of superoxide dismutase (SOD) in rabbits treated with ocular irritant chemicals (acute). Cetyl pyridinium chloride (CPC), sodium salicylate (SS), imidazole (IMI), acetaminophen (ACT), and nicotinamide. (a) SOD in liver and (b) SOD in brain.

chosen [11–14]. These chemicals were chosen so as to slightly breach the corneal surface and make it more permeable. These chemicals were exposed to the eyes of rabbits as per the Draize test [15]. The chemicals solutions were added onto the conjunctival sac and the eye lids were held together for a few seconds to ensure contact with the ocular tissue. From the Draize scoring chart, it was observed that the chemicals showed moderate to severe irritation. Hence they were able to cause trauma to the ocular tissue. To assess whether the

trauma resulted in inflammation, histopathological analysis of the eye and expression of proinflammatory cytokines were carried out. Inflammation has an enhancing effect on penetration into the systemic circulation [16]. The hallmarks of acute inflammation are rubor (redness), tumor (swelling or edema), calor (heat), dolor (pain), and “functio laesa” (loss of function). These responses increase the blood flow, cause migration of neutrophils and macrophages, increase the local temperature, and cause pain in the affected area

[17]. In the current study, it was evident that there was mild inflammation and congestion of blood vessels in the ciliary body but not in the cornea in all treated groups (CPC, SS, ACT, and NIC) except IMI. This might be because of the anti-inflammatory properties of IMI and its derivatives [14]. In a study by Weng et al. [18], it was observed that corneal graft rejection took place five days after surgery. This was the time required for the immune cells to reach the cornea. As the exposure and experimental period in rabbits lasted 72 h, it was not enough time to cause pronounced inflammation in the cornea. Moreover, the ciliary body is a highly vascularized structure and evidence of inflammation will be more in that region during the initial period of ocular irritant chemical exposure. However, proinflammatory markers like IL-1 α , IL-1 β , and TNF- α and chemokines like IL-8 will be released following injury [17]. To assess the cytokine release profile, ELISA assay was carried out. It was found that the cytokine levels were significantly high in all treated animals except for IMI. As mentioned earlier this observation can be attributed to the anti-inflammatory properties of IMI. The production of cytokines by CPC, SS, ACT, and NIC shows that these chemicals were able to injure the ocular tissue and cause inflammation.

Since inflammation was observed in the ocular irritant treated animals, there is an increased chance that these chemicals can enter the systemic circulation and onto the various vital organs. The results from the blood hematological parameters suggested that the animals were not anemic and there was no irregular immune reaction or platelet activation. The biochemical parameters also suggest that the animals had a normal liver and kidney function. Further, the ability of the chemicals to evoke any immune response was assessed by looking at the splenocyte proliferation. The results suggest that the chemicals did not have any immunomodulatory effect on the spleen. Oxidative stress in the vital organs like liver and brain was also assessed. Liver is the site of detoxification and it is prone to insult and injury by foreign substances that enter the body. However, it is well equipped with myriad means to combat the offenses. On the contrary, the brain is a delicate organ that is rich in lipids and relatively low in antioxidant defense mechanisms [19]. However it is well protected by the blood brain barrier. But under certain circumstances, both liver and brain can become susceptible to oxidative damage by the foreign substances. Oxidative stress occurs when there is an imbalance between the prooxidants and antioxidants in favor of the prooxidants [20]. Lipid peroxidation is a result of oxidative damage to lipids [21]. In this study, it was observed that the ocular irritant chemicals did not induce a significant lipid peroxidation suggesting that there was no oxidative stress. The glutathione redox cycle was monitored by looking at concentration of GSH and activity of GPx and GR. In the presence of reactive oxygen species (ROS) such as hydrogen peroxide in the biological system, GSH acts as an electron donor and reduces them with the help of the enzyme GPx. GSH is converted to its oxidized form GSSG during this process. GR helps to replenish GSH from GSSG [22]. So when there is an overproduction of free radicals, ideally there will be a variation in the levels and activities of these antioxidants. In the present study, there

was no significant change in the concentration of GSH and the activities of GPx and GR, suggesting that these chemicals were not able to generate prooxidants. In the presence of superoxide free radicals, SOD is stimulated and catalyzes the dismutation reaction forming hydrogen peroxide and oxygen. Since the activity of SOD in the present study was almost similar to control in the liver and brain, hence it is suffice to say that there was no superoxide radical production [23]. These suggest that the ocular irritant chemicals did not cause ROS generation and oxidative stress in the liver and brain. This can be because of the limited time (acute) exposure of the chemicals to the eye or insufficient amount of chemicals that reached these vital organs.

5. Conclusion

In this present study, the ocular irritant chemicals (CPC, SS, IMI, ACT, and NIC) induced inflammatory response in the eye. However, the systemic response of these chemicals was not conspicuous. Hence it can be concluded that the ocular irritant chemicals did not cause any systemic side effects in the present scenario, when exposed via the ocular route.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

The authors would like to convey their sincere thanks to ICMR (Project no. 5/20/4(Bio)/10-NCD-1), for sanctioning the grants for carrying out this work. The authors are also grateful to the Director and Head, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, for providing the infrastructure to successfully carry out this work. The technical support of Mr. Shaji S. and Mr. Harikumar G. is kindly acknowledged.

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