Protective effects of apigenin against methyl methanesulfonate induced hsp70 expression in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ)Bg

Sir,

Hsp70 are a family of predominantly expressed heat shock proteins of ubiquitously expressed heat shock proteins. In recent years, hsp70 has been considered to be one of the candidate genes for predicting cytotoxicity against environmental chemicals. Methylmethanesulfonate (MMS) is not only classified as a mutagen, but also as a carcinogenic agent. Exposure to MMS appears to be limited to laboratory research persons.

Apigenin is one of the several active ingredients found naturally in many fruits and vegetables. It is recognized in traditional or alternative medicine for its pharmacological activity. Now-a-days the use of animals in toxicological/pharmacological research and testing has become an important issue for both science and ethics. As a result, the emphasis has been given to the use of alternative to mammals in testing, research, and education. The European Centre for the Validation of Alternative Methods (EVCAM) has recommended the use of Drosophila as an alternative model for scientific studies. In our present study, an attempt has been made to validate this model for the evaluation of the chemotherapeutic/natural plant products for their protective action.

A transgenic Drosophila melanogaster line expressing bacterial β-galactosidase as a response to stress was used in this study. The flies and larvae were cultured on standard Drosophila food containing agar, cornmeal, sugar, and yeast at 24°C. MMS at 0.5 and 1.0 µl/ml of food concentration alone and along with 0.1, 0.5, and 1.0 µl/ml of apigenin were established. The third instar larvae were allowed to feed on them for different time intervals (12, 24, and 48 h). For quantifying the β-galactosidase activity, the method as described by Nazir et al. was followed. The extent of tissue damage in larvae exposed to different concentrations of MMS alone and along with apigenin was assayed by a dye exclusion test. Statistical analysis was carried out by Student’s ‘t’-test. P<0.05 was considered statistically significant.

The results of this study reveal that the exposure of 0.5 and 1.0 µl/ml of MMS to the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg for the duration of 12, 24, and 48 h showed an increase in the activity of

| Treatments                  | OD (Mean ± SE) after 12 h | OD (Mean ± SE) after 24 h | OD (Mean ± SE) after 48 h |
|-----------------------------|---------------------------|---------------------------|---------------------------|
| MMS (µl/ml) + Apigenin (µl/ml) |                           |                           |                           |
| 0.5 + 0.1 µl/ml             | 0.265 ± 0.012a            | 0.282 ± 0.014a            | 0.294 ± 0.015a            |
| 1.0 + 0.1 µl/ml             | 0.313 ± 0.023a            | 0.324 ± 0.028a            | 0.332 ± 0.033a            |
| MMS (µl/ml)                 |                           |                           |                           |
| 0.5 µl/ml                   | 0.243 ± 0.011ab           | 0.262 ± 0.012ab           | 0.275 ± 0.13ab           |
| 1.0 µl/ml                   | 0.238 ± 0.009ab           | 0.251 ± 0.011ab           | 0.263 ± 0.011ab           |
| 0.5 + 1.0 µl/ml             | 0.232 ± 0.009ab           | 0.248 ± 0.012ab           | 0.241 ± 0.012ab           |
| 1 + 0.1 µl/ml               | 0.189 ± 0.014ab           | 0.301 ± 0.015ab           | 0.314 ± 0.024ab           |
| 1 + 0.5 µl/ml               | 0.272 ± 0.013ab           | 0.293 ± 0.014ab           | 0.304 ± 0.022ab           |
| 1 + 1 µl/ml                 | 0.261 ± 0.010ab           | 0.283 ± 0.013ab           | 0.292 ± 0.19ab           |
| Apigenin (µl/ml)            |                           |                           |                           |
| 0.1 µl/ml                   | 0.223 ± 0.008             | 0.223 ± 0.009             | 0.234 ± 0.010             |
| 0.5 µl/ml                   | 0.220 ± 0.007             | 0.214 ± 0.005             | 0.221 ± 0.008             |
| 1.0 µl/ml                   | 0.221 ± 0.007             | 0.228 ± 0.008             | 0.230 ± 0.011             |
| Untreated                   | 0.212 ± 0.006             | 0.220 ± 0.009             | 0.218 ± 0.007             |
| DMSO (5 µl/ml)              | 0.223 ± 0.008             | 0.222 ± 0.009             | 0.221 ± 0.009             |

*aSignificant at P <0.05 compared to untreated. bSignificant at P <0.05 compared to MMS treatment
hsp70 expression [Table 1]. The exposure of third instar larvae of MMS along with the 0.1, 0.5, and 1.0 μl/ml of apigenin for 12 h results in the reduction of the activity of hsp70 expression [Table 1]. Similar results were obtained for 24 and 48 h of exposure [Table 1]. The exposure of third instar larvae to 1.0 μl/ml of apigenin for 12 h results in the reduction of the expression of hsp70 activity [Table 2]. Similar results were obtained for the 24 and 48 h of exposure to 1.0 μl of MMS along with the 0.1, 0.5, and 0.1 μl/ml of apigenin [Table 1]. The regression analysis was also performed to study the dose and duration effects for the expression of third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg\(^6\) to MMS and apigenin in combination [Table 2]. The exposure of third instar larvae for 12 h to 0.5 μl/ml of MMS along with 0.1, 0.5 and 1.0 μl/ml of apigenin was associated with the β-coefficient of −1.0 (F = 3346.680). The reduction in the β-coefficient values was also observed for other durations of exposure and combinations [Table 2]. The reduction in the value of β-coefficient demonstrates the reduction in the β-galactosidase activity. Trypan blue staining was performed to study the tissue damage induced by MMS in the larval tissue exposed to different doses of MMS alone and in combination with apigenin. About 92% of the larvae of untreated were negative to trypan blue staining even after 48 h of the treatment. About 85% of the larvae showed staining in midgut of the larvae exposed to 0.5 and 1.0 μl/ml of MMS to 12 h. For higher duration of exposure, i.e. 24 and 48 h, the damage was observed in the brain ganglia, midgut, salivary glands, malpighian tubules, and the hind gut. The damage was reduced when the apigenin was mixed in the diet. About 43% of larvae show light staining in the midgut exposed to 12 h of duration to 1.0 μl/ml of MMS along with the 1.0 μl/ml of apigenin. The damage was further reduced 24 and 48 h of exposure. The damage was observed only in the salivary gland, and no damage was observed in midgut, malpighian tubule, and hind gut. The results of this study reveals that the apigenin is potent enough in reducing the toxic effects of MMS in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg\(^6\). Drosophila may be used as a system for ADR detection and management. The same system may also be used for faster drug development which could be cost efficient. Although the mammalian system may represent more accurate evaluation tools of short terms and safety, they are frequently laborious and costly at early stages of drug discovery and development.[7]

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Sir,

Drug utilization research is defined by World Health Organization (WHO) as the marketing, distribution, prescription and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences. To increase the therapeutic efficacy and minimise the development of resistance drug utilization pattern needs to be evaluated periodically. Inappropriate use of drugs and dosage forms result in potential health hazard to the patients and cause financial burden. To avoid such problems every member of the healthcare system should practise rationally. The five important criteria for rational drug use are accurate diagnosis, proper prescribing, correct dispensing, suitable packing and patient adherence.

Studies have been conducted with the aim of improving prescription writing pattern.

In the recent past, the development of new antibiotics has declined and the limited available antibiotics cannot compete with the rapid increase of antibiotic resistance. Therefore, it is the need of the hour that we utilise the available antibiotics with much care. The area where a medical professional can play a role is to improve in prescription writing. Hence, this study was designed with the aim to investigate the antibiotics utilization pattern in a tertiary care hospital.

This study was conducted in the outpatient department of Ophthalmology of a tertiary care hospital. Data were collected retrospectively from the medical records of the outpatient who had visited the OPD from 8.30 am to 3.00 pm. The study period was from 12/01/2011 to 19/04/2011. Medical records of 200 patients were audited using a proforma to record the required information. All antibiotics prescribed were recorded including its dosage form, frequency of administration, duration and indications. Also patients details such as age and sex were also taken into account.

These forms were then used to analyze the average utilization of antibiotics in different formulations, grouping of patients based on age, sex and whether the drugs were prescribed in generic or proprietary names.

During the study period out of 200 OPD patients 28.5% and 31.00% were male and female respectively. Among geriatric patients 14.00% were male and 18.00% were female. In pediatric patients 4% and 4.5% were boys and girls respectively.

The total number of drugs prescribed were 234 in 200 prescriptions. The most commonly prescribed formulation was found to be eye drops (24). Fluoroquinolone (ofloxacin) was the commonly prescribed antibiotic which coincides with findings of earlier studies.

Of the 200 prescriptions the antibiotics with proper dosage form, frequency and duration mentioned were 92%, 90.5% and 74.5% respectively. Duration was not mentioned in 25.5%, frequency in 9.5% and dosage form in 8%. Drugs were prescribed by generic names in 11.96% and brand names in 88.03%. Similar findings were reported by Yasmeen et al.

In this study, the antibiotics were commonly prescribed for postoperative complaints followed by viral conjunctivitis, swelling of the eye, foreign body, injuries and iritis. Prescribing of antibiotics was rightly indicated according to the diagnosis except for viral conjunctivitis whose treatment is nonspecific, but here we presume that antibiotics were prescribed to prevent secondary infection. One prescription with a diagnosis of orbital cellulitis was irrationally prescribed with ciprofloxacin which is not an indicated antibiotic for the same diagnosis.

The common prescription writing errors were minimum and there was no evidence of polypharmacy. Errors of omission and commission if correctly dealt with in prescription writing the outcome of therapy can be improved and also reduce the development of antibiotic resistance. Prescriptions of generic drugs could facilitate cheaper treatment for patients. Periodical auditing of the prescriptions will help to measure the impact of intervention.

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