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Preliminary Biological study of Polystyrene (PS) Beads and Derived Styrene Maleic Anhydride

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Abstract:
This preliminary study preclude the biomedical uses of maleic anhydride and polystyrene polymers. Styrene maleic anhydride copolymers and polystyrene has the various applications in biomedical and other fields. The anhydride structure of styrene maleic anhydride shows the various drugs polymer conjugates. The polymer SMA and PS shows bio-medical property as it effects on the biological impact. The aim to study is biological impact of polystyrene beads and derived styrene maleic anhydride.

Key words: Polystyrene Beads, Derived SMA, Biomedical, Polymer

Introduction

Polystyrene and SMA have been widely used for packaging and in biomedical science [1]. In biological system polystyrene latex beads are utilized in the study of clotting factors, enzyme array and solid phase radio- immunoassay [2]. The toxicity of its monomer (styrene) is well known [3,4]. Workers employed in polystyrene industries generally shows carcinoma [5]. Studies utilizing mammals such as rats and mice suggests that styrene has embrotoxicity and teratogenic effects [6,7,8]. Hence the earlier work on exposure on human being in industries to polystyrene has led to the belief that polystyrene or its derivatives cannot be safely used for in vivo biological studies. In order to develop biomaterials for eventual use in humans, It is necessary to asses toxic effects of styrene polymer in experimental animals. So far the for toxicity studies on the polymer it is powered and covered in to the form of films and discs [9]. But polystyrene is used in the solid form various applications in human systems. Therefore it is important to study the toxic effects of the polymer when used in the solid bead form.

Materials:

Styrene (Aldrich) was used its small amount was purified for further use. Maleic anhydride was supplied by(s-d fine chemicals) and was also purified before use. Benzoyle peroxide (BPO) of analytical grade (s.d-fine chemicals) was purified by $CHCl_3$ and alcohol mixture.

Pure DMSO (Dimethyl sulfoxide) (s-d fine chemicals) was used for the synthesis. The solvent acetone was of analytical grade (s-d fine chemicals) and used as such.

Experimental Protocol:

Infra red spectra were recorded on Perkin-Elmer Spectrum RX1 in the range 4000-450 cm^{-1}, Pellets were prepared with KBr disc. ^1H NMR spectra were recorded on Buker Avance 400 MHz FTNMR in $(CD_3)_2SO$. Elemental analysis was done using Carlo Erba 1108. Purity of polymer was by tested the method of Hiller[10] and by infra red spectroscopy Silverstein [11].

Method: 2.0g (0.01mole) styrene maleic anhydride was mixed with 1ml dimethtyl sulfoxide and kept with P_2O_5 in a desiccator for 15 days. SMA-DMSO complex (derived SMA)was obtained in the form of a colourless gel. **Analysis:** $C_{14}H_{16}O_4S$:Cal. (%): C: 59.98, H: 5.75, S: 11.43,Found (%):C: 59.62,H: 5.55, S: 11.30, **Spectral data- IR (KBr cm^{-1}) :** 3002 (C-H *str*.)aromatic,1742 (C=O *str*.) ester, 1650 (C=C *str*.) aromatic , 1461 (C-H bend) methylene, 1259 (O-C *str*.), 3343 (O-H *str*.) H bond, 762 mono substituted.**^{1}HNMR $(CD_3)_2SO$(δ ppm):**1.7 (s,4H), 3.5 (s,2H), 2.4 (t,2H), 9.8 (s,1H) O-H, H-bond, 1.4 (d,2H), 7.1 (s,5H, Ar).

Biological study: Healthy male and female albino rats of wt. 180 ± 10 gm. were selected for study. They were kept under ideal biological conditions. Photo period of 13 hrs light and 11 hrs dark were maintained through the entire duration of studies. Food and water were allowed throughout the study. Two sample of polystyrene and derived styrene maleic anhydride wt. 5.0 mg. Each was insert on the left and right side of the abdominal region in two groups of the male rats. The rats were monitored for their behaviour able changes, growth and inflammatory response at the site of implantation.

Urine sample were collected at interval of 12, 24, 48, 96 hrs. After implantation of the polystyrene and derived SMA metabolites such as styrene oxide, styrene glycol, mandelic acid, uric acid was produced for the study. The rats were distributed in two groups

A(PS) and B(SMA). The rats in group A and B were kept with females for mating. The implanted polystyrene beads rats were removed after two weeks, while the gel of derived styrene maleic anhydride was dissolved, First the left side and then the right side. The weight of beads was recorded after removal from the body. Two week after removing the beads, the rates were satisfied. Sample of tissue histology were collected from testis, kidney and liver. The tissues were fixed in Bouvin's fixative and that was stained with haematotoxyline and eosin. For Urine analysis, each sample was subjected to Soxhelect extraction, first with ether-ethanol (9:1) V/V) and subsequently with ethyl acetate. The Extract were evaporated to dry and chromatograph on TLC Plated impregnated with silica gel-G. Different solvent systems, such as petroleum ether, benzene, butanol, acetic acid, ammonia and water were used to extract the metabolites. Various combinations were used and it was found that the resolution was better if the following mixture of solvents were used as n-butanol: acetic acid: water (3:1:2), benzene: acetic acid: water (1:1:2).For calibration controls or standard of various metabolites were run simultaneously. Iodine vapours and concentrated sulphuric acid were used to develop colour on TLC plates. In addition to TLC the urine samples were examined by infra red spectra with Perkin-Elmer Spectrum RX1 in the range 4000-450 cm^{-1}, Pellets were prepared with KBr disc.

Result and discussion

Implantation of polystyrene beads and derived styrene maleic anhydride did not cause any change in behaviour and weight of rats when compared to the normal control rats. There was no growth of abnormal tissues at the site of beads implantation. The weights of polystyrene beads were found unchanged after removal from the body. Tissue reaction to implants of polystyrene beads was not significant as seen in histological sections from the tissue removed from the implant site and near vicinity up to 5mm. The morphological shape of cells was in normal limits. The histological profile of kidney, liver, and testis showed no significant changes in structure or infiltration of cells by the polymer. Results of urine analysis by TLC indicated that neither the polystyrene components nor its depolymerised products were ever excreted by the kidney. These findings were supported by infrared spectra of urine. The spectra of urine was obtained from controlled treated rats were super imposable. The major absorption peaks were due to C-H stretching vibrations at 2935 and 2860 cm^{-1}, which could be assigned to -COOH group. The hydroxyl absorption peaks were observed at 3460-3100 cm^{-1}. The spectra also revealed absorption band at 1740 and 1640, 1612 cm^{-1} which could be attributed to amide bands at I and II. The vibrational bands at 1140 and 1030 cm^{-1} were assigned to C-N stretching and bending. All the above absorption bands at various frequencies were due to organic compounds such as urea, uric acid commonly found in the urine. Details about behaviour and observations are as present in following tabulation (Table:1).

Table:1

Group No.	Nature of Study	Observation	Results
I.	Long term toxicity in Rats	Histopathology of kidney, Liver, testis Behaviour changes	No Significant changes in structure or infiltration cell No abnormalities in Have been noticed.
II	Local toxic effects of derived SM Metabolites In Rats	Implant SMA for 2 weeks Implanted derived SMA was dissolved After 2 weeks	No inflammation of Cells was observed. No significant change in Weight was observed No morptological abnormalities were recorded.
III	Polystyrene (PS) Metabolites	Implant PS for 2 weeks Implanted PS was removed After 2 weeks Urine analysis on samples collected 12 24, 48 and 96 hrs aft implantation	No inflammation of Cells was observed. No significant change in Weight was observed. No morptological abnormaliti were recorded.The analysi of urine metabolities like styrene Glycol, mandelic acid and hippuric acid. No metabolities were detected

Conculsion

In the above studies it has been observed that the weight of polystyrene beds remains unchanged and derived styrene maleic anhydride was dissolve and also the urine analysis by chromatography and IR spectra suggest the absence of polystyrene and derived SMA metabolites. Histological profile in tissues of treated rats shows no pathological and morphological abnormalities. Thus polystyrene and derived SMA is neither excreted in its original form and not in it's degraded form in the body . Polystyrene beads and derived SMA do not cause any adverse toxic effects in rat.

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