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Edible oil adulterants, argemone oil and butter yellow, as aetiological factors for gall bladder cancer

Vivek Mishra [a], *Manjari Mishra* [a], *Kausar M. Ansari* [a], *Bhushan P. Chaudhari* [a], *Raj Khanna* [b], *Mukul Das* [a,*]

[a] *Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Post Box #80, Lucknow 226 001, India*
[b] *Department of Biochemistry, University of Lucknow, Lucknow, India*

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ABSTRACT

Carcinogenic potential of argemone oil (AO) and butter yellow (BY), the adulterants encountered in edible oil, in gall bladder of Swiss albino mice was undertaken to investigate the potential aetiological factors of gall bladder carcinoma (GBC) in the Indo-Gangetic basin. Twice weekly intraperitoneal (ip) administration of AO (5 ml/kg body wt) and BY (25 mg/kg body wt) to Swiss albino male and female mice for 30 and 60 days indicated that females were more vulnerable to these adulterants in terms of responses to inflammatory markers. Subsequent experiments with dietary exposure of AO (1%) and BY (0.06%) for 6 months in female mice showed symptoms related to cachexia, jaundice and anaemia. High levels of total cholesterol, low density lipoprotein (LDL), TG, bilirubin and low level of high density lipoprotein (HDL) as well as gallstone formation was shown by AO exposure only, leading to the development of adenocarcinoma. BY exposure resulted in adenoma and hyperplasia without stone formation. The cyclooxygenase (COX-2) overexpression was found to be related to prostaglandin E2 (PGE2) production in AO treated mice but not in BY exposed animals, thereby indicating a differential pathway specific carcinogenicity. PGE2 stimulates the secretion of secreted mucins (MUC5AC), which is involved in stone formation following AO exposure. Enhanced secretion of membrane bound mucins (MUC4) in BY and AO exposed mice resulted in the activation of ErbB2 and downstream signalling such as p-AKT, p-ERK and p-JNK, which ultimately affects the target proteins, p53 and p21 leading to adenoma and adenocarcinoma, respectively. The study suggests that AO and BY are responsible for producing GBC in mice along with stone formation in the AO exposed animals.

1. Introduction

Carcinoma of gall bladder is the sixth most common cancer affecting the gastrointestinal tract.[1] The majority of gall bladder cancer (GBC) cases is diagnosed in the advanced stages, leading to extremely poor prognosis. The prognosis is mainly dependent on histological subtype, grade and stage of the tumour at the time of presentation. The overall mean survival rate for patients with GBC is 6 months, with a 5-year survival rate of 5%.[1] GBC has a female predilection, especially women >65 years of age.[2] The association of GBC is with gallstones formation contributing to increased risk.[3] GBC is a common gastrointestinal malignancy in the Asia–Pacific region.[4] The incidence in the northern parts of India, mainly along the Gangetic plains, is 4.5 cases per 100,000 population in men and 10.1 cases per 100,000 in women.[5] This is

* *Corresponding author:* Tel.: +91 522 2613786; fax: +91 522 2628227.
E-mail address: mditrc@rediffmail.com (M. Das).
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comparable to the data from Chile, which has the highest GBC incidence in the world (7.5 cases per 100,000 people).[6] Several possible aetiological factors have been implicated for GBC, which includes chronic infection of the biliary tract (cholelithiasis), typhoid carrier state, dietary factors, genetic predisposition, chemical carcinogens, cigarette smoking, high parity, post-menopausal state and obesity.[2,3]

Amongst the dietary factors in Indian subcontinent, type of edible oil consumption differs drastically including peanut oil in west and coconut oil in south. Mustard oil from *Brassica nigra* seeds is the preferred edible oil in the northern and eastern parts of India especially, the Gangetic basin.[7] Mustard oil is also used conventionally for skin and hair massage in this region. Data suggest that this commodity is often adulterated with argemone seed oil and consumption of such oil even for a short duration leads to a clinical condition collectively referred to as Epidemic Dropsy.[8] The toxic effects of argemone oil (AO) have been attributed to the presence of benzophenanthridine alkaloids, sanguinarine and dihydrosanguinarine.[9] AO causes dilatation of the smaller arterioles and capillaries, leading to the leakage of serum albumin with a concomitant increase in globulin resulting in increased capillary permeability.[10] Experimental and clinical studies suggest that skin, liver, lungs, kidneys and heart are the target sites for argemone oil intoxication.[11]

Light pale cheaper oils, dyed with synthetic colours like butter yellow (BY; 3′-methyl-4-dimethyl-aminoazobenzene) along with the addition of mustard pungency factor, allyl isothiocyanate, are sometimes sold under the pretext of mustard oil. An earlier study on the analysis of edible oils from the state of Uttar Pradesh (India) showed that almost 3.5% samples were adulterated with a non-permitted fat-soluble azo dye, BY.[12] However, in a set of over 100 unpacked mustard oil samples collected during July 2002 from different markets of Lucknow, (India) showed that more than 50% samples were artificially coloured with BY.[12] BY interacts with macromolecules like DNA thereby causing genotoxic and mutagenic responses.[13] It has been found to produce hepatic and skin tumours as well as cancer in respiratory tract.[13,14] Due to its highly reactive and toxic properties, this colour has been banned for food usage.

It has been observed that the incidence of hepatobiliary/gall bladder cancers is statistically more in the states of the Gangetic basin than in other states of India.[15] Since mustard oil is one of the prime differences and preferred choice in the dietary habits of the population of the Gangetic basin, it was therefore argued that consumption of adulterated oil could be one of the aetiological factors for higher incidences of GBC. In the present investigation, AO and BY induced gall bladder carcinogenicities were evaluated in Swiss albino mice, with an objective to underline the molecular events involved therein.

2. Materials and methods

2.1. Chemicals

Primary antibodies (p-ErbB2, ErbB2, p-AKT, p-ERK, p-JNK, COX-2, Actin) and secondary antibody were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, United States of America. *Argemone mexicana* seeds were procured from the outskirts of Lucknow city, Uttar Pradesh, India. The seeds were crushed and the oil was extracted with the help of Soxhlet apparatus using n-hexane.[16] The hexane containing AO was filtered under vacuum through a Buchner funnel containing glass wool and the solvent was distilled at 30 °C under vaccum using Buchii Rotavapor-R. The oil obtained was stored in amber glass bottle under nitrogen atmosphere. The yield of AO from its seeds was 35% (v/w). All the chemicals used were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise specified.

2.2. Animals and treatment protocol for intraperitoneal administration

Healthy male and female Swiss albino mice (20 ± 3 g), obtained from the animal breeding colony of Indian Institute of Toxicology Research (Lucknow, India), were acclimatised under standard laboratory conditions for 1 week prior to the experiment. Animals were housed in an air-conditioned room in plastic cages and maintained at 22 ± 2 °C under standard laboratory conditions of light/dark cycle (12–12 h) and had free access to food and water *ad libitum*. Mice were randomly divided into six groups of 10 each. Intraperitoneal (ip) injections of AO (5 ml/kg bwt)[17] and BY (25 mg/kg bwt)[18] were given twice weekly for 30 and 60 days. The animals of control group received 100 μl injections (ip) of mustard oil twice weekly. During the treatment schedule, the animals were observed for daily food intake and the body weight of animals was recorded weekly. After 30 and 60 days of treatment, mice were sacrificed by cervical dislocation as mentioned in the guidelines for the care and use of laboratory animals of IITR. Gall bladder from each animal was dissected out and weighed.

2.3. Preparation of diet

Commercial stock pellet diet from Ashirwad Industries, Chandigarh, India, was used in the entire study. After making powder from pellet diet, AO and BY were mixed in the powdered diet, to obtain 1% and 0.06% concentrations, respectively, as described earlier.[19,20] AO and BY containing diets were prepared after every 15 days. Control diet was prepared from powdered pellet diet containing 1% of mustard oil.

2.4. Animals and treatment protocol for dietary exposure

Female Swiss mice (20 ± 3 g) were randomly divided into three groups of 25 each in controls, AO and BY. The mice were given powder diet as mentioned above for 180 days. During the treatment schedule, the animals were daily observed and food intake was recorded. Body weight of animals was recorded weekly. After 180 days of treatment, mice were sacrificed by cervical dislocation as mentioned in the guidelines for the care and use of laboratory animals of IITR. Gall bladder was removed from animals and weighed.

2.5. Specimen collection and processing

Blood was collected in dry test tubes containing sodium citrate as anticoagulant. A portion of the blood was allowed to clot at