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Research Article

PHARMACOLOGICAL EFFECT OF POLYSORBATE 80 IN CANCER CHEMOTHERAPY

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ABSTRACT

Objective- Tween 80 (Polysorbate 80) is a classical non-ionic surface-active detergent. It is widely used as an additive in pharmaceuticals and in the food industry. Many of the Tweens are considered to be carcinogens. Here in this study an attempt was made to find out whether the tween 80 used as an excipient in an anti cancer drug, Methotrexate affected its therapuetic efficiency or not.

Method- Hepatocellularcarcinogenesis (HCC) was induced in the wister rats by the administration of a single dose chemical carcinogen DENA (150 mg/kg i. p.). The animals exposed to DENA were treated with pure Methotrexate (MTX) and marketed MTX (0.14mg/kg by oral route through force feeding) and Tween 80 (0.14mg/kg by p. o.).

Result- Exposure of DENA and Tween 80 elevated the levels of SGOT, SGPT, ALP, and AFP in Wistar rats. Histopathological examinations of the liver tissue showed marked carcinogenicity of the chemical carcinogen and excipients, while pure MTX treated group showed normal liver cell architecture.

Conclusion- The study revealed that the excipient polysorbate 80 used in the marketed formulation of Methotrexate decreased the efficacy of the pure drug (pure Methotrexate).

Keywords: Polysorbate80 , Excipient, Efficacy, Diethylnitrosamine(DENA), Hepatocarcinogenesis, Methotrexate (MTX).

INTRODUCTION

Polysorbate 80 (tween 80) is a stabilizer used in a wide variety of food products and medical products like vaccines and anti-cancer medications [1].It is an excipient that is used to stabilize aqueous formulation of medications for parental administration and is also used as an emulsifier in various dosage forms [2,7]. Tween 80 belongs to the polyoxyethylene family of nonionic detergents. Tween 80 (polyoxyethylene sorbitan monooleate), an emulsifier, is a commonly used excipient being implicated in the suppression of the immunological response[4].The immunosuppression caused by Tween 80 is restricted to the primary humoral response[3,4,6]. It was also founded to disrupt the blood brain barrier and change the receptor affinity[3].In a detailed study it was also found out that treatment with Tween 80 accelerated maturation, prolonged oestrous cycle and induced persistant vaginal oestrus[5,10]. In another study it was also proved that repeated subcutaneous injection of polysorbate 80 caused sarcoma at the site of injections[11,12,13].[15].These results can raise the possibility of polysorbate 80 to be a carcinogen or a co-carcinogen. Methotrexate is a classic antifolate used in cancer chemotherapy and polysorbate 80 is a usual excipient used in its various dosage forms[8,17,18].

MATERIALS AND METHODS

Drugs and chemicals

Methotrexate was provided as a gift sample from Dabur pharmaceuticals, New Delhi. DENA was procured from Sigma-Aldrich Chemicals co., St. Louis, USA. Chloroform and Diethyl ether was obtained from S. D. Fine Chem. Ltd., Mumbai. All the chemicals were of analytical grade.

Animals

Male Wistar albino rats weighing 100–125 g were procured from the animal house facility of Siddhartha Institute of Pharmacy for the present protocol. The rats were housed in polypropylene cages under controlled conditions of temperature (22+ 3 °C) and light (14:10 h light and dark cycle) and provided with balanced pallet diet and water. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) with approval no SIP/IAEC/09A/2011 under the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA); Ministry of Social Justice and Empowerment, Government of India.

Induction of hepatocarcinoma (hcc)

Animals were subjected to subnecrogenic dose of DENA 150 mg/kg body weight, IP in phosphate buffer when associated with fasting/refeeding [20,21].

Experimental design

The rats were acclimatized and randomly divided into 8 groups each having 6 rats for a 16 week study. Group-I, Normal control (NC) rats served as vehicle control and were administered with saline orally. Group-II, DENA control (DC) rats were administered with a single dose of DENA (150 mg/kg). Group-III, Marketed MTX control (MC) rats were administered Methotrexate (0.14 mg/kg) only. Group-IV, DENA + Marketed MTX (DM) rats were administered with DENA (150 mg/kg) as a single dose and after 7th day treated with Marketed MTX (0.14mg/ kg). Group-V, Pure MTX control (PC) rats were administered with Pure MTX (0.14 mg/kg) only. Group-VI, DENA + Pure MTX control (DP) rats were administered with DENA (150 mg/kg) as a single dose and after 7th day treated with pure MTX treatment (0.14 mg/ kg). Group-VII, Tween 80 control (TwC) was treated with tween 80 (0.14mg/kg) only. Group-VIII, DENA + Tween 80 (DTw) rats were administered with DENA (150 mg/kg) as a single dose and after 7th day with Tween 80 (0.14 mg/ kg).

Estimation of biochemical parameters

Blood samples were collected on the termination day of the experiment from the retro-orbital plexus under light ether anesthesia without any anticoagulant and were allowed to stand for 30 min at room temperature, centrifuged at 2500 rpm for 10 min to separate the serum. The serum obtained was kept at 2–4 °C for further use. The blood glucose level was measured using a digital glucometer (Abbott Diabetes care Inc., Alameda, USA). Estimation of serum SGOT, SGPT, ALP, and AFP were performed using standard kits (Nicholas India Pvt. Ltd.) with semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd).

Histopathological examination

The liver samples were preserved in phosphate-buffered 10% formalin, embedded in paraffin and used for histopathological examination. Five-µm-thick sections were cut, deparaffinized, hydrated and stained with hematoxylin and eosin. The sections were examined blindly for tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis in all treatments.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA). The results were expressed as mean ± SEM. Statistical significance between more than two groups were tested using one-way ANOVA followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were regarded as significant.

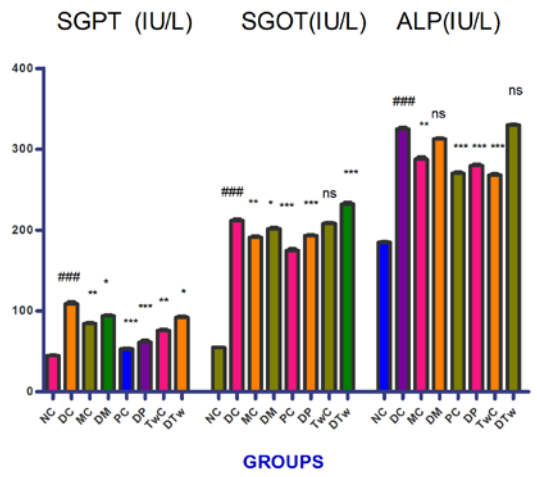


Fig. 1: Graphical representation of the effect of various pharmacological interventions on SGPT, SGOT and ALP of animals.

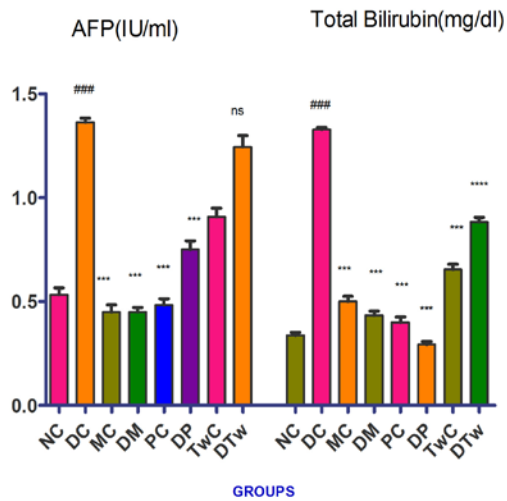


Fig. 2: Graphical representation of the effect of various pharmacological interventions on AFP and Total Bilirubin of animals.

Table 1: Effect of various pharmacological interventions on Body Weight, SGPT, SGOT, AFP, ALP and Total Bilirubin of animals

S. No.	Groups	Body weight (g)	SGPT (IU/L)	AFP (IU/ml)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
1	Normal control (NC)	123.28 ± 0.37 a	44.83 ± 0.30 a	0.53 ± 0.03 a	54.83 ± 0.30 a	185.0 ± 0.03 a	0.33 ± 0.014 a
2	DENA control (DC)	100.71 ± 1.22 a/***	108.83±2.24a/***	1.36 ± 0.01 a/***	211.3 ± 3.18a/***	326.3 ± 4.20a/***	1.32 ± 0.009 a/***
3	Mtx control (MC)	98.05 ± 2.65 a/***	87.83 ± 1.19a/***	0.45 ± 0.03 ns	194.2 ± 0.60a/***	289.7 ± 0.03a/***	0.50 ± 0.025 a/*
4	DENA+ Mtx (DM)	122.19 ± 1.29ns	93.83 ± 1.24 a/*	0.45 ± 0.02 ns	203.8 ± 1.44a/***	319.5 ± 0.02a/***	0.43 ± 0.021ns
5	Pure Mtx control (PC)	109.52 ±1.92 a/***	53.00± 1.09a/***	0.48 ± 0.03 ns	177.2 ± 1.92a/***	265.0 ± 0.03a/***	0.40 ± 0.02ns
6	DENA+ Pure Mtx (DP)	99.85 ± 1.28 a/***	61.5 ± 1.94a/***	0.50 ± 0.02ns	190.2 ± 2.18a/***	280.0 ± 0.02a/***	0.51 ± 0.04a/*
7	Tween 80 control (TwC)	111 ± 1.02 a/***	74.32 ± 2.40a/***	1.05 ± 0.01 a/***	213.7 ± 1.45a/***	267.0 ± 0.01a/***	0.65 ± 0.04a/***
8	DENA+Tween 80 (DTw)	96.01 ± 1.10 a/***	91.17± 2.30a/***	1.4 ± 0.02 a/***	238.7 ± 1.22a/***	330.5 ± 0.02 a/***	0.86 ± 0.05 a/***

Values are expressed as mean ± SEM (N= 6). a p<0.0001, as compared to vehicle control.

RESULTS

Alkaline phosphatases (alp)

In DENA treated group the ALP level was significantly increased (*p<0.001*) as compared to NC group. While in MTX treated group MC, PC & DP the ALP level were significantly decreased (*p<0.001*) as compared to DC group and DM group showed a less significant decrease(p<0.05). In excipient treated group TwC the ALP level were significantly decreased (*p<0.001*) and in DTw group there was slight increase(no significant)when compared to DC group. (Table-1)

Serum glutamate oxaloacetic transaminase (sgot)

In DENA control (DC) group the SGOT level were significantly increased *(p<0.001)* as compared to NC group. While in MTX treated group, MC& DM the decrease of SGOT level was less compared to the PC & DP group. In the excipient treated group TwC showed no significant change but DTw group showed a significant increase(p<0.001) in the SGOT level when compared with the DC group. (Table-1)

Serum glutamic pyruvate transaminase (sgpt)

In DENA treated group the SGPT level were significantly increased (*p<0.001*) as compared to NC group. While in MTX treated group MC& DM the decrease in SGPT level was slightly decreased(p<0.01)while in PC & DP group the SGPT level were significantly decreased (*p<0.001*) as compared to DC group. The TwC& DTw group showed significantly decreased (*p<0.001*) SGPT level when compared to DC group. (Table-1)

Alfa feto protein (afp)

In DC group the AFP level was significantly increased (*p<0.001*) as compared to NC group. While in MTX treated group MC, DM, PC & DP the AFP level were significantly decreased (*p<0.001*) as compared to DC group. In excipient treated group TwC the level of AFP was slightly decreased (p<0.01) than the DC group and the DTw group

showed only a slight increase (ns-not significant) in the AFP level. (Table-1)

Serum bilirubin (tbr)

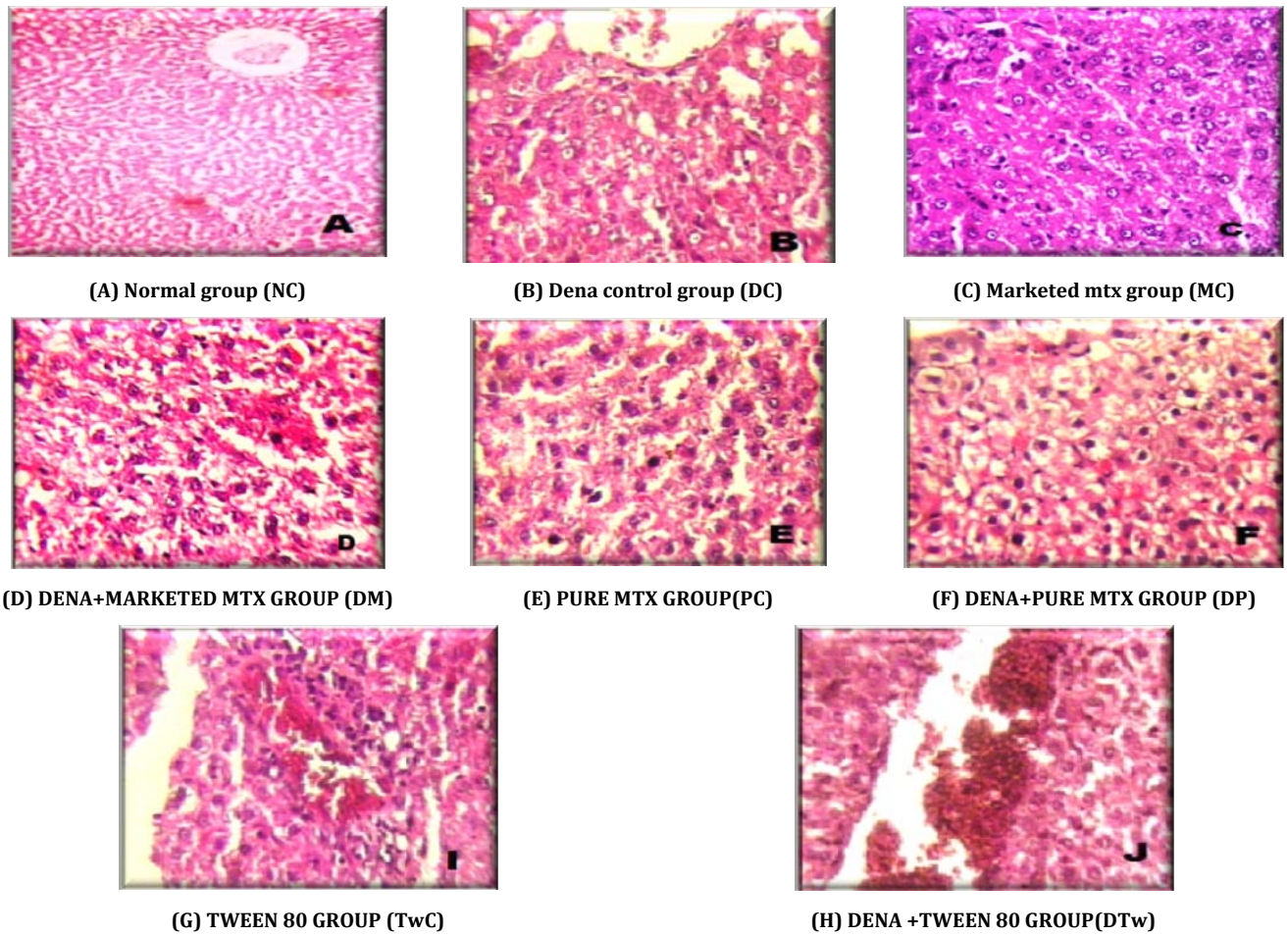
In DC group the Direct Bilirubin level were significantly increased *(p<0.001)* as compared to NC group. Where as in MTX treated MC, DM, PC & DP group, the TBR level were significantly decreased *(p<0.001)* as compared to DC group. In the excipient treated group TwC &DTw showed significantly decreased *(p<0.001)* level of TBR when compared to DC group. (Table-1)

Histopathology

Insert figure 3

Liver sections from the NC group showed normal hepatic architecture with unremarkable central veins, no evidence of hepatocyte injury or fibrosis or dysplasia or malignancy noticed. While Liver of DC exhibited centrilobular necrosis, hepatic steatosis, macrovaesicular fatty changes and disturbed portal vein architecture, cholestasis, ballooning degeneration and marked atypia. Comparatively in the treatment groups MC, DM, PC and DP restored the abnormal architecture of the liver. DP was having more pronounced effect as compared to other treatment groups. From the histological studies it can be inferred that DP group restored the normal architecture of liver and is proved to be the most efficacious strategy for the treatment of HCC.

TwC group exhibited centrilobular necrosis, macrovaesicular fatty changes and DTw group showed cholestasis, abnormal architecture, ballooning degeneration, macrovaesicular fatty changes and presence of centrilobular necrosis as compared to the normal group which gives the clear indication that Tween-80 may promote HCC.



(A) Normal group (NC) **(B) Dena control group (DC)** **(C) Marketed mtx group (MC)**

(D) DENA+MARKETED MTX GROUP (DM) **(E) PURE MTX GROUP(PC)** **(F) DENA+PURE MTX GROUP (DP)**

(G) TWEEN 80 GROUP (TwC) **(H) DENA +TWEEN 80 GROUP(DTw)**

Fig. 3: Representative Photo micrographs of histopathology (45X) examination.

(A) Liver of Normal group (NC) showing normal hepatic architecture with absence of centrilobular necrosis, hepatic steatosis.

(B) Liver of DC exhibited centrilobular necrosis, hepatic steatosis, macrovaesicular fatty changes and disturbed portal vein architecture.

(C) Liver of MC group shown mild abnormal architecture of histopathological observations.

(D) Liver of DM group restored normal architecture of histopathological observations.

(E) Liver of PC group rats had shown mild abnormal architecture of histopathological observations.

(F) Liver of DP group rats had shown restored normal architecture of histopathological observations.

(G) Liver of TwC group rats had showed the abnormal architecture, atypia, councilman body.

Liver of DTw group rats showed the abnormal architecture, ballooning degeneration, councilman body and presence of centrilobular necrosis.

DISCUSSION

In the present study, DENA induced hepatocellular damage is clearly evidenced by the marked elevation in serum SGPT, SGOT, AFP and ALP enzymes which are indicators of tumor response [22].

Serum alkaline phosphatase increases to some extent in most types of liver injury [23]. The highest concentrations are observed with cholestatic injuries, elevations occur as a result of both intrahepatic and extrahepatic obstruction of bile flow [24]. Further ALP is used as a specific tumor marker during diagnosis in the early detection of cancer [25]. In present study there were significant increases in ALP levels in all groups exposed to DENA as compared to the NC group. While for the treatment groups with marketed and pure MTX the ALP levels were brought towards the normal levels.

Among all treatment groups, DP group showed the significant reduction in the serum ALP levels as compared to DC group. TwC& DTw, showed elevated ALP level when compared with the NC group, DTw group showed elevated ALP level when compared with the DC group.

The SGOT&SGPT enzyme levels are found to exceed in toxic hepatitis, viral hepatitis, chronic active hepatitis and cholestatic hepatitis [28]. In current study it was observed that there was a consequent significant increase in the levels of SGPT and SGOT in DC group when compared with the normal control which indicates the hepatocellular damage induced by the chemical carcinogen DENA. Treatment with pure and marketed MTX significantly reduced the level of these enzymes as compared to DC group. Here also pure methotrexate showed a better result than the marketed formulation. The TwC& DTw treated rats showed significantly increased level of the SGOT when compared with NC group, which further added to the hepatotoxic activity of the excipient.

It has been confirmed on numerous occasions that AFP serum concentration increases in parallel with HCC tumor size. For this reason AFP has to be considered as 'the gold standard' for HCC serum markers [26]. Elevated serum concentration of this protein can be achieved by exposure to hepatotoxic agents like DENA [27]. In the present study, serum AFP level of DENA treated rats showed significant elevation as compared to the normal control group, proving the premalignant changes due to DENA. Treatment with MTX significantly reduced the AFP level that showed the anti carcinogenic effect of the drug. However TwC&DTw group showed an elevated level of AFP when compared with the NC control thus acting as a promoter for HCC.

The Total bilirubin (TBR) level is one of the best tests for liver function. The degree of increase in TBR values has prognostic significance in chronic liver injuries, but not in acute injuries [25]. It is studied that if the direct or conjugated bilirubin is low, while the TBR is high, it reflects liver cell damage or bile duct damage [27]. TBR level of DC group of rats showed a significant increase($p<0.001$) than that of normal control group. Such elevated level may be due to hepatocellular or obstructive jaundice in hepatocellular carcinoma [29]. In MC, DM, PC, and DP groups the level of TBR were decreased significantly as compared to DC group. The DP group showed better decrease in TBR levels as compared to other group. There was significant increase in the TBR level of TwC and DTw group which indicate the liver cell damage.

Further from the histopathological results it was also proved that the excipient treated groups showed more liver cell damage and resembled tumour promoting cells.

CONCLUSION

From the results of the study it was concluded that the excipient polysorbate 80 used in the marketed formulation of Methotrexate decreased the efficacy of the pure drug (pure Methotrexate). Polysorbate 80 used in the marketed formulation was found to be promoting hepatocarcinogenisis, hence decreasing the therapeutic efficacy of the pure drug (pure Methotrexate).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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