J.Natn.Sci.Foundation Sri Lanka 2010 **38** (2):145-149

SHORT COMMUNICATION

Hepatotoxicity of Schiff bases derived from benzoin salicylaldehyde, aminophenol and 2,4 dinitrophenyl hydrazine

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Revised: 21 October 2007 ; Accepted: 21 December 2007

Abstract: Hepatotoxicity of three Schiff bases *viz.* PDH [N-(1-phenyl-2-hydroxy-2-phenyl ethylidine)-2/,4/ dinitrophenyl hydrazine],PHP[N-(1-phenyl-2-hydroxy-2-phenylethylidine) 2/ hydroxy phenyl imine] and HHP [N-(2-hydroxy benzylidine)-2/ hydroxy phenyl imine] in both mice with and without Ehrlich Ascites Carcinoma (EAC) was studied. The parameters selected were serum level of the enzymes alanine transaminase, aspartic transaminase, alkaline phosphatase, glucose, blood urea and cholesterol. In mice with no carcinoma there was a modest increase in all the above parameters during the treatment period (10 consecutive days at the dose of 2 mg/kg). After treatment the enhanced values gradually decreased to normal levels. In EAC bearing mice, the toxic effects due to EAC cells in all cases were found to be nullified by treatment of the test compounds. No significant abnormalities in histology of the various organs of the mice were detected due to such treatments.

Keywords: EAC cells, Hepatotoxicity, HHP, histopathology, PDH, PHP.

INTRODUCTION

In recent years Schiff bases have been widely used in formulating various types of drugs with diversive biological activities[1–5]. The antimicrobial activities of three such bases, namely PDH [N-(1-phenyl-2-hydroxy-2-phenyl ethylidine)-2/,4/ dinitrophenyl hydrazine], PHP [N-(1-phenyl-2-hydroxy-2-phenyl ethylidine) 2/ hydroxy phenyl imine] and HHP [N-(2-hydroxy benzylidine)-2/ hydroxy phenyl imine] have been previously studied[6]. It has also been found that these three compounds could be successfully utilized (*unpublished data*) as antitumour agents against Ehrlich Ascites Carcinoma (EAC) cells in swiss albino mice. They have also been found to reduce tumour weight, inhibit cell growth and enhance life span of EAC bearing mice. In addition they can effectively restore the depleted haematological parameters like white blood cells (WBC), red blood cells (RBC) and haemoglobin content. Before claiming these compounds to be potent antitumour agents, more investigations have to be carried out with other cell lines from higher animal models and subject them to extensive clinical trials. In the present investigation the toxic effect of any of these compounds on the host was evaluated with the aim of determining whether the test compounds while functioning as antitumour agents could also exert unacceptable toxic side effects, especially on the liver function. For this purpose the effect of the test compounds on a number of biochemical parameters which are directly related to liver damage *viz.* serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) as well as the serum levels of glucose, cholesterol and urea were evaluated in EAC treated and normal mice. In addition, the histopathology of various organs of the mice treated with these compounds was also investigated.

METHODS AND MATERIALS

All chemicals and reagents used throughout the investigation were of reagent grade (BDH, England). The compounds, PDH, PHP and HHP used for the present study were synthesized and duly characterized by methods described earlier[6]. Swiss albino mice, 6 to 8 wks old (25 ± 5g body weight), were collected from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) and used throughout the studies. The mice were fed with standard mice food-pellets (collected from ICDDR,B) and water was given *ad libitum*.

The protocol used in this study for the use of mice as animal model for cancer research was approved by the Animal Ethics Committee of Rajshahi University.

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Ehrlich Ascites Carcinoma (EAC) cells were obtained from the Indian Institute of Chemical Biology (IICB), Kolkata, India. The cells were maintained as ascites tumours in swiss albino mice by intraperitoneal inoculation (bi-weekly) of 2×10^6 cells/mouse.

Acute toxicity was determined by dissolving the compounds in 2% dimethyl sulfoxide (DMSO) and injected intraperitoneally (i.p.) to 8 groups of mice (each containing 6 in number) at different doses. LD_{50} values were determined by recording mortality after 24 h. The LD_{50} values were found to be 15, 16 and 15.5 mg/kg respectively for PDH, PHP and HHP. The details of the procedure have been described previously[7].

The parameters (*viz*. ALT, AST, ALP, serum glucose, cholesterol, urea) were determined for both normal and EAC bearing mice. Mice with no carcinoma were treated with the dose of 2 mg/kg (i.p.) of each compound and the treatment continued for 10 consecutive days. For tumour bearing mice similar treatments were given after 24 h of EAC cell transplantation (2×10^6cells/mouse). Eight groups of mice (24 in each) were used. Groups 1 to 3 included mice with no carcinoma treated with the compounds while groups 4 to 6 included tumour bearing mice. Group 7 included EAC bearing mice without any treatment and the 8th group was used as the control group (normal mice). On day 5, 10, 15 and 25, mice from each group were sacrificed. Blood was collected from the heart in plastic centrifuge tubes. These were then allowed to clot at room temperature for half an hour and centrifuged at 4000 rpm for 15 min using a WIFUNG centrifuge LABOR-50M. The clear straw coloured serum was then collected from the upper part of the tubes in vials with a Pasteur pipette. All parameters were determined according to the procedures[8–13] established earlier.

The major body organs *viz*. brain, liver, kidney, heart, lungs and spleen, were collected from the experimental animals (both mice with carcinoma and compound treated mice) and processed by the techniques recommended by Gurr[14]. Histopathological examination of the processed sections were examined with a light microscope.

The experimental results have been expressed as mean ± S.E.M. Data have been calculated by one way ANOVA followed by Dunnett "t" test using SPSS software of 10 version.

Table 1: Dependency of cholesterol and urea contents of mice on treatment time

Treatment	Days			Normal mice	Tumour bearing mice
		Cholesterol (mg/dL)	Urea (mg/dL)	Cholesterol (mg/dL)	Urea (mg/dL)
Untreated (control)	0	150.0 ± 0.42	52.0 ± 0.73	–	–
EAC cells	5	–	–	156.0 ± 0.73**	55.0 ± 0.73***
	10	–	–	160.5 ± 0.67***	60.1 ± 0.59**
	15	–	–	167.0 ± 0.97	70.0 ± 0.52
	25	–	–	175.0 ± 0.73***	73.0 ± 0.63***
PDH	5	180.0 ± 1.15***	46.0 ± 0.68***	152.0 ± 0.58***	53.2 ± 0.31***
	10	190.0 ± 0.89***	44.0 ± 0.36**	155.0 ± 0.81***	56.0 ± 0.58***
	15	175.0 ± 0.58***	44.5 ± 0.62***	157.0 ± 0.82**	58.5 ± 1.05***
	25	162.2 ± 0.83***	45.0 ± 0.36***	158.0 ± 0.81	61.9 ± 0.70***
PHP	5	160.0 ± 0.58***	48.2 ± 0.30	149.0 ± 0.58***	55.0 ± 0.73***
	10	190.0 ± 1.15***	47.0 ± 0.37	153.0 ± 0.52***	57.9 ± 0.81***
	15	173.0 ± 0.73***	48.0 ± 0.52	154.0 ± 0.73***	59.0 ± 0.73**
	25	167.0 ± 0.97***	49.0 ± 0.52	155.5 ± 0.88***	63.2 ± 0.71***
HHP	5	179.7 ± 0.08***	50.8 ± 0.40*	151.0 ± 0.73***	54.0 ± 0.73***
	10	180.7 ± 0.79***	47.0 ± 0.36	156.3 ± 1.20***	60.0 ± 0.73***
	15	165.0 ± 0.86***	48.0 ± 0.45	158.0 ± 0.81	65.0 ± 0.73*
	25	150.5 ± 0.99***	48.5 ± 0.56**	160.4 ± 0.42**	68.0 ± 0.73***

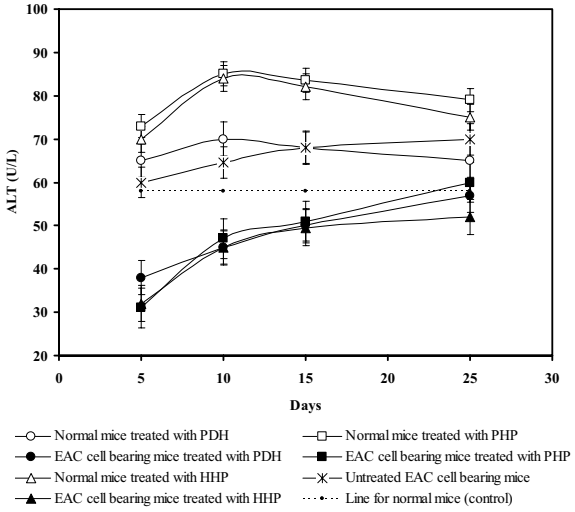
Treatment was continued for 10 consecutive days at the dose 2 mg/kg (i.p.) (number of mice in each group = 6). For tumour bearing mice, similar treatment was started 24 hours after EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 10 days from the start. Results are shown as mean ± SEM, where significant values are * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared with control.

RESULTS

Effects of the test compounds on the enzyme activities (ALT, AST and ALP) have been presented in Figures 1–3.

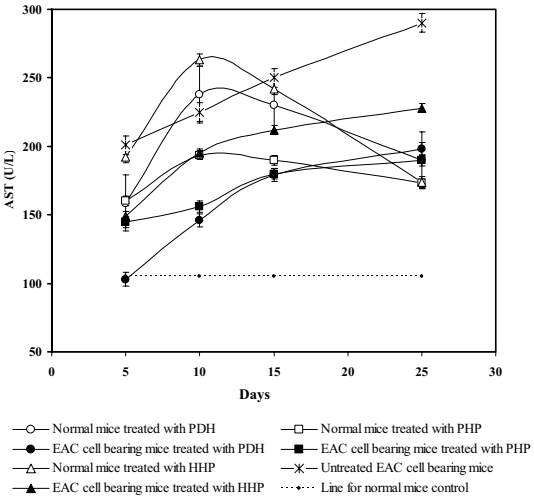
In mice without carcinoma the treatments were found to moderately increase the enzyme activities during the treatment period (10 consecutive days at the dose 2 mg/kg i.p.) after which these were found to gradually decrease towards normal values.

Figure 1: Variation of ALT of normal and EAC cell bearing mice on treatment with the test compounds



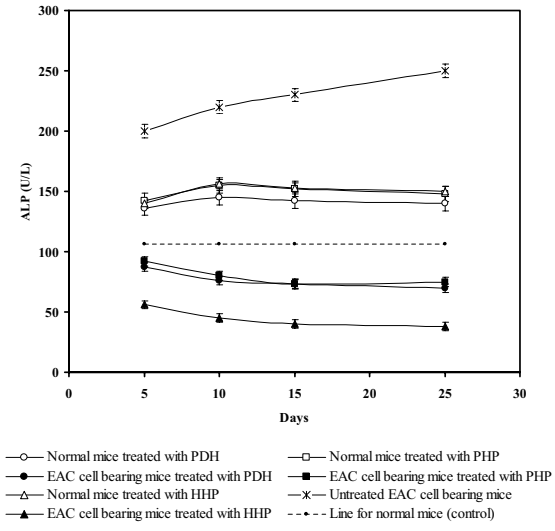
Treatment was continued for 10 consecutive days at the dose 2 mg/kg (i.p). For tumour bearing mice, similar treatment was started 24 hours after EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 10 days from the start.

Figure 2: Variation of AST of normal and EAC cell bearing mice on treatment with the test compounds.



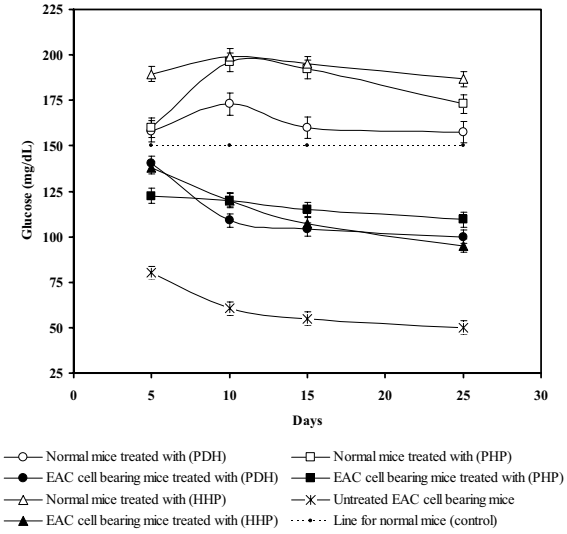
Treatment was continued for 10 consecutive days at the dose 2 mg/kg (i.p). For tumour bearing mice, similar treatment was started 24 hours of EAC cell transplantation (2×106 cells/mouse). Treatment was discontinued after 10 days from the start.

Figure 3: Variation of ALP of normal and EAC cell bearing mice on treatment with the test compounds



Treatment was continued for 10 consecutive days at the dose 2 mg/kg (i.p). For tumour bearing mice, similar treatment was started 24 hours after EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 10 days from the start.

Figure 4: Variation of glucose level of normal and EAC cell bearing mice on treatment with the test compounds



Treatment was continued for 10 consecutive days at the dose 2 mg/kg (i.p). For tumour bearing mice, similar treatment was started 24 hours after EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 10 days from the start.

Table 2: Histopathological changes in major body organs

Body parts	Untreated (normal)	Changes observed with Schiff bases at the dose 2 mg/kg/day with PDH	PHP	HHP
brain	no abnormality detected	no abnormality detected	no abnormality detected	no abnormality detected
heart	no morphological change or congestion found in cardiac muscles	no morphological change or congestion found in cardiac muscles	no morphological change or congestion found in cardiac muscles	no gross abnormalities detected
liver	no abnormality detected in cell architecture	no gross abnormality detected	no gross abnormality detected detected	no gross abnormality
kidney	glomerulus structure occurred	glomerulus structure occurred	no evidences of pathological changes	no evidences of pathological changes
lung	alveoli, no congestion occurred	alveoli, no congestion occurred	alveoli, no congestion occurred	alveoli, no congestion occurred
spleen	no abnormality detected	no gross abnormality detected	no gross abnormality detected	no gross abnormality detected

Treatment was continued for 10 consecutive days at dose 2 mg/kg (i.p). For tumour bearing mice, similar treatment was started 24 hours of EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 10 days from the start.

For EAC bearing untreated mice, all such values increased almost linearly with time. The treatment of the test compounds, however, diminished ALT and ALP values significantly. After treatment, the ALT values returned to normal levels with time (Figure1) while the ALP values remained almost the same (Figure 3). In case of AST, the test compounds partially reduced the rate of its increment and did not reverse to normal values (Figure 2).

Figure 4 shows the effect of test compounds on serum glucose content of mice with and without EAC. The glucose content of mice with no EAC was found to increase slightly during the treatment period, after which it slowly reversed towards normal. On EAC bearing mice, the glucose content reduced abruptly. The treatment with test compounds increased the values close to normal levels.

Variations were also observed in the case of cholesterol and blood urea (Table 1). For mice with no EAC, the cholesterol level enhanced modestly during the treatment time and reversed towards normal with time. The cholesterol content of untreated EAC bearing mice enhanced gradually with time. The treatment with all three test compounds restored the cholesterol levels more or less to normal levels.

Urea levels, on the other hand, diminished slightly during the treatment period in mice with no EAC. On the contrary, in 3 untreated EAC bearing mice these values were increased gradually with time. On treatment the rate of increase slowed down.

The data for the histopathological tests are shown in Table 2. No significant abnormalities were detected.

DISCUSSION AND CONCLUSION

The three test compounds behave almost identically in restoring the depleted biochemical parameters of the EAC bearing mice but are less effective in increasing the depleted ALP values. The slight host toxicity observed in mice during the treatment period is mostly reversible. This means that, treatments with these compounds do not cause any acute or permanent damage to the liver.

Some interesting features are seen from the results presented here. In almost all cases the toxic effects of EAC cells on biomolecules were found to be nullified by such treatments. In some cases antagonistic effects were observed instead of additive effects. Further elevation of glucose levels of EAC bearing mice due to treatment with these compounds probably indicates their partial recovery from tumour growth.

The major organs of the treated mice do not show any histopathological abnormalities. These findings together with those obtained from the estimation of serum biomolecules indicate the necessity to conduct further research to formulate novel anticancer drugs.

Acknowledgement

The authors gratefully acknowledge the IICB, Kolkata, India for providing the EAC cells and also the ICDDR,B, Dhaka, Bangladesh for kindly supplying swiss albino mice and standard mouse pellets.

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