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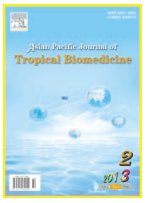

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Hepatoprotective effect of acetone semicarbazone on Ehrlich ascites carcinoma induced carcinogenesis in experimental mice

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PEER REVIEW

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Comments

This piece of work is a good research, in which the authors evaluated the hepatoprotective and host toxic effects of acetone semicarbazone, a simple Schiff base to find out an effective anticancer drug with better safety profile. The results are interesting and suggested that ASC posses protective effects on liver against the toxicity generated by EAC cell bearing mice.
(Details on Page 109)

ABSTRACT

Objective: To determine the hepatoprotective effect of acetone semicarbazone (ASC) *in vivo* in normal and Ehrlich ascites carcinoma (EAC) bearing male Swiss albino mice. **Methods:** Drug-induced changes in biochemical and behavioral parameters at dose of 2.0 mg/kg body weight for 14 d and nullifying the toxicity induced by EAC cells were studied. The histopathology studies of the protective effects of ASC on vital organs were also assessed. **Results:** The administration of ASC made insignificant changes in body weight and behavioral (salivation, diarrhea, muscular numbness) changes during treatment period due to minor toxicity were minimized after the treatment in normal mice. The biochemical parameters, including serum glutamate pyruvate transaminase, glutamate oxaloactate transaminase, alkaline phosphatase, serum glucose, cholesterol, urea, triglyceride and billirubin changed modestly in normal mice receiving ASC. Though the treatment continued, these values gradually decreased to normal level after the treatment. In EAC bearing mice, the toxic effects due to EAC cells in all cases were nullified by treatment with the ASC. Significant abnormalities were not detected in histology of the various organs of the normal mice treated with ASC. **Conclusions:** ASC can, therefore, be considered safe in formulating novel anticancer drug, as it exhibits strong protective effect against EAC cell bearing mice.

KEYWORDS

EAC cell, Host toxicity, Acetone semicarbazone, Schiff bases, Histopathology, Subacute toxicity

1. Introduction

Schiff bases are an important and interesting group of chemical compounds because they exhibit a number of biological activities and play an important role in the regulation of many biochemical processes. Due to these properties, these compounds are potentially useful for the design and production of novel anticancer, antimalarial, antiviral and antimicrobial drugs[1]. The more significant bioactivities of Schiff bases are found in a variety of semicarbazones (anti-protozoa, anticonvulsant) and thiosemicarbazones (antibacterial, antifungal, antitumoral, antiviral) and their metal complexes, such as hydroxysemicarbazide showed potential anticancer activity against L1210 murine leukemia cells[2]. Nickel (II) complexes of semicarbazone derivatives showed potent anticancer activity against MCF-7 cell lines[3]. Semicarbazones, thiosemicarbazones demonstrated potent cytotoxicity against different cancer cell lines[4]. The antitumor activity of vanillin semicarbazone have also been reported in the literature[5].

Most of the reported compounds have complex structure

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with many difficulties to solubilize them for use and their host toxic effects have not been well reported. However studies with simple Schiff bases have not been done extensively. For this, we have synthesized a series of compounds and evaluated their different biological properties. It has been found that these compounds have potential anticancer, antimicrobial and insecticidal activities[6-9]. In these compounds, acetone semicarbazone (ASC), a simple water soluble compound synthesized from acetone and semicarbazide, showed significant anticancer activity against Ehrlich ascites carcinoma (EAC) cells *in vivo*[10]. ASC also exhibits potent antibacterial activity against both pathogenic gram positive and gram negative bacteria[11].

As a part of our research focused bioactive compounds to find a novel host friendly anticancer agents, we here report the host toxic effects of ASC in normal mice with the aim of determining whether the test compound while functioning as an antitumour agent can also exert any unacceptable toxic side effects to the host and also its protective effects in EAC bearing mice. For this purpose, we studied the different biochemical profiles and histological investigation of organs with a view to assess its safety profile which may be helpful for its application.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used throughout the investigation were of reagent grade and from BDH, England and Sigma Aldrich, USA.

2.2. Test compound

The compound, ASC used for the present study was synthesized and duly characterized by the methods described earlier[10].

2.3. Test animals

Adult male Swiss albino mice, six to eight weeks old with (25±5) g body weight were collected from animal resource branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B) and used throughout the studies. Animals were housed in polypropylene cages containing sterile paddy husk as bedding material under hygienic conditions with a maximum of ten animals in a cage. They were maintained under controlled conditions (12:12 h light-dark), temperature of (22±5) °C. The mice were fed with standard mice food-pellets (collected from ICDDR'B and water was given *ad libitum*.

2.4. Ethical clearance

Protocol used in this study for the use of mice as animal model for cancer research was approved by the University Animal Ethical committee (27/08/RUBCMB).

2.5. Cell line

EAC cells were obtained by the courtesy of Indian Institute of Chemical Biology, Kolkata, India. The cells were maintained as ascites tumour in Swiss albino mice by intraperitoneal inoculation (bi-weekly) of 2×10^6 cells/mouse.

2.6. Acute toxicity

The LD_{50} value was determined following conventional methods[12]. The test compound was dissolved in distilled water and injected intraperitoneally to six groups of mice (n=6) at different doses (10, 20, 30, 40, 50 and 60 mg/kg). LD_{50} was evaluated by recording mortality after 24 h. LD_{50} value in Swiss albino mice was 30 mg/kg (*i.p.*).

2.7. Grouping and administration

The effects of ASC on both normal and EAC bearing mice were studied. For this purpose, four groups of mice each containing 24 animals were used. Group 1, normal mice treated with ASC, Group 2, EAC bearing mice treated with ASC. Group 3 and 4 served as tumor control (EAC bearing without treatment) and normal control (normal mice without treatment) respectively. Group 1 were treated with ASC at the dose of 2 mg/(kg·day) (*i.p.*) for 14 consecutive days. For Group 2, similar treatment were done after 24 h of EAC cell inoculation (2×10^6 cells/mouse).

The mice were observed daily very keenly to notify the general features such as behavior, central nervous system (CNS) excitation, CNS depression, food intake, salivation, diarrhea, muscular weakness and reflexes. The body weight of each mice was measured before administration of ASC and at the completion of the treatment prior to sacrifice the animals. The weights of individual mice of Group 1 were compared with mice of Group 4 and Group 2 with Group 3.

2.8. Measurement of biochemical parameters

The parameters *viz.* serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatases (ALP), serum glucose, cholesterol, urea, billirubin, triglyceride *etc.*) were determined for both normal and EAC bearing mice. For this experiment, on day 5, 10, 15 and 25, six mice from each group were sacrificed. Blood sample was collected from heart in plastic centrifuge tubes. These were then allowed to clot at room temperature for half an hour and centrifuged at 4 000 r/min for 15 min using a WIFUNG centrifuge LABOR-50M. The clear straw colored serum was then collected from the upper part of the tubes in vials with a Pasteur pipette. All the parameters were determined according to the procedures established earlier[13].

2.9. Histopathology

The major body organs like brain, liver, kidney, heart, lung and spleen, were collected from the experimental animals on day 15 and processed by standard methods[13] to

prepare slides of tissues by hematoxylin and eosin staining. The slides were viewed under Motic Advanced system microscope (B, series) with the help of Motic J. 1 software in a Macintosh computer. Drug induced hepatotoxicity, nephro- toxicity and spleen toxicity, neuro-toxicity, cardio-toxicity and lung toxicity are insignificant in normal mice and furthermore, ASC also nullifying efficacy of hepatotoxicity generated by EAC cell in EAC cell bearing mice were observed.

2.10. Statistical analysis

The experimental results have been expressed as the mean±SEM. Data have been calculated by one way ANOVA followed by Dunnett *t* test using SPSS software of 10 version.

3. Results

During the whole experimental period, general behavior, CNS excitation, CNS depression, reflexes, muscular weakness, salivation, diarrhea and food intake of all the mice were observed. The control group and group 1 mice did not show any abnormalities and their food intake was also observed to be normal. No muscular numbness of the hind and fore legs, salivation or diarrhea was observed. But EAC bearing mice showed some noticeable signs such as tremor, convulsion and reflex abnormalities, muscular numbness of the hind and fore legs, salivation or diarrhea due to the tumorogenesis while mice of Group 2 (EAC bearing mice treated with ASC) minimize such toxicities a great extent owing to antitumoric activity of ASC. Table 1 shows the average body weights of all the mice before and after the treatment and the data were compared. No significant changes in body weight of Group 1 and 4 mice were observed. However, the body weights of EAC bearing mice increase remarkably due to tumor growth which is also limited to the mice treated with ASC.

Effects of the test compound on the enzyme activities of GPT, GOT and ALP have been presented in Figures 1-3. Comparing with normal mice, these activities were found to be moderately increased during the treatment period of 14 consecutive days at dose 2 mg/(kg·day) *i.p.* and after which these were found to gradually return to the more or less normal level.

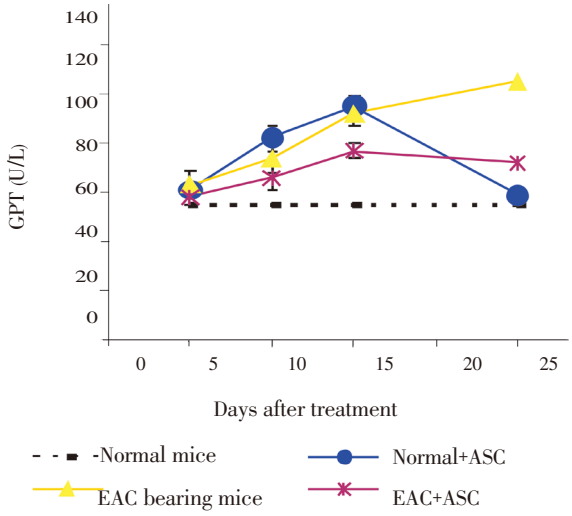

Figure 1. Effects of ASC on serum GPT level in experimental mice. Results are shown as mean±SEM.

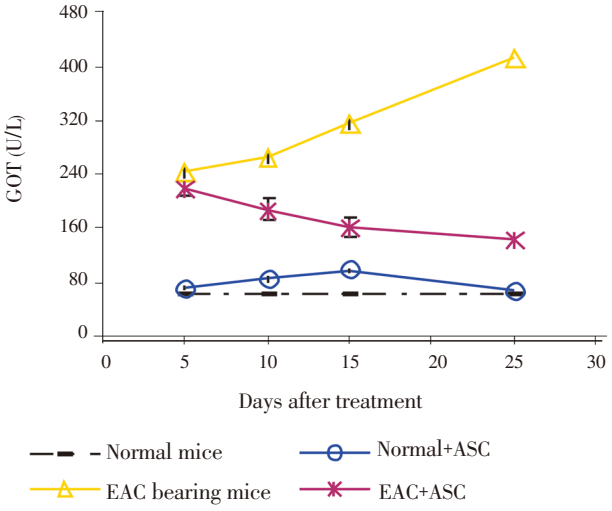

Figure 2. Effects of ASC on serum GOT level in experimental mice. Results are shown as mean±SEM.

For EAC bearing untreated mice, all such values increased almost linearly with time while EAC bearing mice treated with ASC however, diminished GPT, GOT and ALP values significantly. After treatment, the GPT values returned to normal levels with time (Figure 1). In case of GOT, the test compound partially reduced the rate of its increment and could reverse it back to near normal (Figure 2). While the ALP values

Table 1

Effects of ASC on body weight of experimental mice.

Days after treatment	ASC treated (Group 1)		EAC +ASC (Group 2)		Control EAC (Group 3)		Control (Group 4)	
	Body weight (g)	% change	Body weight (g)	% change	Body weight (g)	% change	Body weight (g)	% change
0	25.03±2.61	–	30.49±2.10	–	30.93±1.00	–	24.90±1.84	–
5	27.26±2.76	8.92	31.73±2.60	5.76	34.75±2.50	12.35	26.26±1.80	5.46
10	29.17±3.07	16.51	34.09±1.70	11.70	36.43±3.10	21.01	27.57±1.18	10.71
15	31.53±3.06	25.95	34.82±1.80	14.20*	39.62±1.50	28.41	29.40±1.65	18.07
25	32.60±3.75	30.23	35.60±1.50	16.75**	44.81±3.10	44.87	31.40±2.03	26.10

Treatment was continued for 14 consecutive days at dose 2 mg/kg (*i.p.*) (number of mice in each day=6). For tumour bearing mice, similar treatment was started 24 h of EAC cell transplantation (2×10^6 cells/mouse). Treatment was discontinued after 14 d from the start. Results are shown as mean±SEM, where significant values are *: $P < 0.05$; **: $P < 0.01$ when compared with control. –: No change.

remained almost the same with some backdrop (Figure 3).

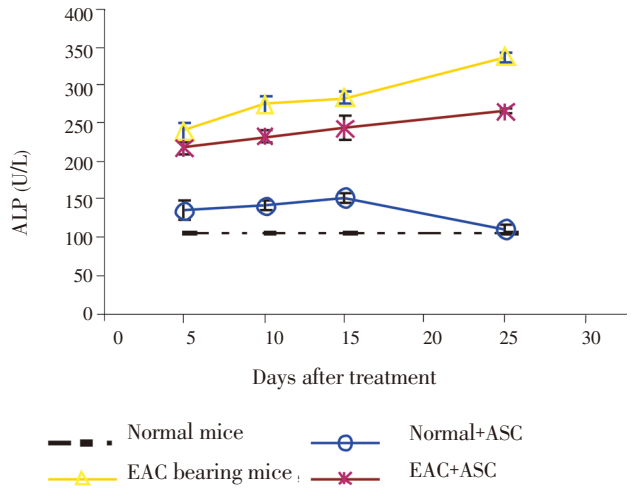

Figure 3. Effects of ASC on serum ALP level in experimental mice. Results are shown as mean±SEM.

Table 2 shows the effects of ASC on serum glucose, cholesterol, urea, triglyceride and billirubin content of both normal and EAC bearing mice. All this parameters except glucose were increase in both normal and EAC bearing mice but they regain to its normal range in ASC treated normal mice after the treatment period whereas this values return back to more or less normal level in EAC bearing mice treated with ASC. The glucose content of normal mice was found to be increased to some extent during the treatment period, after which it slowly reversed back towards normal. For EAC bearing mice, the glucose content was found to be reduced abruptly. The treatment with ASC increased the value close to normal.

Histology of liver, kidney, heart, lung, spleen and brain were performed to observe any changes in the cellular structures (infiltration, inflammation, congestion, degradation and regeneration *etc*.) of the mice receiving the test compound for 14 consecutive days with respect to control group (normal mice). In mice of the treated group, no abnormalities in the histopathology of kidney, spleen, lung and brain were detected in comparisons with control group under microscope. Liver tissues showed very little infiltration with no central vein dilation, fatty generation or nodule formation in normal mice whereas major organs of EAC bearing mice showed significant cellular degeneration/regeneration. The hepatotoxicity generated by EAC cells were partially nullified by the protective effects of ASC treatment (Figure 4).

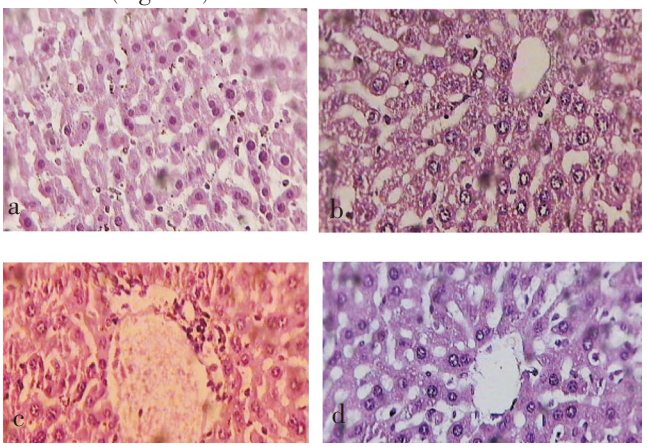

Figure 4. Histopathological examinations of experimental mice. a. Liver tissues from control mice with no abnormality; b. Liver tissue from ASC treated normal mice showed very little infiltration with no central vein dilation, fatty generation or nodule formation; c. Liver tissues from untreated EAC bearing mice necrosis, central vein dilation; d. Liver tissues from EAC bearing ASC treated mice with very little necrosis and no central vein dilation.

Table 2

Effect of ASC on biochemical parameters in experimental mice (mg/dL).

Group	Days	Serum glucose	Serum cholesterol	Serum urea	Serum triglyceride	Serum bilirubin
Normal mice	0	144.3±1.2	141.5±3.5	36.6±1.7	105.5±1.0	1.3±1.9
	5	145.7±2.1	180.0±3.6	22.0±2.4	112.4±2.3	1.5±0.2
Normal+ASC	10	157.3±4.0	217.0±2.5*	24.0±4.2	120.6±1.0	1.4±0.6
	15	161.0±4.4	239.0±3.1*	26.3±2.1	131.0±6.1*	1.7±0.5
	25	132.0±5.1	149.2±4.2	29.0±1.9	96.2±5.4	1.4±1.2
	5	87.0±2.3*	152.0±3.7	64.1±1.8*	188.6±6.2	1.9±0.7
EAC control	10	73.0±3.7**	156.7±2.6	77.0±0.8*	192.0±3.1	2.1±1.2
	15	65.0±2.4**	165.0±3.7	84.1±1.2**	205.0±5.2**	2.7±1.3
	25	54.3±3.0	183.0±3.8**	93.0±3.1***	231.0±3.1***	3.6±0.8**
	5	89.0±4.2	149.4±5.8	59.8±1.1	165.2±3.2*	1.6±0.4
EAC+ASC	10	91.0±2.7	154.0±5.2	47.3±3.7*	177.3±6.3	1.8±1.6
	15	111.0±4.6	160.0±4.1	42.3±1.7	181.8±8.1*	2.0±0.8
	25	113.4±1.7	168.0±4.2*	31.2±1.9	137.2±3.7	2.2±1.1*

Treatment was continued for 14 consecutive days at dose 2 mg/kg (*i.p.*) (number of mice in each day=6). For tumour bearing mice, similar treatment was started 24 h of EAC cell transplantation (2×10^{6} cells/mouse). Treatment was discontinued after 14 d from the start. Results are shown as mean±SEM, where significant values are *: $P<0.05$, **: $P<0.01$; ***: $P<0.001$ when compared with control.

4. Discussion

All the results mentioned before showed that the compound ASC at dose 2.0 mg/(kg·day) for consecutive 14 dson Swiss albino mice have no significant abnormalities in comparison to control. Normal mice receiving the compound did not show any tremor, convulsion, reflex abnormalities or muscular disorder. It shows only insignificant changes of body weight due to normal growth and development of the animals. Whereas the compound all most nullified the physiological abnormalities (tremor, salivation, diarrhea, muscular problem *etc*.) of EAC bearing mice, which is

produced owing to the toxicities of carcinogenesis. As we reported earlier[10], the tumor burden of EAC bearing mice is remarkably reduced by ASC which is ratifying here as we find very limited body weight gain of EAC bearing mice treated with ASC.

Literature survey reveals that progression of tumor was accompanied by the following hematological changes compared to normal gradual decrease in hemoglobin content, erythrocyte count and gradual increase in leukocytes which was observed in control EAC bearing untreated mice[14]. The RBC count was almost restored back to normal range on treatment with ASC which is described earlier[10]. It could also improve the WBC count efficiently. The hemoglobin levels were in the nor-mal range in the ASC treated group. Recovery of the hemoglobin content, RBC and WBC cells count in the experimental mice indicates the protective action of ASC on the hemopoietic system.

It is well known that there are significant elevations in the levels of serum GPT, GOT and ALP in liver diseases and disorders and in hepatocellular damage caused by a number of agents. An increase in these enzyme levels is also observed with cardiac damage due to myocardial infarction and with liver disorders[15]. Biochemical measurements of these parameters in normal mice treated with ASC showed some extent of increase due to little hepatotoxicity during treatment period but they become normal after completion of treatment schedule. The slight host toxic effects observed in mice during treatment time are mostly reversible. This means that, the treatments of the compound do not cause any acute or permanent damage to the liver. But in case of tumor bearing mice, these parameters were found to be increase more drastically with time due to the acute and permanent toxicities induced by EAC cells on host. After treatment with ASC in the EAC bearing mice these values remain near the normal range in the treated group. From this it follows that the damage generated by EAC was prevented by ASC supplementation.

Treatment of normal mice with ASC slightly changed blood glucose, cholesterol, urea, triglyceride and billirubin level which also rectified more or less to normal after treatment. This indicates that after short term treatment the compound did not cause any extreme abnormality at the dose used in this study.

The development of hypoglycaemia and hyperlipidaemia in experimental animals with carcinoma has been previously reported[16-20]. In this experiment, the reduced glucose level and elevated cholesterol, triglycerides and serum urea were returned to more or less normal levels in ASC-treated mice, thereby indicating a potent antitumour efficacy of ASC.

The histopathology studies of major organs also revealed the relatively less toxic nature of ASC as compared to control group when viewed under microscope. The histopathology of kidney tissues of ASC treated mice did not show any cellular and glomerular infiltration, and there is no sign of tubular necrosis, casts and glomerular congestion. Tissues from brain and lung did not shown any cellular degeneration or regeneration in the treated mice and this is why they have no signs of neurotoxicity and pulmonary toxicity. Treated mice also have not any change in the splenic architecture. The histology of liver showed very little infiltration(inflammation) with no central vein dilation, fatty generation or nodule formation and due to this mild hepatotoxicity some biochemical parameters were deteriorate during treatment period which become normal after closing treatment whereas tissues from EAC bearing mice showed major abnormalities and it is interesting that the hepatic damage induced by EAC cells were nullified by ASC supplementation. All these slight host toxic effects observed in normal mice during treatment time are mostly reversible and so treatment with ASC do not cause any acute or permanent damage to the host.

The aim of this study was to determine the hepatoprotective effects and sub-acute toxicity of the compound to find out less host toxic potential anticancer agents and did not attempt to identify the specific mechanism involved. This study revealed some interesting features have been presented here. Almost in all cases the effects of EAC cells on biomolecules have been found to be nullified by such treatment. In most cases antagonistic effects have been found instead of additive effects. Further elevation of glucose levels of EAC bearing mice by the treatment of the compound probably indicates their partial recovery from tumour growth.

As the major organs of the treated mice do not show any histopathological abnormalities, these findings in conjunction with those obtained from the measurement of serum biomolecules definitely give positive support to conclude that ASC is an effective antineoplastic agent with comparatively less toxic effects in our experimental model. However, further chronic toxicological studies and its anti-tumor activity should be carried out against other tumor cell lines which may bring promising results in cancer chemotherapy.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to IICB, Kolkata, India authority for providing the EAC cells and also to ICDDR'B, Dhaka, Bangladesh for kindly supplying swiss albino mice and standard mouse pellets. We are also thankful to the Head, Department of pathology, Rajshahi Medical College, and Dr. Anower Habib, Associate professor, Department of pathology, Rajshahi Medical College, for providing technical support in this study.

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Comments

Background

Cancer continues to represent the second largest cause of mortality in the world and claims over six million lives every year. An extremely promising strategy for treatment of cancer prevention today is chemotherapy. For developing chemo-agents, Schiff bases have been drawn the attention of many investigators owing to their potential applications such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-tubercular, anti-HIV, antileprosy, herbicidal etc activities. In some cases, these compounds have been proved to possess anti-leukemic effects. Schiff bases of semicarbazone and their derivatives also possess potential anticancer activities. Anticancer activity of hydroxysemicarbazide

against L1210 murine leukemia cells showed higher inhibitory effect than hydroxyurea. Some thiosemicarbazones have been shown to have anticancer and antiviral activities. Nickel (II) complexes of semicarbazone derivatives showed potent anticancer activity against MCF-7 cell lines. Semicarbazones, thiosemicarbazones and acetyl-hydrazones of phthalimide, o-benzosulfinide, napthalimide and diphenimide demonstrated potent cytotoxicity against different cancer cell lines. The antitumor activity of pyridine-2-carboxaldehyde thiosemicarbazones and a series of di-2-pyridyl ketone thiosemicarbazone ligands have also been reported in the literature. Thus, Schiff bases are an important and interesting group of chemical compounds and they exhibit a number of biological activities and play an important role in the regulation of many biochemical processes.

Research frontiers

In this study, the host toxic effects of the interested compound acetone semicarbazone has been examined to find out a more convenient and safe anticancer chemopreventive drug. The toxicity induced by the EAC cells in Swiss albino mice was studied particularly on liver by monitoring main candidate biochemical parameters of liver function test. Toxicity of the compound on normal mice has also been reported in this article.

Related reports

The experimental methods and materials used in this study are conventionally applied to measure the protective effects as well as host toxic effects of an emerging drug before going clinically trial. The results are quite similar to other anticancer agents with some interesting exception such as most of the chemoagents are insoluble in water and have profound toxic effects on hemopoitic system and on liver but acetone semicarbazone possess none of these problems.

Innovations and breakthroughs

Most of literature regarding biological activity of Schiff base involve the compounds possess large structures and are mostly insoluble in common solvents. Much difficulty has been encountered to solubilize them for use. Anticancer activity on different cell lines specifically EAC cell have not been done with simple Schiff base like acetone semicarbazone. As acetone semicarbazone is a water soluble compound, we have selected it to evaluate its antineoplastic activity against EAC cells in Swiss albino mice.

Applications

Data obtaining from this experiment is very much interesting and promising. We found that the compound has strong hepatoprotective effects and nullified the toxicity induced by EAC cell in mice. It also found that the compound has no major toxic effects on host. Thus, the compound has been used to develop potential chemo-preventive drug after further research with others cell line and also with higher animal.

Peer review

This piece of work is a good research endeavor, in which the authors evaluated the hepatoprotective and host toxic effects of acetone semicarbazone, a simple Schiff base to find out an effective anticancer drug with better safety profile. The results are interesting and suggested that ASC posses protective effects on liver against the toxicity generated by EAC cell bearing mice.

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