



# Cytotoxic Activity of Sulphonamide with Selenium to Decrease Raised Lipid Profile in the Treatment Hepatocarcinogenesis

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## ABSTRACT

**AIM-** The aim of this study was to investigate the cytotoxic activity of sulfamethoxazole hydrochloride (30mg/kg) with Selenium in the form of sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) (4mg/kg) orally on lipid parameter in the treatment of hepatocarcinogenesis.

**MATERIALS AND METHODS** -Wistar rats were divided into 7 groups and 6 animals in each group: Group 1 control group (normal saline), Group 2 positive control (DEN), Group 3 (positive control for SMX), Group 4 (positive control for sodium selenite), Group 5 (positive control for SMX + Se combination) Group 6 (post SMX + Se) (THR) Group 7 (Pre SMX + Se) (PRH). Single intraperitoneal administration of chemical carcinogen diethylnitrosamine (DEN) (150 mg/ kg) in single dose elevated the levels of total cholesterol, triglycerides and the HDL level was decreased in the DEN-administered animals of group 2 when compared to group 1 animals.

**RESULTS-** The levels of Total cholesterol and Triglycerides were significantly elevated and the serum HDL level was decreased in the DEN-administered animals of group II when compared to group 1 animals. These parameters were maintained to normal by the treatment of (SMX+SE) in the group 6, group 7 and group 5 control groups.

**CONCLUSION-** The study result suggests that at a reduced dose of combination of sulfamethoxazole and sodium selenite at doses 30mg/kg and 4mg/kg can act as chemopreventive agent in DEN induced Hepatocarcinoma.

**Keywords:** Sulfamethoxazole, Sodium selenite, Hepatocarcinogenesis, Lipid profile.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. Worldwide, the incidence of HCC in developing nations is twice than that in developed countries<sup>1</sup>. In 2000, the age-adjusted incidence of HCC in men was 17.43 per 100,000 population in developing countries compared with only 8.7 per 100,000 population in the United States. In high-income countries the number of liver incidence accounts 2.7 % of 285,804 global economic burden<sup>2</sup>. Diethylnitrosamine (DEN) is frequently used to induce hepatocarcinogenesis in experimental animals<sup>3</sup> possibly by causing oxidative stress and cellular injury with enhanced formation of detrimental free radicals. DEN metabolizes to its active ethyl radical, which can interact with DNA causing mutation and subsequent carcinogenesis<sup>4,5</sup>.

Selenium (Se) is an essential dietary component for animals including humans, and there is increasing evidence for the efficacy of certain forms of selenium as cancer chemopreventive compounds. It is shown to affect the functions of intracellular selenoproteins as it is itself a major component of their essential constituent selenocysteine (SeCys). These selenoproteins include glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), which have important antioxidant and detoxification functions<sup>6</sup>. Mechanisms for anticancer action of selenium are not fully understood; however, several have been proposed: antioxidant protection, enhanced carcinogen detoxification, enhanced immune surveillance, modulation of cell proliferation (cell cycle and apoptosis), inhibition of tumor cell invasion, and inhibition of angiogenesis<sup>7,8</sup>.

A large number of novel sulfonamide derivatives have been reported to possess in-vitro and in vivo antitumor activity<sup>9</sup>. Sulfonamides are compounds that bearing amino acid moieties were reported

to possess antitumor activity<sup>10</sup>. They inhibit the synthesis of purine nucleotides and nucleic acids and also affect the proliferation of normally growing cells. Sulfonamides competitively inhibit dihydrofolate reductase and block the formation of THF providing hindrance in the biosynthesis of purines, thymine nucleotides, and hence DNA is impaired resulting in blockade of cell proliferation. Sulfamethoxazole being the drug of sulfonamides acts as a competitive inhibitor of the enzyme dihydropteroate synthase (DHPS) which catalyzes the conversion of para-aminobenzoate (PABA) to dihydropteroate (AHHMD), a precursor of folate synthesis necessary for the synthesis of nucleic acids that are essential as building blocks of RNA or DNA. As a result, it is possible to inhibit the synthesis of nucleic acids and proteins<sup>11</sup>. SM-NHOH is prepared as an intermediate product from sulfamethoxazole after oxidation via CYP P-450. When it is further oxidized, it produces nitroso-compounds, SM-NO which exerts direct cytotoxic effects<sup>12</sup>. Sulfamethoxazole serves as the best representative of the sulfonamide group. Therefore, the present experiment was designed to observe, synergize, and utilize the cytotoxic property of sulfamethoxazole and selenium in combination for the treatment of DENA-induced hepatocarcinogenesis rats.

## MATERIALS AND METHODS

### Drugs and chemicals

Sulfamethoxazole was procured from Asoj pharmaceutical, Ahmadabad and Selenium, DEN were Sigma-Aldrich Chemicals Co. St. Louis, USA. Chloroform and diethyl ether were procured from S.D. Fine Chem. Ltd., Mumbai. Disodium Hydrogen Phosphate was purchased from

Merk Specialities Pvt. Ltd., Mumbai. All the chemicals were of analytical grade.

### Animals

This experiment was carried out on 30 albino wistar rats procured from the animal house facility of Siddhartha Institute of Pharmacy for these experiments. Animals were caged in group of five under-controlled conditions of temperature (2°C + 3°C) and light (14:10 h light and dark cycle) and provided balanced pellet diet and water ad libitum. The Protocol was approved from the institutional animal Ethical committee (IEAC) (Reg. no. SIP/IAEC/17/2011) for usage of animal in the experiment was obtained as per 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 and taken for conducting research studies.

### Experimental Design

After 1-week acclimatization adult, healthy, experimental Wistar albino rats were assigned randomly into five groups ( $n = 6$ ):

#### Group I rats (NC)

Served as control normal and were treated with saline orally [NC].

#### Group II rats (DEN)

Were administered a single dose of DEN (150 mg/kg body weight, i.p.) to induce hepato cellular carcinogenesis (HCC) and served as disease control.

#### Group III rats (SMX)

Were administered sulfamethoxazole (SMX=30 mg/kg orally) and served as sulfamethoxazole control group.

#### Group IV rats (Se)

Were administered Sodium selenite (Se= 4 mg/kg orally) and served as selenium control group.

#### Group V rats (SMX+Se)

Served as SMX + Se control group.

#### Group VI rats (THR)

Served as therapeutic group (post SMX+Se) and

#### Group VII rats (PRH)

Treated as prophylactic group (pre SMX+ Se).

### Dose selection of DEN, sulfamethoxazole and sodium selenite for induction and treatment of hepatocarcinogenesis induction

The selections of the doses of DEN, sulphamethoxazole hydrochloride and sodium selenite were as reported respectively by Ghosh A. S.<sup>13</sup>, Lawrence D. Mayer *et al*<sup>14</sup> and Alwahaidi *et al*<sup>8</sup>.

### Sampling and analysis of biochemical parameters

All the rats were withheld diet for 12 h before sampling and humanely sacrificed by cervical decapitation. Conjunctival blood samples were collected before sacrifice under light ether anesthesia, allowed to stand for 30 min at room temperature, and centrifuged at 2500 rpm for 10 min to separate the serum. The serum obtained was kept at 2-4°C for further use. The TC, TG, HDL were obtained using a standard kit (Nicholas India Pvt. Ltd.) with semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd)<sup>15</sup>.

### Statistical analysis

The results were expressed as mean  $\pm$  SEM. Statistical significance among more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparison test or an unpaired two-tailed

student's *t* test as appropriate using computer-based fitness program (Prism, Graphpad). Differences were considered to be statistically significant ( $p < 0.05$ ).

## RESULTS

The effect of SMX+Se on total cholesterol, triglyceride and high density lipoprotein (HDL) was presented in Tables 1.

### Lipid profile

Total cholesterol (TC) and triglycerides (TG) levels were found to be significantly ( $p < 0.01$  and  $p < 0.001$  respectively) increased and the serum high density lipoprotein (HDL) level was found to be significantly ( $p < 0.01$ ) decreased in the animals of group 2 as compared to the animals of group 1. However, the animals of group 6 showed a significant ( $p > 0.001$ ) decrease in total cholesterol levels as compared to those of group 2. Whereas, triglycerides level was significantly ( $p < 0.01$ ) decreased in the animals of group 6 as compared to the animals of group 2. In contrast, HDL level was significantly increased ( $p < 0.001$ ) in the animals of group 6 as compared to the animals of group 2 and no significant changes were observed in the lipid profile parameter in the animals of groups 3 and 7. (Table 1)

## DISCUSSION

In the present research protocol it was observed that SMX+Se (30mg/kg + 4mg/kg) improved the levels of total cholesterol, triglycerides. These findings suggest that SMX+Se can be used as a potential chemotherapeutic agent in the treatment of HCC. Researchers worked on the cancers and their findings suggest that the levels of total cholesterol increases in the rapidly multiplying cells<sup>16,17</sup>.

The liver plays a major role in cholesterol metabolism in mammals. During

tumour growth, the animals progressively developed marked changes in the liver and distribution of total cholesterol<sup>18</sup>. Similar elevation in cholesterol levels was reported in hepatoma cells in N-nitrosodiethylamine<sup>19</sup> and in aflatoxin induced hepatocarcinoma<sup>20</sup>. The increased total cholesterol level in the animals of group 2 may be due to decreased uptake of cholesterol from blood. Cholesterol metabolism in the body is regulated by continuous exchange of cholesterol between tissues and blood. Blood supply to hepatoma is decreased and hence 80% decrease in uptake of blood born substances occurred in hepatoma conditions<sup>21</sup>. Deregulated cholestrogenesis observed in tumours, implicated an over production, which could result in the enrichment of tumour cell membranes with cholesterol. This may be capable of inducing cell population into enormously enhanced proliferative activity<sup>22</sup>.

The decreased cholesterol content to normal in the treated groups (group 6) can be due to hypocholesteromic activity of selenium are reported by earlier studies<sup>23,24</sup>. These observations clearly indicate the effect of selenium in correcting the abnormalities of lipid metabolism in tumor-induced rats.

A significant increase ( $p < 0.001$ ) in TG was also observed in the animals of group 2 and this may be due to the peroxidation of unsaturated membrane lipids by free radicals<sup>23</sup>. In the hepatocellular carcinomic rats treatment with SMX+Se combination caused a significant ( $p < 0.001$ ) reduction in TG levels. Triglycerides are probably metabolized by lipoprotein lipase (LPL) and the reaction products, free fatty acid (FFA) and glycerol may then be translocated into the liver readily crossing the liver cell membrane<sup>25</sup>. Increased fatty acid mobilization from peripheral adipose tissues and decreased triglycerides clearance from blood circulation are considered as causes for the hepatoma induced hypertriglyceridemia<sup>26</sup>. Hypertriglyceridemia which is frequently observed in

various degrees in tumour bearing animals in combination with increased VLDL and decreased HDL, which is very suggestive of a defective catabolism rather than elevated hepatic synthesis of triglycerols rich lipoproteins<sup>27</sup>. Triglyceride levels were found to be greatly reduced in the animals of group 6 as compared to the animals of group 1.

HDL is considered to be a beneficial lipoprotein<sup>28</sup>. It helps in the scavenging of cholesterol from the extra hepatic tissues in the presence of lecithin cholesterol acyl transferase and brings it to the liver<sup>29</sup>. Decreased levels ( $p < 0.001$ ) of serum HDL was seen in the rats of group 2 as compared to the control rats. The lowered HDL levels can be attributed to the decreased serum lipoprotein lipase and lecithin cholesterol acyl transferase activity. It has been observed from our study that serum HDL level was significantly ( $p < 0.001$ ) similar to what was observed for the animals of normal control. This may be due to selenium supplementation, it has also been reported that selenium supplementation protect LDL from oxidation and other atherogenic changes<sup>30-32</sup>.

## CONCLUSION

Results from the study suggest that selenium and sulfamethoxazole together can possess synergistic chemopreventive action. Combination of SMX+Se suppresses the tumor lesions and decreases the biochemical markers which are elevated in HCCs. The present investigation will open new perspectives that selenium with sulfonamide are chemopreventive compounds to prevent, slow, or treat the occurrence of liver cancer due to its antioxidant, apoptotic activity, and by providing hindrance to the formation of essential nucleotides for the formation of new cells. Moreover, further exploration of the combination is required to be done by the researchers in terms of toxicity and adverse effect.

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**Table 1.** Effect of combination of sulfamethoxazole and selenium on serum lipid profile

| S.No. | Group   | TC (mg/dl)        | TG (mg/dl)     | HDL (mg/dl)     |
|-------|---------|-------------------|----------------|-----------------|
| 1     | NC      | 91.80 ± 1.655     | 82.50 ± 6.96   | 24.76 ± 4.26    |
| 2     | DEN     | 147.40 ± 2.87 ### | 176.4±3.98 ### | 15.09 ±0.38 ##  |
| 3     | SMX     | 96.243 ± 2.11     | 70.23 ± 1.23   | 22.34± 3.21     |
| 4     | Se      | 81.40 ± 2.48      | 82.76 ± 1.83   | 24.72 ± 4.26### |
| 5     | SMX +Se | 95.6 ± 5.701**    | 76.60 ±1.28**  | 25.05 ± 1.08*   |
| 6     | THR     | 102.40 ± 4.567*** | 85.75±1.06***  | 25.54 ± 4.33*** |
| 7     | PRH     | 107.4 ± 2.441     | 92.4 ± 3.12    | 30.22 ± 2.08    |

Values are expressed as Mean ± SEM ( $N = 6$ ), one-way ANOVA followed by the Bonferroni multiple comparison test.

Significance level: ##  $p < 0.01$ , ###  $p < 0.001$  as compared to normal control of animals.

Significance level: \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  as compared to disease control of animals.