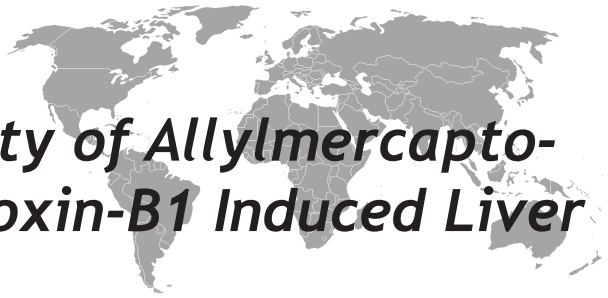

# *Anticarcinogenic Activity of Allylmercapto-captopril Against Aflatoxin-B1 Induced Liver Carcinoma in Rats*

Shrinivas Sharma, Lakshmi KS, V. Chitra, I. Aparna Lakshmi, M. Pharm

*SRM University, SRM College of Pharmacy, Department of Pharmacology, Kattankulathur, Kanchipuram District-603203. Tamilnadu, India.*

*Eur J Gen Med 2011;8(1):46-52*

*Received: 13.05.2010*

*Accepted: 15.06.2010*

**ABSTRACT**

***Aim:*** *The present study was aimed to investigate the cellular and molecular mechanisms of protective effects of allylmercaptocaptopril (AMC) against liver carcinoma induced by Aflatoxin B1, a potent inducer of liver cancer.*

***Method:*** *In this study we determined the protective effect of AMC on liver tissue, as well as on enzymatic liver functions by estimating glycolytic enzymes like hexokinase, phosphoisomerase and aldolase, gluconeogenic enzymes like glucose-6-phosphatase and fructose 1,6 biphosphatase. Determination of total protein, DNA and RNA content also made to elucidate its action.*

***Result:*** *Aflatoxin B1 treatment to rats resulted in significantly elevated levels of glycolytic enzymes like hexokinase, phosphoglucoisomerase and aldolase and along with significant decrease in serum total protein, gluconeogenic enzymes and DNA and RNA content when compared to the control rats. The administration of AMC to the hepatocellular carcinoma bearing rats resulted in restoration of most of enzymatic liver functions and also total protein content, DNA and RNA content.*

***Conclusion:*** *Allylmercaptocaptopril has an ability to modulate the function of glycolytic and gluconeogenic enzymes, DNA and RNA synthesis in hepatocellular carcinoma which proved its anticarcinogenic activity.*

***Key words:*** *Hepatocellular carcinoma, aflatoxin B1, allylmercaptocaptopril, glycolytic enzymes, gluconeogenic enzymes*

***Correspondence:*** *Shrinivas Sharma*
*Department of Pharmacology,*
*SRM College of Pharmacy, SRM University,*
*Tel: 919381446030, Fax: 914427455717*
*E-mail: shrinivas.sharma29@gmail.com*

***Ratlarda Aflatoksin-B1'in Oluşturduğu Karaciğer Kanserinde Aliyilmerkaptokaptopril'in Antikanserojen Etkisi***

***Amaç:*** *Bu çalışma güçlü bir karaciğer kanser nedeni olan aflatoksin B1 tarafından oluşturulan karaciğer kanserine karşı aliyilmerkaptokaptopril'inin (AMC) koruyucu etkisinin hücresel ve moleküler mekanizmasını araştırmak amacıyla yapıldı.*

***Metod:*** *Bu çalışmada, biz heksokinaz, fosfoizomeraz ve aldolaz gibi glikolitik enzimlerin ve glukoz-6-fosfataz ve fruktoz 1,6 bifosfataz gibi glukoneogenik enzimlerin saptanması ile enzimatik karaciğer fonksiyonlar ile birlikte karaciğerde AMC'nin koruyucu etkisini saptamaktır. Total protein, DNA ve RNA içeriğinin saptanması etkisini açığa çıkarmak için yapıldı.*

***Bulgular:*** *Ratlarda aflatoksin B1 tedavisi control grubuyla karşılaştırıldığında belirgin olarak heksokinaz, fosfoglukoizomeraz ve aldolaz gibi glikolitik enzimlerin düzeylerinde artma ve serum total protein, glukoneogenik enzimler, DNA ve RNA içeriğinde belirgin azalma ile ile sonuçlanmaktadır. AMC'nin uygulanması enzimatik karaciğer fonksiyonları ve total protein, DNA ve RNA içeriğinde yenileme ile sonuçlandı.*

***Sonuç:*** *AMC hepatoselüler kanserde glikolitik ve glukoneogenik enzimler, DNA ve RNA sentez fonksiyonlarını modüle etme yeteneğine sahip olduğu gösterilmiştir.*

***Anahtar kelimeler:*** *Hepatoselüler kanser, aflatoksin B1, allylmerkaptokaptopril, glycolytic enzimler, glukoneogenik enzimler*

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant neoplasm of hepatocytes and constitutes more than 80% of primary malignant liver neoplasms in the world (1). Given that the burden of chronic liver disease is expected to rise owing to increasing rates of alcoholism, hepatitis B and C prevalence and obesity-related fatty liver disease and chemicals like aflatoxin, CCl4, nitrosodiethylamine, etc (2). Cancer of the liver is the sixth most common type of cancer worldwide, with 625,000 cases recorded. Globally, liver cancer accounts for 5.6% of all cancers in humans with more cases diagnosed in males (where it accounts for 7.5% of all cancers) than females (3.5% of all cancers) (3).

Aflatoxin is a toxin produced by Aspergillus flavus and A. Parasiticus. The different types of aflatoxins produced were designated as follows due to their blue and yellow-green autofluorscence B1, B2, and G1, G2. Aflatoxin B1 is the most potent. The International Cancer Institute identified Aflatoxin B1 as a class I carcinogen. Aflatoxin B1 is metabolized by Cytochrome p450 into exo-8, 9 epoxide. Aflatoxin B1 epoxide forms adducts with DNA predisposing to DNA mutations. The favourite site for Aflatoxin induced adducts is a hotspot in the p53 gene. P53 is a tumour suppressor gene (4). Presumably as a result of the sulfhydryl group in its molecular structure, Captopril is a potent free radical scavenger and this action is responsible for its clinical use in the management of systemic hypertension and congestive heart failure, pulmonary hypertension, diabetic renovascular disease, rheumatoid arthritis and its anticarcinogenic activity has also been documented (5).

The antitumoral effects of garlic (Allium sativum) have been recorded since very early times. In addition, experimental studies have demonstrated the ability of garlic to reduce chemical carcinogenesis in different animal models (6). The present study was sought to investigate the advantages of both molecules, captopril and allicin, which operate by different mechanisms. The covalently bonded reactive product of captopril and allicin leads to allylmercaptocaptopril (AMC), a nonsymmetric disulfide.

## MATERIALS AND METHODS

Captopril was kindly provided by WOCKHARD Ltd, Aurangabad, Maharashtra, India. Aflatoxin B1, diallyl sulfide was provided by Sigma chemical company, St. Louis, Missouri. All other chemicals and AR grade solvents were procured from SRL, Mumbai, India.

### *Synthesis of Allylmercaptocaptopril*

Allylmercaptocaptopril was synthesized by previous reported method by Miron et al (8), with some major modifications. First, Allicin (diallyl disulfide oxide, Fig. 1) was synthesized by a modified method of Stoll and Seebeck (7). Allylmercaptocaptopril (AMC) was synthesized by the addition of captopril solution (1 mmol in 7.5 ml water, pH 5.5) to an aqueous solution of 0.55 mmol allicin. The reaction was monitored by HPLC analysis until captopril was no longer detected. The reaction mixture was then acidified by hydrochloric acid and extracted by ether to remove non-reacted allicin. The water phase was extracted with ethyl acetate. The organic phase was dried by Na2SO4, filtered and evaporated, re-dissolved in ethanol, and dried by speed vacuum concentrator. The reaction product was analyzed by HPLC and its structure confirmed by IR,

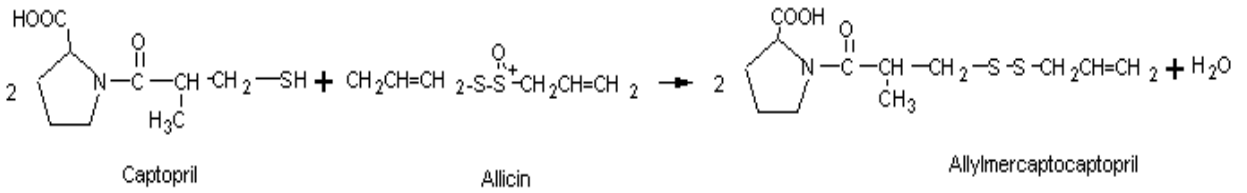


***Figure 1**. Schematic illustration of the chemical reaction between captopril and allicin that forms allylmercaptocaptopril.*

NMR and mass spectrometry. IR (KBr) spectra were recorded on a Perklin Elmer FTIR spectrometer (vmax in cm-1) and 1H NMR spectra were recorded in CDCl3 on a Bruker 500 MHz Avance spectrometer using TMS as internal reference (Chemical shift in δ ppm). Mass spectra were recorded using Agilant HP5937 spectrometer. The purity of synthesized compound was checked by HPLC. The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-visible detector. The data acquisition was performed by Spincotech 1.7 software. The system was equipped with reverse phase column Gemini C18 (150 mm × 4.6 mm i.d., 5 µm) (Phenomenex, Torrance, USA). The mobile consisted of 60% methanol in water containing 0.1% formic acid at a flow rate of 0.8 mL/min. The retention time was found to be 11.6 min. Spectral data (1H NMR, IR and mass) of the synthesized compound was in full agreement with the proposed structure. IR (KBr) v cm-1: 3459 (OH), 2984 (C-H), 1728 (C=O), 939 (S=S). 1H NMR (DMSO) δ (ppm): 10.02 (s, 1H, OH), 5.02 (t, 2H, SCH2), 1.24 (m, 3H, CH3). MS: m/z 289 (M+). The reaction between captopril and allicin to form allylmercaptocaptopril is illustrated in Figure 1.

### *Pharmacological studies*

### *Grouping of animals*

Male albino rats of Wistar strain (weighing 170 - 200 g) were used for the study. Rats used for the study were obtained from the Central Animal House facility of SRM University, Kattankulathur, Chennai, India. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" (IAEC) (Proposal. No. IAEC 67/2009) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules and were in accordance with the guidelines of IAEC (Reg. No.662/02/C/CPCSEA). Animals were allowed to free access to water and standard chow diet up to the end of the experimental period and divided into following groups. Each group consists of six rats. Group I: Normal group of rats received a single intraperitoneal (i.p) dose of dimethyl Sulphoxide (DMSO; 0.5 ml). Group II: Control group of rats bearing hepatocellular carcinoma was induced by single dose of AFB1 in DMSO (2 mg/kg, i.p). Group III: Test group of rats bearing hepatocellular carcinoma induced as in Group II along with allylmercaptocaptopril (AMC). AMC was dissolved in saline (20 mg/ml saline) and administered at a dose of 50 mg/kg/day i.p started before 7 days and continued for 14 days. Group IV: drug control animals received the same dosage of allylmercaptocaptopril (50mg/kg/i.p) as group III animals.

### *Preparation of liver homogenate*

At the end of the experimental period on the 15th day, the animals that were fasted over night were killed by cervical decapitation. Blood was collected in heparinized tube. Plasma was separated by centrifugation at 3000 rpm for 20 min. Liver tissue was immediately excised from the animals, weighed and carcinomatous tissue was carefully dissected free of haemorrhagic, necrotic and non-tumour material. Ten percent homogenate was prepared in 0.1M, Tris HCI buffer (pH 7.4) using a Potter Elvehjem homogenizer.

### *Biochemical analysis*

The protein content (9) and glycolytic enzymes like hexokinase (10), phosphoglucoisomerase (11), aldolase (12) and gluconeogenic enzymes like glucose-6phospha-

***Table 1**. Effect of AMC on DNA, RNA and protein content in liver cancer induced rats*

| | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| DNA, mg/g | 6.06±0.24* | 8.71±0.31 | 5.76±0.17** | 6.23±0.38$^{ns}$ |
| RNA, mg/g | 3.83±0.16* | 5.13±0.52 | 4.02±0.47* | 3.93±0.24$^{ns}$ |
| Protein, mg/g | 158.46±9.83* | 89.33±8.47 | 150.37±9.01* | 153.52±9.27$^{ns}$ |

*Values are expressed as Mean ± S.D; n=5. Statistical significance: (*p<0.01 and NS-Not Significant). One way ANOVA followed by Tukey test.*

tase (13-14) and fructose-1,6-biphosphatase (15) were assayed. The nucleic acids from the tissues were extracted by the method of Schneider with trichloroacetic acid. RNA and DNA were estimated by the method of Burton and Rawal et al respectively (16-17).

### *Statistical analysis*

All data were expressed as mean±SD. The groups were compared using one-way ANOVA followed by Turkey multiple comparison test.

## RESULTS

### *Biochemical changes*

The variations of DNA, RNA and protein content in different groups of animals were depicted in table 1. An increase in the level of DNA and RNA was observed in case of group II animals in which hepatocellular carcinoma was untreated. In group III, the levels of both DNA and RNA were decreased to near normal levels in which the animals were treated with AMC (50mg/kg). In group IV there was no significant change in the DNA and RNA levels when compared to normal group. In contrast there was a steep fall in the protein content in group II animals when compared to group I. But in group III, which consists of drug treated animals the protein content was restored to its normal levels.

In group IV there is no observable change in protein content.

Table 2 and 3 shows the activities of glycolytic and gluconeogenic enzymes in different group of animals. There was an increase in the activity of hexokinase, phosphoglucoisomerase and aldolase in liver homogenate in group II animals when compared to normal group of rats. The drug treated animals has shown a decrease in the activities of glycolytic enzymes when compared to group II and were nearer to normal group, due to the drug treatment in group III. While in group IV animals there was no significant change in activity of glycolytic enzymes.

On the other hand there was a fall in the activity of gluconeogenic enzymes in group II animals when compared to normal group. But in case of drug treated animals group III these levels were elevated when compared to cancer induced rats and were approximately parallel to the normal group of rats. Thus, the deviation in the enzyme activities in cancer induced rats were counteracted by the drug treatment and were almost similar to the normal group of animals. In group IV the values were approximately nearer to normal group and there is no significant change.

## DISCUSSION

It is commonly observed that cancer cells possess an abnormal pattern of energy metabolism when compared to normal cells. Studies on experimental rat hepatomas have shown that metabolic alterations occurring in the

***Table 2**. Effect of AMC on glycolytic enzymes in Aflatoxin B1 induced liver carcinoma in rats*

| Parameters | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Hexokinase | 9.93±0.48* | 15.03±1.53 | 10.56±0.84* | 10.02±0.57$^{ns}$ |
| Phospho-glucoisomerase | 12.47±0.34* | 16.39±1.87 | 11.18±0.81* | 11.97±0.86$^{ns}$ |
| Aldolase | 11.56±0.29* | 14.80±1.81 | 10.96±0.53* | 11.01±0.72$^{ns}$ |

*Values are expressed as Mean ± S.D; n=6. Statistical significance: (*p<0.01, and NS-Not Significant). One way ANOVA followed by Turkey test. Units are expressed as follows: Hexokinase- Nanomoles of glucose-6-phosphatase liberated; Phosphoglucoisomerase - Nanomoles of fructose liberated; Aldolase - Nanomoles of glyceraldehyde liberated.*

***Table 3**. Effect of AMC on gluconeogenic enzymes in Aflatoxin B1 induced liver carcinoma in rats*

| *Parameters* | *Group I* | *Group II* | *Group III* | *Group IV* |
|---|---|---|---|---|
| *Glucose-6- phosphatase* | *31.83±1.60** | *19.89±1.93* | *26.44±1.53** | *29.13±0.83NS* |
| *Fructose 1,6- biphosphatase* | *23.05±1.02** | *18.76±2.01* | *21.08±1.21** | *22.13±0.97NS* |

*Values are expressed as Mean ± S.D; n=6. Statistical significance: (*p<0.01, and NS-Not Significant). One way ANOVA followed by Tukey test. Units are expressed as follows: Glucose-6-phosphatase and fructose- 1,6-biphosphatase - Nanomoles of inorganic phosphorus liberated per min per mg protein.*

tumours are often accompanied by the changes in the activities of various enzymes, including key enzymes of carbohydrate metabolism (18). The present study depicts that there was an increase in the activities of glycolytic enzymes i.e. hexokinase, phosphoglucoisomerase and aldolase in cancer induced rats. This infers that the rate of glycolysis was enhanced during tumour growth (19). There exists a direct correlation between glycolytic activity and hexokinase in a variety of tumour cell lines (20).

The function of hexokinase is to metabolize glucose to glucose-6-phosphatase. It is present in liver and plays an important role in phosphorylation of glucose in glycolysis, glucose transport. Hence, the fast growing cells like tumours catabolise large amounts of glucose and its metabolic pathway is activated more in the direction of hexose monophosphate shunt rather than gluconeogenesis (21). The levels of hexokinase in carcinoma bearing animals group II greatly exceed than normal group of rats group I. Phosphoglucoisomerase is helpful for the conversion of glucose-6-phosphate to fructose-6-biphosphate and it regulates the growth of several types of cells. This is an indicator of metastatic growth and increases specifically after metastasis. This may be one of the reasons for its elevated levels in the liver (22). The phosphoglucoisomerase activity was found to be more in group II hepatocarcinoma induced animals when compared to group I. The function of aldolase is to convert fructose-1,6-biphosphate to glyceraldehyde-3-phosphate and is an important step in glycolysis. Since glycolysis is most favourable pathway that promotes the invasion and metastasis of tumour cells. Aldolase levels were elevated in cancer induced animals (group II) but the extent of increase is less when compared to other enzymes (23).

Gluconeogenesis takes place in liver, this reveals that any disease that affect liver also alters the levels of gluconeogenic enzymes (24). Glucose-6-phosphatase hydrolyses glucose-6-phosphate before being liberated as glucose into the circulation. There will be a decreased rate of glucose-6-phosphate mediated dephosphortylation in malignant cells (25). The result also depicts that there is a marked fall in glucose-6-phosphatase levels in diseased rats (group II) when compared to normal.

Fructose-1,6-biphosphatase is an gluconeogenic enzyme and is helpful for the synthesis of smaller sub-

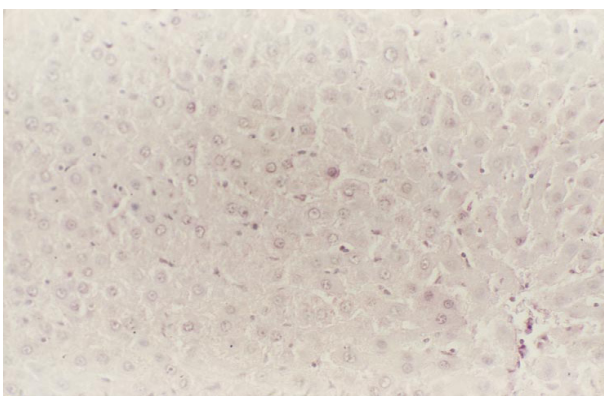

***Figure 2**. Liver from control rats. Haematoxylin and eosin (400x)*

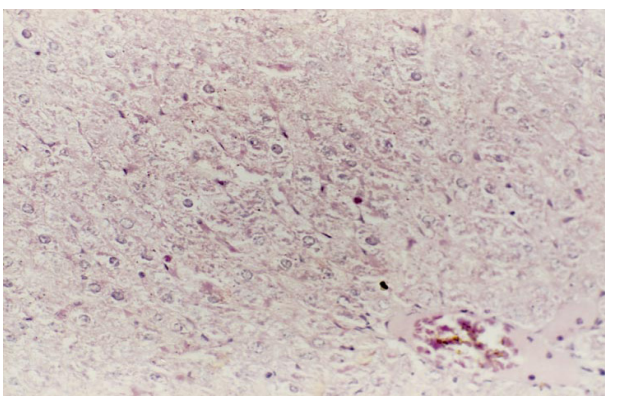

***Figure 3**. Liver from Group II HC-bearing rats. Haematoxylin and eosin (400x)*

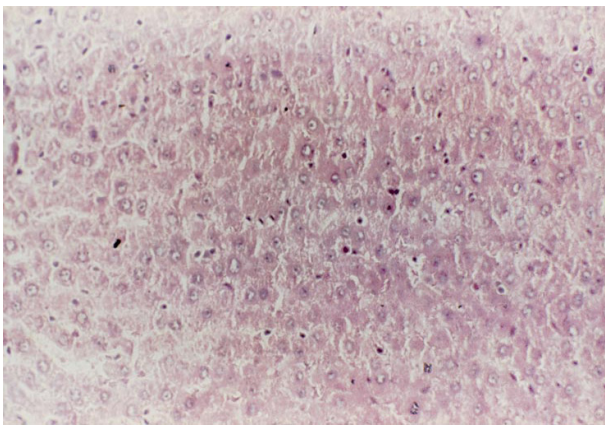

***Figure 4**. Liver from Group III drug-treated rats. Haematoxylin and eosin (400x).*

strates from which glucose is synthesized. It synthesizes glucose-6-phosphate from pyruvic acid. Fructose-1,6-biphosphatase levels were found to be decreased in hepatoma induced rats (group II) when compared to normal. Fructose-1,6-biphosphatase levels were found to be decreased during the tumour growth which may block the synthesis of glucose (26).

The DNA content directly relates to the size of the tumour and is an indicator of tumour prognosis (27). There is an increase in the RNA content in tumour bearing animals (group II), but not as significant as DNA. Increase in RNA content may be due to increased transcription. The decreased protein content in cancer induced animals (group II) may be due to decreased RNA polymerase synthase and also due to increase in protein degradation rate and implies the underlying metabolic imbalance. The total protein content was recovered in the group III drug treated animals. Moreover, the histological studies reveals that, the liver sections of group II aflatoxin B1 induced rats (Figure 2) shows marked congestion of central vein and intense cytoplasmic granules in hepatocytes this was found to be due to nuclear segregration (28) . Alteration in the synthesis of ribosomal precursors by various agents which bind to DNA and RNA synthesis and protein synthesis may also be partly responsible for ultrastructural changes observed.

The liver section of group III drug treated rats shows almost normal architecture as depicted in Figure 3 which is due to the anticarcinogenic activity of the AMC, there is hyperplasia of parenchymal cells in group II animals and contributes to the development of hepatocellular carcinoma in two ways. Firstly by cell proliferation and secondly by carcinogen altered hepatocytes appear better to survive in presence of hepatotoxin (29). The liver section of Figure 3 can be compared from Figure 2 which have normal architecture without any signs of carcinoma and Figure 4 which was from drug treated rats shows almost similar structure to Figure 2. In group IV animals there is no significant change in the glycolytic enzymes, gluconeogenic enzymes, DNA and RNA content and protein content. This suggests that the AMC exerts anticarcinogenic activity without causing any alterations in biochemical pathways in normal condition.

From the above experimental results it may be concluded that the allylmercaptocaptopril has an ability to modulate the function of glycolytic and gluconeogenic enzymes, DNA and RNA synthesis in hepatocellular carcinoma with its anticarcinogenic activity. It has a definite beneficial role against aflatoxin B1 induced hepatocellular carcinoma.

*ACKNOWLEDGEMENT*

*The authors are grateful to Prof. Dr. K. Ilango, Vice Principal, SRM College of Pharmacy for providing necessary facilities and invaluable support to carry out this work. One of the author (SS) acknowledges the research fellowship given by SRM University, Kattankulathur, India.*

***REFERENCES***

1. *Path FRC, Ali Abdel S. An update on the pathogenesis and pathology of Hepatocellular Carcinoma. Bah Med Bull 2007;29:1-8.*
2. *Asmaa IG, Shahid AK, Mireille BT, Imam W, Simon D, Robinson T. Hepatocellular carcinoma: Epidemiology, risk factors and pathogenesis. World J of Gastroenterol 2008;14:4300-8.*
3. *Noore A, Monica R, Deborah B. Epidemiology of primary liver cancer. Cancer Forum 2009;33:1-5.*
4. *Daniel MK, Daniel ZV, Misael U, Nahum MS. Hepatocellular carcinoma: An overview. Annal Hep 2006;5:16-24.*
5. *William FW, Agostino M. Captopril as a chemopreventive agent. Free Patent Online 1993; Pt no.5192524.*
6. *Amitai E, Mirelman D, Peleg E, Wilchek M, Miron T, Rabinkov A, MorOron H, Rosenthal T. The Effects of Allicin on Weight in Fructose-Induced Hyperinsulinemic, Hyperlipidemic, Hypertensive Rats. American J Hyper 2003;16:1053-6.*
7. *Miron T, Rabinkov A, Peleg E, et al. Allylmercaptocaptopril: a new antihypertensive drug. Am J Hypertens 2004; 17: 71-3.*

8. Stoll A, Seebeck E. Die synthesis des naturlichen Aliins und senier drei optish aktiven Isomerasen. Hel Chem Acta 1948;31:189-210.

9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagents. J Biol Chem 1951;193:265-75.

10. Darrow RA, Colowick SP. Methods Enzymol 1962; 226-7.

11. Horrocks JE, Ward J, King JA. A routine method for the determination of phosphoglucoisomerase activity in body fluid. J Clin Pathol 1963;16: 248-52.

12. Bergmeyer HU. Methods of Enzymatic Analysis, Second Edition, 1974, Volume I, 430

13. Taussky HH, Shorr EJ. Biol Chem 1953; 202:675-85.

14. Nordlie, RC, Arion WJ. Methods Enzymology 1966;9:619-25.

15. Gancedo JM, Gancedo C. Fructose 1,6-disphosphatase, phospho fructokinase and glucose-6-phosphate dehydrogenase from fermenting and non-fermenting yeasts. Arch Microbiol 1971;76:132-8.

16. Kamali M, Manhouri H. A Modified Orcinol Reaction for RNA Determination. Clin Chem 1969;15:390-2.

17. Burton K. A Study of the Conditions and Mechanism of the Diphenylamine Reaction for the Colorimetric Estimation of Deoxyribonucleic Acid. Biochem J 1956;62:315-23.

18. Bannasch P, Mayer D, Hacker HJ. Hepatocellular glycogenesis and hepatocarcinogenesis. Biochim Biophys Acta 1980;605:217-45.

19. Hertzfeld A, Greengard O. Enzyme activities in human fetal and neoplastic tissues. Cancer 1980;46:2047-54.

20. Parry DM, Pedersen PL. Intracellular localization and properties of particulate hexokinase in the Novikoff ascites tumour. J Biol Chem 1983;258:10904-12.

21. Arora KK, Pedersen PL. Functional significance of mitochondrial bound hexokinase in tumour cell metabolism. J Biol Chem 1988;263:17422-28.

22. Campbell DM, King, EJ. Serum phosphatases and glycolytic enzymes in cancer of the breast. Biochem J 1962; 82:23-8.

23. Hennipman A, Van Oirschot BA, Smits J, Rijksen G,Stacil GEJ. Heterogeneity of glycolytic enzyme activity and isozyme composition of pyruvate kinase in breast cancer. Tumour Biol 1988;9:178-88.

24. Quistorff, B. Gluconeogenesis in periportal and perivenous hepatocytes of rat liver, isolated by a new high-yield digitonin/ collagenase perfusion technique. Biochem J 1985;229:221-6.

25. Graham MM, Spence AM, Muzi M, Abbot GL. Deoxyglucose kinetics in rat brain tumour. J CerebBlood Flow Metab 1989;9:315-22.

26. Weber G, Cantero A. Comparison of carbohydrate metabolism in normal and neoplastic liver. EnzymeStudies. Acta Unio Internat Contra Cancrum 1960;16:1002.

27. Ellis EN, Burnette JJ, Sedlack R, Dyas C, Blackemore WSA. Surgery 1991;173:329.

28. Lancaster MC. Comparative Aspects of Aflatoxin-induced Hepatic Tumors. Cancer Research 1968; 28:2288-92.

29. Roebuck BD, Maxuitenko YY. In: Eaton DL, Groopman JD, editors. Toxicology of aflatoxins. San Diego: Academic Press, 1994:27-43.