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EFFECT OF NUTRIENTS ON LIVER MARKER ENZYMES OF WISTAR RATS

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Key words:

Glucosinolates, Myrosinase, *Brassica oleracea*, thiocyanates, anticarcinogenic

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ABSTRACT: A wide range of compounds are found to be effective against cancer for example Vitamin D_3, lysine, proline, gallate, quercetin, etc. Glucosinolates from cabbage are secondary metabolites hydrolyzed by the enzyme myrosinase [1]. Their breakdown products such as isothiocyanates, nitriles and thiocyanates, are chemically very reactive, and they are known for their bioactive characteristics, such as anticarcinogenic, fungicidal and bactericidal properties [2]. The objective of this study was to formulate a nutrient medium containing glucosinolates from cabbage, Vitamin D_3, proline and selenium and study its effect on the SGOT, SGPT and ALP levels. To see the synergistic effect of these nutrients, along with glucosinolates from cabbage two nutrient medium were formulated, NM1 (100 mg/kg BW of rats of *Brassica oleracea* extract+ 25 mg/kg BW of Vitamin D_3+ 4 µg/kg BW selenium+ 500 mg/kg BW of proline) and NM2 (200 mg/kg BW of rats of *Brassica oleracea* extract+ 50 mg/kg BW of Vitamin D_3+ 6 µg/kg BW selenium+ 1000 mg/kg BW of proline) [3]. These NM were administered to Wistar albino rats, divided into three groups. Each group housed 5 rats (2 males and 3 females). The effect of administration of NM1 and NM2, to above mentioned rats, once daily for 14 days on the liver markers was investigated. On 15th day, the rats were sacrificed, liver was surgically removed. Liver sections were analyzed histopathologically. Decrease in SGPT and SGOT levels as compared to normal control was observed. Histology of liver tissue, also corroborated with the biochemical analysis.

INTRODUCTION: Nutrients are compounds which are required in small quantity by the body but they are essential for various processes. There are various nutrients which plays a important role in various mechanisms of keeping the body healthy. Deficiency of nutrients causes various diseases. In the present study, effect of combinations of glucosinolates from cabbage extract, proline, selenium and vitamin D3 are studied on liver enzymes. The above mentioned nutrients are individually reported to have anticancer activity [1, 2, 3].

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Role of individual component of above mentioned nutrient medium is as follows:

Glucosinolates: Glucosinolates are alkyl-N-hydroximine sulphate esters with a β-D thoiglucopyranoside group attached to the hydroximine carbon in Z configuration relative to the sulphate group [4]. These are secondary metabolites, and are hydrolyzed by the enzyme myrosinase (thioglucosidase). The hydrolysis products of glucosinolates includes Indole-3-carbinol (I3C), di-indolylmethane (DIM) which possess various properties i.e. antibacterial antifungal and anticancer [3]. They act by various mechanisms to prevent cancer. Sulphoraphane increases activity of phase II enzymes, where as I3C increase Phase I isozymes and arrest cancer cells in G1 phase of cell cycle [5]. DIM inhibits the

invasion of cancer cells and tumor neo-angiogenesis [6].

Proline:

Proline is an amino acid. It can be derived from diet and biosynthesized endogenously. An important source of proline in the microenvironment is from degradation of extracellular matrix by matrix metalloproteinases [7]. It is metabolized by its own specialized enzyme, proline oxidase. It is a p53-induced gene and its overexpression can initiate proline-dependent apoptosis by both intrinsic and extrinsic pathways[7].

Selenium:

Selenium is an essential mineral. A small amount (55µg/day) of selenium is needed by the human body. Selenium as a nutrient may help prevent the development and progression of cancer, by activating antioxidant enzyme in the body [8]. Antioxidants are compounds that block the action of free radicals -- activated oxygen molecules that can damage cells. Selenium supplements can be toxic If the levels go beyond 400µg/day [8].

Vitamin D3 (Cholecalciferol):

Vitamin D3 is the naturally occurring form of vitamin D with cholesterol as precursor. It mediates intestinal calcium absorption, bone metabolism, and muscle. It is also reported to exhibit antiproliferative and proapoptotic activity in breast cancer cell lines, and can reduce the development of mammary tumors in carcinogen-exposed rats [(1)]. The metabolite of Vitamin D, 1α, 25-dihydroxyvitamin D3 (also known as calcitriol), is a bioactive compound possessing hormonal activity[9].

Methodology:

Preparation of Nutrient Medium:

To investigate the synergistic effect of these nutrients, along with glucosinolates from cabbage two nutrient mediums (NM) were formulated according to the recommended dose of each constituent [8]. NM1 (100 mg/kg BW of rats of *Brassica oleracea* extract (hot soxhlet extract, extracted with 80% methanol) + 25 mg/kg BW of Vitamin D3+ 4 µg/kg BW selenium+ 500 mg/kg BW of proline) and NM2 (200 mg/kg BW of rats of *Brassica oleracea* extract+ 50 mg/kg BW of Vitamin D3+ 6 µg/kg BW selenium+ 1000 mg/kg BW of proline) [4, 6].

Formulation of Doses:

These NM were administered by oral gavage to Wistar albino rats. The wistar rats were divided into three groups, Control, NM1 group and NM2 group. The doses were administered according to the per kg BW of rats. The dosage to be administered were decided according to the recommended doses of each constituent and BW (body weight) of rats.

TABLE 1: NUTRIENT MEDIUM COMPOSITION

Nutrient Medium	Cabbage Extract	Vitamin D_3	Selenium	Proline
NM1	100 mg	25 mg	4 µg	500 mg
NM2	200 mg	50 mg	6 µg	1000 mg

Selection of Animals:

A total of 15 Albino Wistar rats weighing 150-250 gm were selected and utilized for the study. IAEC clearance was obtained prior to commencement of the experiment. Before the experiment all the rats were checked for any abnormality by palpitation. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature and were supplied standard diet at *ad libitum* and free access to drinking water. All rats were maintained with natural 12 hour light and dark cycle [4].

Experimental Design:

3 groups of albino rats were formed and each group consisted of 3 females and 2 males. One group served as control which was not administered with any doses. Group II received daily doses of NM1 and group III received daily doses of NM2 for 14 days by oral gavage. Blood was withdrawn from orbit plexus on 0, 7^{th} and on 14^{th} day before sacrifice and biochemical enzymatic investigations of SGOT, SGPT and ALP were done. Rats were kept for overnight fasting before blood withdrawal and sacrifice. On 14^{th} day, liver was surgically removed. The same biochemical parameters were studied for the liver homogenates. Also the liver tissue was histopathologically examined [4].

Food and water intake Vs Body weight:

Body weight of each rat was measured on 0, 7^{th} and 14^{th} day of the study. The diet and food

intake was recorded daily for each group.

Biochemical parameters:
Three biochemical parameters were estimated SGOT, SGPT and ALP

Serum Glutamate oxaloacetate Transaminase (SGOT):
SGOT was estimated by Reitmann and Frankle Method [4].

Serum Glutamate Pyruvate Transaminase SGPT:
SGPT was estimated by Reitmann and Frankle method [4].

Alkaline Phosphatase (ALP)
ALP was estimated by Kind & King Method [4].

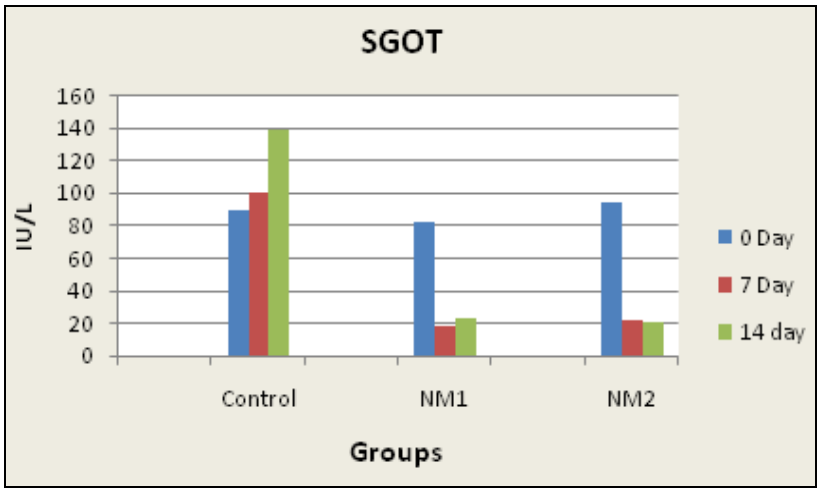
RESULTS:
Effect of Nutrient Medium:

Throughout the experimentation period of 14 days, no mortality was observed in control or in experimental groups.

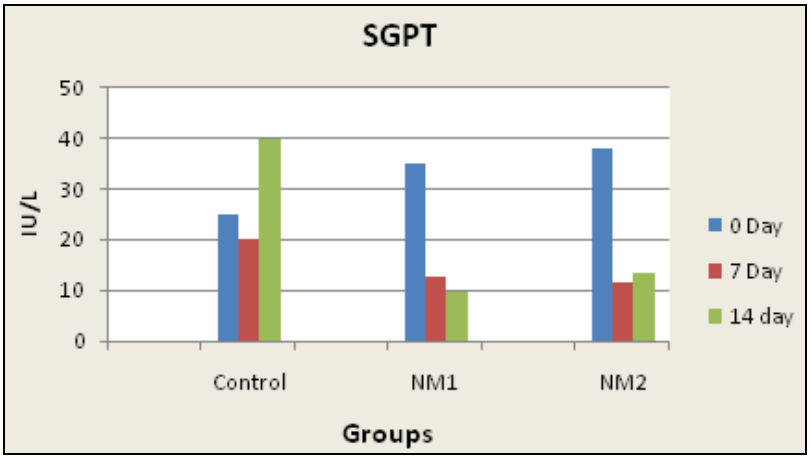
Physical Examination:
No physical changes were observed in any group throughout the study period.

Biochemical observation:
The biochemical parameters SGOT, SGPT and ALP were studied on 0, 7th and 14th day. Upon analysis of observations recorded, it is clear that SGOT, SGPT and ALP levels decrease from normal control. SGOT levels decrease (approx 80 IU) as we compare with control to NM1 and NM2. SGPT follows the same trend and it also decreases (approx 20 IU) from control to NM2.

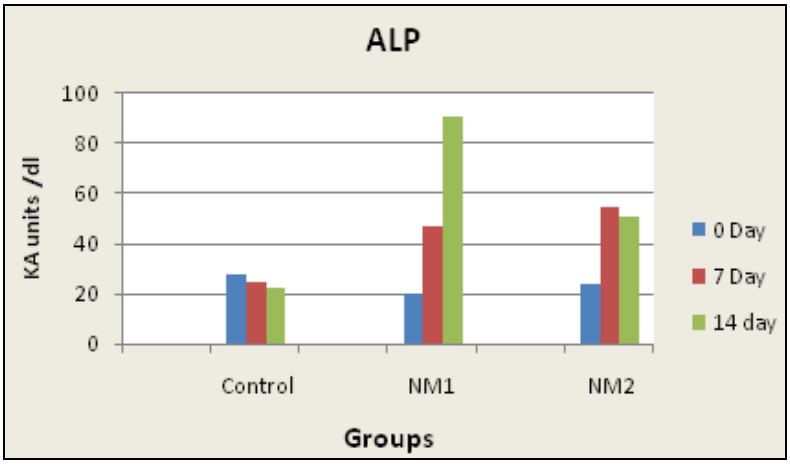
ALP increases (approx 20 Ka units) from control to NM1 and NM2. Increase is more prominently seen in NM1 as compared to NM2.



GRAPH 1: SERUM SGOT VALUES ON 0, 7TH AND 14TH DAY OF ADMINISTRATION OF NM1 AND NM2.



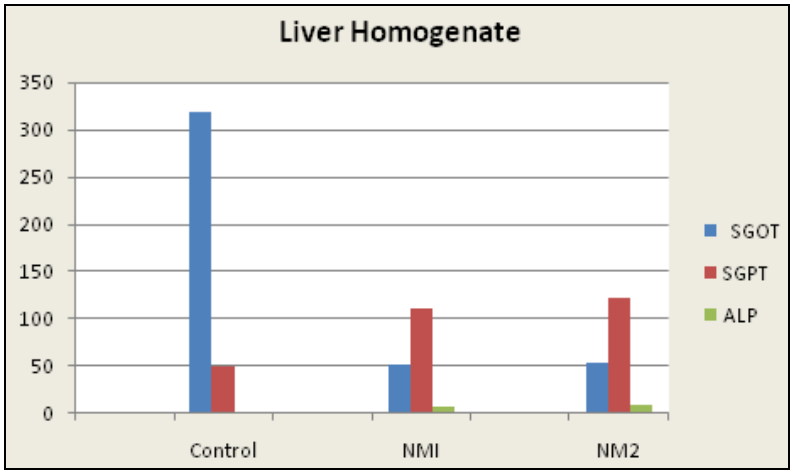
GRAPH 2: SERUM SGPT VALUES ON 0, 7TH AND 14TH DAY OF ADMINISTRATION OF NM1 AND NM2



GRAPH 3: SERUM ALP VALUES ON 0, 7TH AND 14TH DAY OF ADMINISTRATION OF NM1 AND NM2

Liver Biomarker Estimation:

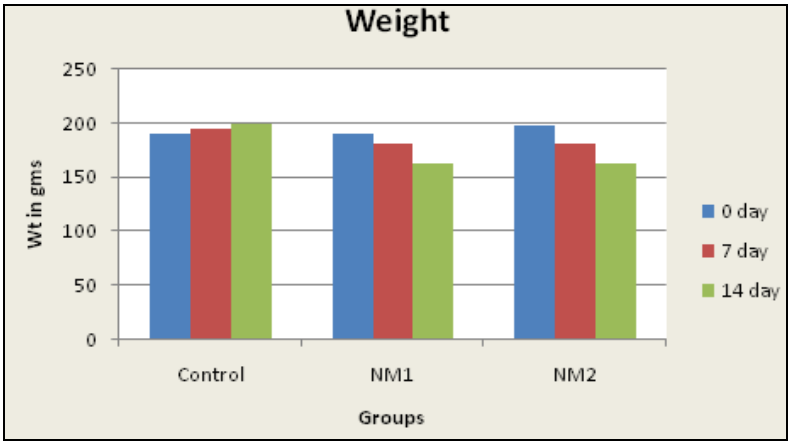
The liver biomarker estimation show SGOT levels decrease (approx 250 IU) from control group to NM2. SGPT increases (approx 50 IU) from control group to NM1 and NM2, but the increase is not very much remarkable. There is no significant difference in ALP, from control to NM2 group.



GRAPH 4: LIVER HOMOGENATE BIOCHEMICAL PARAMETERS.

Food, water intake Vs Body Weight:

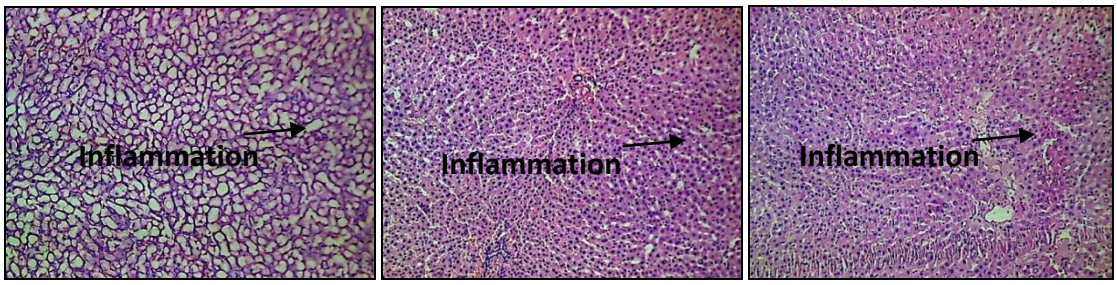
No significant variation was observed in food and water intake during entire study period. Weight decreased in every group except in control throughout the experimental period.



GRAPH 5: EFFECT OF NM1 AND NM2 ON BODY WEIGHT

Histology:

Upon histopathological examination of the liver tissue, it was observed that inflammation prevailed in NM1 and NM2 treated groups.



CONTROL | **NM1 AT 100 mg/kg BW OF EXTRACT** | **NM2 AT 200 mg/kg BW OF EXTRACT**

Statistical analysis:

Data obtained from this work, was analyzed statistically by two-way analysis of variance (ANOVA) (with replication). Two parameters, dosage and time of biochemical analysis were studied. Values of $P<0.05$ were regarded as significant.

DISCUSSION: When the nutrient mixture containing cabbage extract, vitamin D_3, proline and selenium was administered orally to wistar albino rats, they showed decrease in liver enzyme activity (SGOT and SGPT). Only ALP levels slightly increased. SGOT and SGPT are sensitive markers for liver diseases (for example: cirrhosis, Hepatitis) whereas ALP is also produced by liver, bone and also excreted by bile. This may be the reason that the ALP is not following the same trend as SGOT and SGPT. In liver homogenate investigation, SGOT and SGPT decrease after NM supplementation which may be due to synergistic effects of the nutrients, but ALP increases as in serum. The liver marker enzyme showed decrease in their levels, which shows that NM1 and NM2 can bring enzymatic levels back to normal level. So these nutrient medium can be effective in diseases in which liver markers enzymes increases. In histology studies, there was no significant difference in NM1 and NM2. Inflammation is observed in both the groups as well as control.

CONCLUSION: As observed in the study, after administration of nutrient medium (NM1 or NM2), the decrease in the liver enzyme levels was observed. This implies that in diseases (cirrhosis, Hepatitis and cancer) in which the liver enzyme levels elevate, this nutrient medium may be given as Treatment as an adjunct to bring down the enzyme levels. Specifically NM1, has been found to be more effective in bringing enzyme levels near to normal. Also, the component of the nutrient medium i.e. Vitamin D_3, proline, cabbage extract and selenium all are reported to have anticancer activity [8, 9]. Since NM1 can be utilized as supplementation therapy in liver diseases and cancer.

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