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IN VIVO TOXICITY PROFILE OF *BRASSICA OLERACEA L. VAR.CAPITATA* (CABBAGE)

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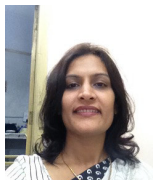
ABSTRACT

The cruciferae, Brassicaceae family, are characterized by the presence of a group of secondary metabolites called glucosinolates. More than 30 glucosinolates are present in the *Brassica* species. They are hydrolyzed by the enzyme myrosinase. Their breakdown products are chemically very reactive, and for a long time been known for their bioactive characteristics, such as anticarcinogenic, fungicidal or bactericidal properties. However, there is little information available about its toxicity profile. The present study was undertaken to investigate the potential toxic effects of methanolic extracts of *Brassica oleracea L.var capitata (*cabbage*) in vivo*. The toxic effects of extracts of *Brassica oleracea* were evaluated at different doses of hot soxhlet and cold macerated extracts. Alteration in SGOT, SGPT and ALP levels in a dose and concentration dependent manner was observed there by portraying its therapeutic and toxic concentrations.

KEYWORDS: **Liver toxicity, SGOT, SGPT, ALP, *Brassica oleracea* extracts, glucosinolates.**



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INTRODUCTION

The use of plants for healing purposes is popular since they are beneficial and free of side effects [1]. Plants commonly used in traditional medicine are frequently promoted as natural and harmless. This assessment is based on their usage in the treatment of diseases over centuries. However, some medicinal plants must be used with caution because they can cause adverse reactions, especially if taken in excessive doses, or if they interact with the conventional drugs[2]. The Brassicaceae family comprises many commonly consumed vegetables, condiments, forages and oil containing plants, such as cabbage, broccoli, cauliflower, Brussels sprouts and rape. They are rich in glucosinolates[3]. Glucosinolates (alkyl-N-hydroximine sulphate esters with a β-D thioglucopyranosid group attached to the hydroximine carbon in Z-configuration relative to the sulphate group) have been reported to have detrimental activity against cancers of breast, lung and colon [3]. These are also reported to have antibacterial and fungistatic activity[4]. Cabbages are low in calories, high in complex carbohydrates and are rich in antioxidants, which play an important role in health maintenance [5]. It is a good source of vitamin C and vitamin K. Sulphoraphane and indole-3-carbinol are secondary metabolites found in cabbage and these include detoxification of carcinogens, limit production of cancer related hormones, block carcinogens and prevent tumor growth and therefore it is suggested to consume a diet rich in fruits and vegetables, and especially cruciferous vegetables such as broccoli, collard greens, Brussels sprouts and kohlrabi to reduce risk of developing several types of cancers [6]. Despite the exposure of this plant to the population, there is rarely any information in the scientific literature on its toxicity profile. The present investigation was therefore undertaken to evaluate the toxicity in rodents following the administration of methanolic extract of *Brassica oleracea.*

METHODOLOGY

Collection of Brassica oleracea (Cabbage)

Brassica oleraceae was purchased from a local grocer. The leaves were washed and air dried in shade and ground to a fine powder using an electric grinder [5].

Preparation of extract

Extraction

The *Brassica Oleraceae* powder thus obtained was used for extraction purposes. Extraction was performed by the following 2 methods:

a) Soxhlet Extraction

8 gm Brassica oleracea powder was extracted by soxhletion with 80% methanol (250ml). After extraction, the solvent was evaporated and extracts were preserved at 4^0C. For phytochemical screening, extracts were dissolved in distilled water [5].

b) Cold Maceration

10 gm of powdered cabbage was kept for 3 days in 80% methanol on a rotary shaker. The supernatant obtained was utilized for phytochemical screening [5]

Formulation of doses

Pure glucosinolates are known to be toxic above 10 mg /kg of BW[6]. Hence, five concentrations of extracts ranging from 100 mg/kg BW to 500 mg/kg of BW were decided for the toxicity study.

Selection of Animals

A total of 60 Albino Wistar rats weighing 150-250 gm were selected and utilized for the study. IAEC clearance was obtained prior to commencement of the experiment. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature of 25^0 C and were supplied with standard pellets and fresh drinking water. All the rats were kept in clean cages and were maintained with natural 12 hour light and dark cycle[7].

Experimental Design

6 groups (n=5) of Wistar albino rats having 3 females and 2 males per group were formed. The first group served as control which was administered 80% methanol 0.5 ml/kg BW. The other 5 groups received daily doses of hot soxhlet and cold macerated cabbage extracts in methanol by oral gavage for 14 days respectively. The determined doses are mentioned in table 1 below:

Table 1

Dose formulation for methanolic soxhlet and cold macerated extracts of Brassica oleracea

S.no	Groups	Doses	Hot/Cold Extract
1	Control	0.5 ml/kg of BW of methanol	Methanol
2	Group I	100 mg/kg of BW	Hot Extract
3	Group II	200 mg/kg of BW	Hot Extract
4	Group III	300 mg/kg of BW	Hot Extract
5	Group IV	400 mg/kg of BW	Hot Extract
6	Group V	500 mg/kg of BW	Hot Extract
7	Control cold	0.5 ml/kg of BW of methanol	Methanol
8	Group VI	100 mg/kg of BW	Cold Extract
9	Group VII	200 mg/kg of BW	Cold Extract
10	Group VIII	300 mg/kg of BW	Cold Extract
11	Group IX	400 mg/kg of BW	Cold Extract
12	Group X	500 mg/kg of BW	Cold Extract

Methanolic extracts of *Brassica oleracea* were administered to wistar albino rats for 14 days as mentioned in table 1 above. The blood was withdrawn from oribito plexus on 7th day, after overnight fasting. Blood was collected into plane screw cap bottles. Serum was collected by allowing blood to stand for 2 hours for clotting to take place. The serum biochemical parameters (SGOT (Serum Glutamate oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase) and ALP (Alkaline Phosphatase) were studied. On 15th day, the blood was withdrawn for biochemical parameters and the animals were sacrificed by CO_2 asphyxiation and liver was surgically removed. The same biochemical parameters were studied for liver homogenates. Liver tissue was histopathologically examined.

Food and water intake Vs Body weight

Body weight of each rat was measured on 0, 7th and 14 day of the study. The diet and food intake was recorded daily for each group[1].

Biochemical parameters

Three biochemical parameters were estimated SGOT, SGPT and ALP.

1. Serum Glutamate oxaloacetate Transaminase (SGOT)

SGOT was estimated by Reitmann and Frankle method. In this method, the amount of oxaloacetate released, is determined after incubation, colorimetrically; by the formation of hydrazones with DNPH reagent which is highly colored in alkaline medium. The colored final product was read at 540 nm [8].

2. Serum Glutamate Pyruvate Transaminase SGPT

SGPT was estimated by Reitmann and Frankle method. In this method the amount of pyruvate released, is determined after incubation, colorimetrically; by the formation of hydrazones with DNPH reagent which is highly colored in alkaline medium. The colored final product was read at 540 nm [8].

3. *Alkaline Phosphatase (ALP)*

ALP was estimated by King & King Method. In this method, the serum is incubated with the buffer substrate under optimum, conditions of temperature and pH to release phenol. This reacts with 4- animoantipyrine in alkaline medium to give a red coloured compound which is estimated at 520nm against a reagent blank [9].

RESULTS

A.Toxicity Profile Soxhlet Brassica oleracea Extract

Throughout the 14-day feeding study, there was no mortality recorded in either the control or treated groups at the administered doses.

Physical Examination

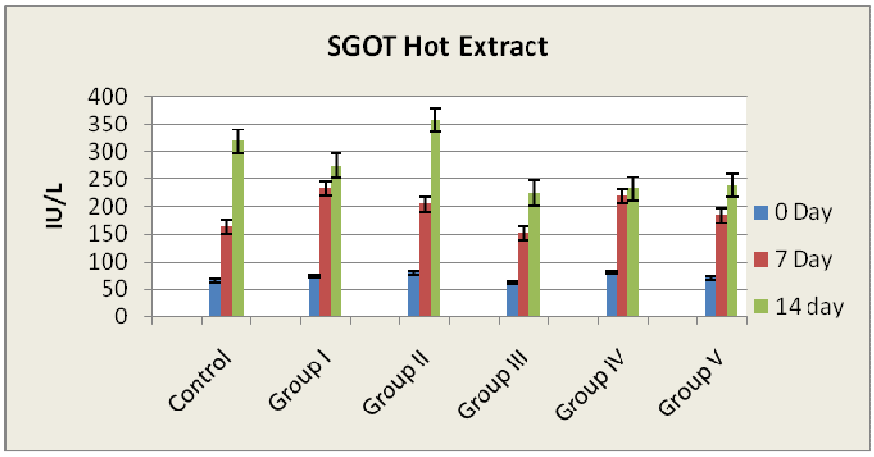
Some clinical signs observed in rats after the 7th day were abdominal contractions (observed after gavage), reduced activity and hunched posture. Reduction in the physical activity is more prominently observed with increase in concentration of the extract i.e. group V showed more sluggishness.

Biochemical Observation

The biochemical parameters SGOT, SGPT and ALP increase with the increase in dose concentration. SGOT levels increase (approx 50 IU) as the concentration of the extract increases. Sharp increase is seen in group II. Also, the 14th day SGOT levels are approximately doubled than the 7th day in this group. The SGPT concentration increases as the dose increases. But 7th day SGPT levels are more than the 14th day. Again a sharp increase is seen in group II. The ALP, levels increases up to 25 Ka units up to group III. Group I to Group III level of ALP increase steadily up to 7th day.

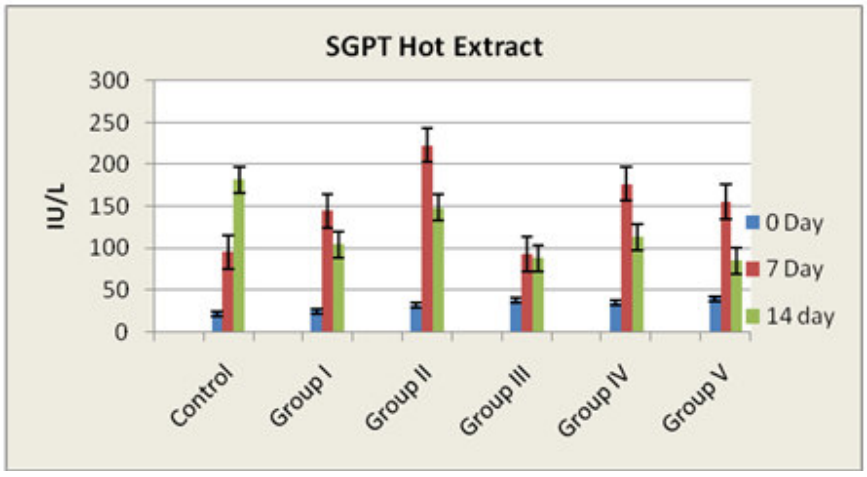
Graph 1

Serum SGOT values on 7th and 14th day of administration of soxhlet extract.



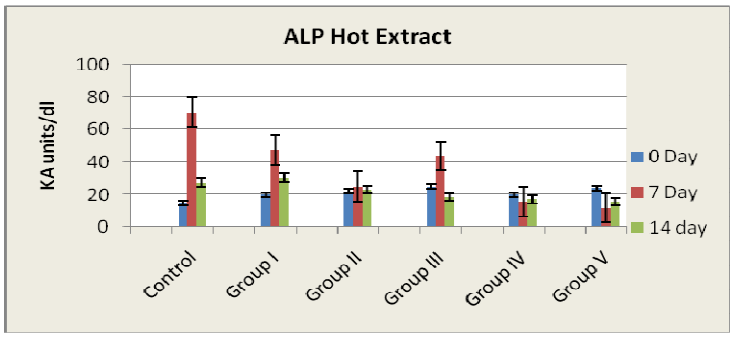
Graph 2

Serum SGPT values on 7th and 14th day of administration of soxhlet extract.



Graph 3

Serum ALP values on 7th and 14th day of administration of soxhlet extract.

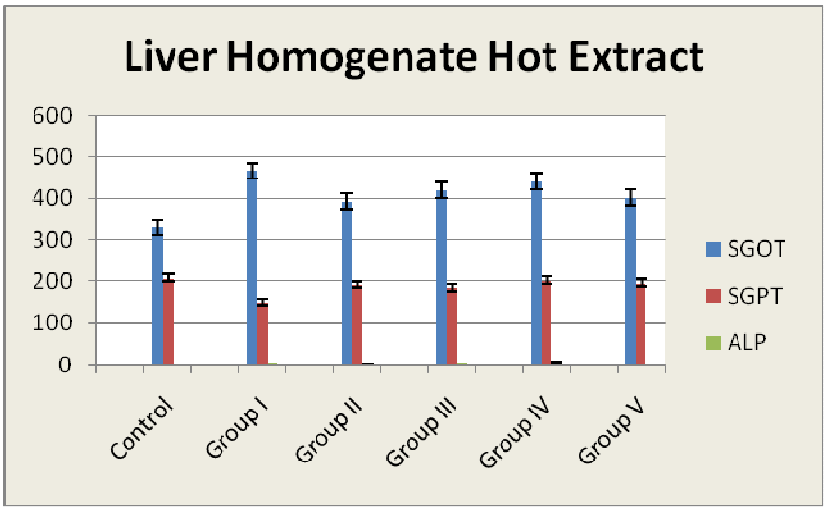


Liver Homogenate estimation

On 15th day, the rats were sacrificed, and liver SGOT, SGPT and ALP were investigated. It was observed that SGOT increased from 200 to 400 IU units, SGPT increased from 100 to 200 IU units. ALP increased by 10-15 ka units. These observations indicate no significant rise in ALP levels.

Graph 4

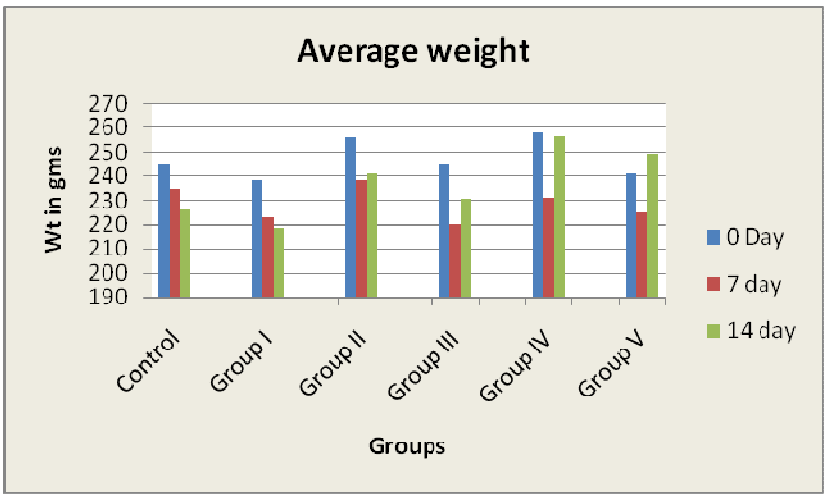
Liver homogenate biochemical parameters.



Food, water intake Vs Body Weight

No significant variation was observed in food and water intake during entire study period. But, weight decrease in every group throughout the experimental period.

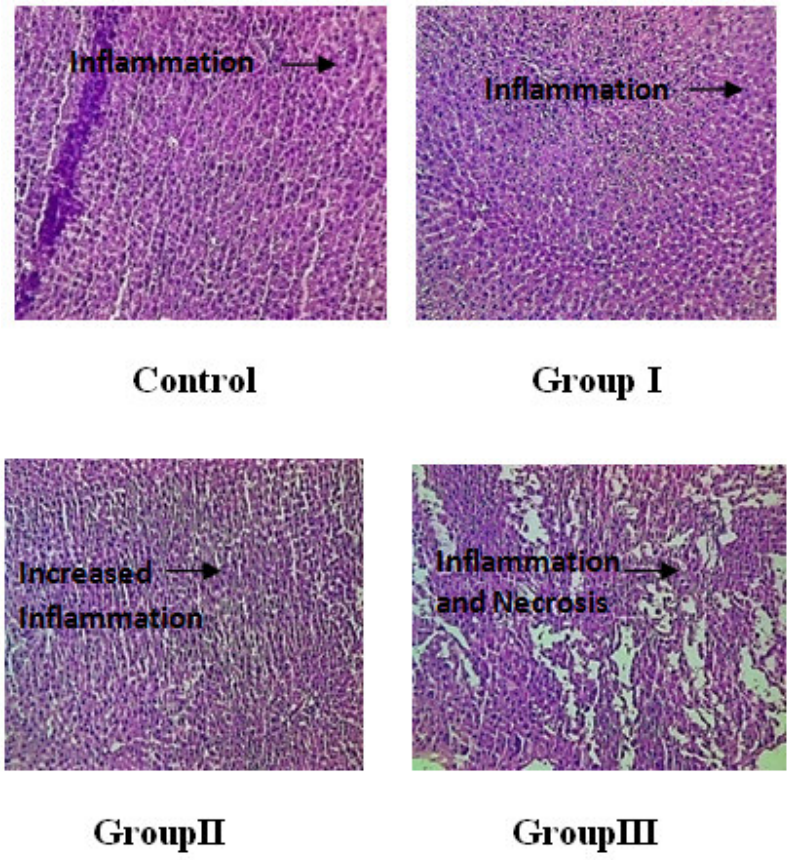
Graph 5
Comparative weight of rats during experimental period.

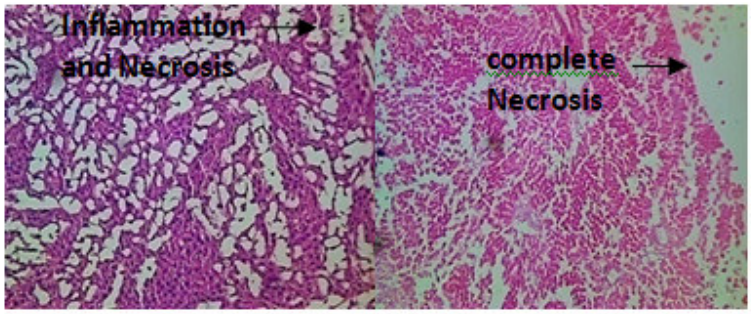


Histology

Liver sections were analyzed histologically. As the concentration of the extract increases more inflammation, necrosis and lobular disarray was observed in the hepatocytes indicating the deterioration of the liver. The damage to the liver cells was more prominently observable from group III i.e. 300 mg/kg BW concentration onwards.

Figure 1
Liver histology of Experimental Animals administered Hot Soxhlet Brassica oleracea





Group IV Group V

B. Toxicity Profile Cold Macerated Brassica oleracea Extract:

Physical Examination

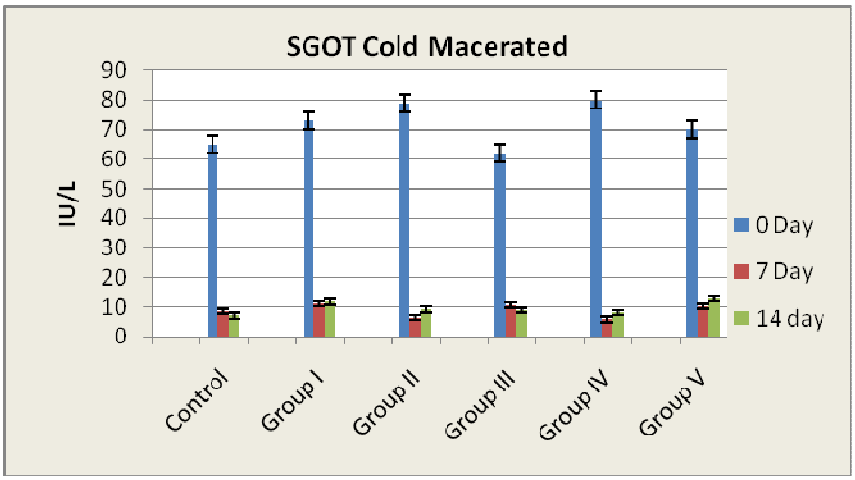
The clinical symptoms observed for cold macerated *Brassica oleracea* extract were similar to hot soxhlet *Brassica oleracea* extract.

Biochemical Parameters

Administration of cold macerated extract to rats for 14 days resulted in changes in the biochemical parameters viz; SGOT, SGPT and ALP levels increase with the increase in the dose concentration. SGOT levels increased (8 IU units) as concentration of the extract increases from 100-500 mg/kg BW. Also, the 14th day SGOT levels are 5-8 IU units higher than the 7th day. SGPT analysis showed that, concentration increase in a dose dependent fashion. But 7th day SGPT levels are 5 IU more than the 14th day. ALP, increased as the concentration of the extract increased. 14th day levels were higher than the 7th day levels (10-15 ka units).

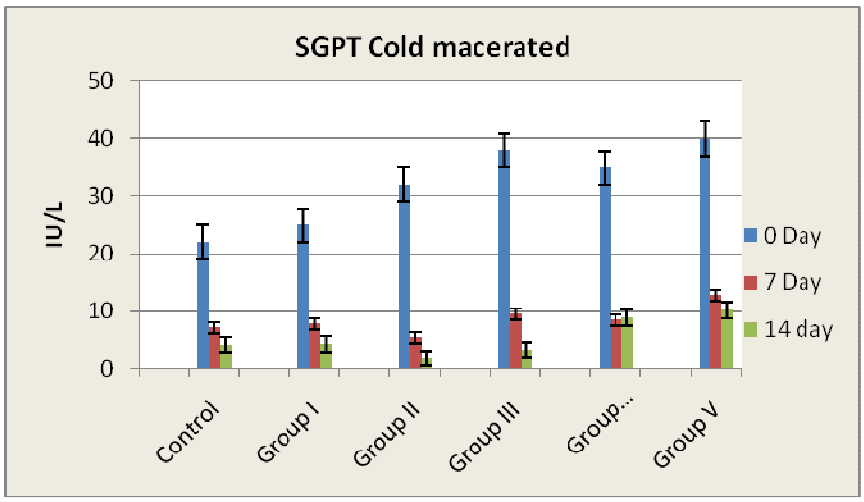
Graph 6

Serum SGOT values on 7th and 14th day of administration of cold extract.



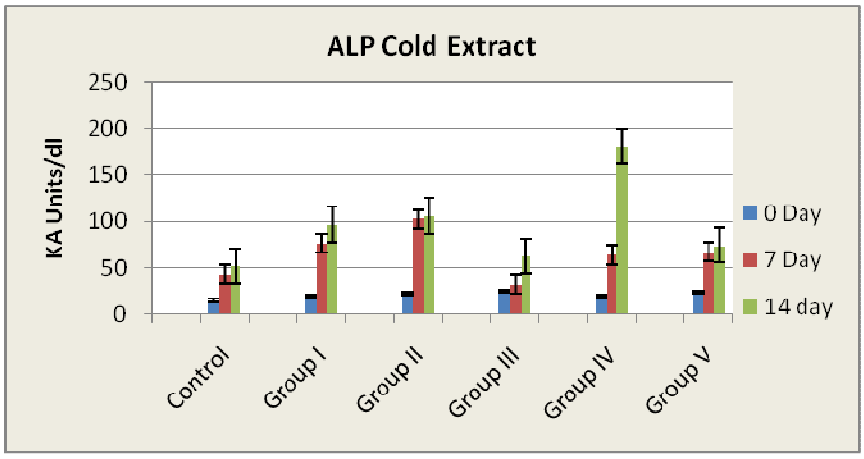
Graph 7

Serum SGPT values on 7th and 14th day of administration of cold extract.



Graph 8

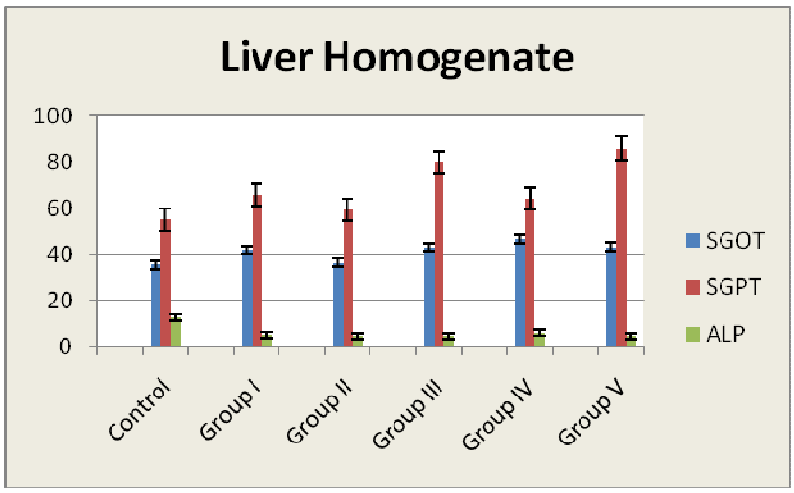
Serum ALP values on 7th and 14th day of administration of cold extract.



Liver Homogenate estimation

Changes in the levels of SGOT, SGPT and ALP were observable parallel to administration of hot soxhlet extract in liver homogenates. The SGOT increased from 50 to 80 IU units, SGPT increased from 30 to 45 IU units. The level of ALP increase (5-10 ka units) is not significant.

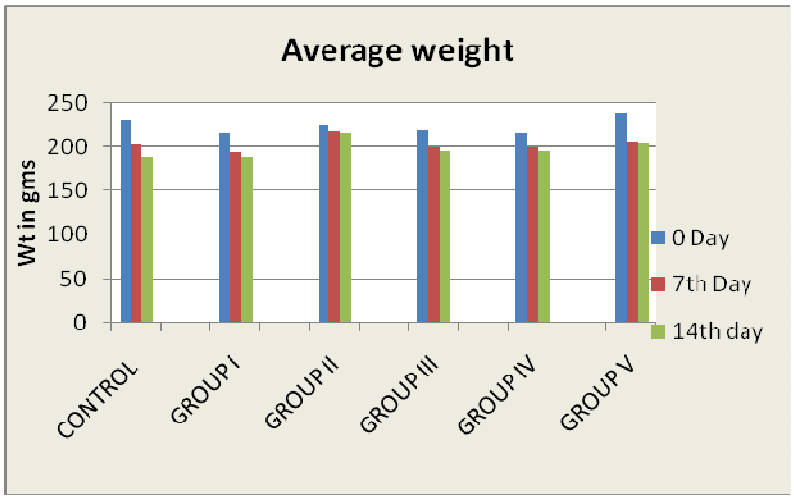
Graph 9
Liver homogenate biochemical parameters.



Food, water intake Vs Body Weight

Not much variation was observed in food and water intake. But weight decreased in every group throughout the experimental period. The results were similar to hot soxhlet extract administration results.

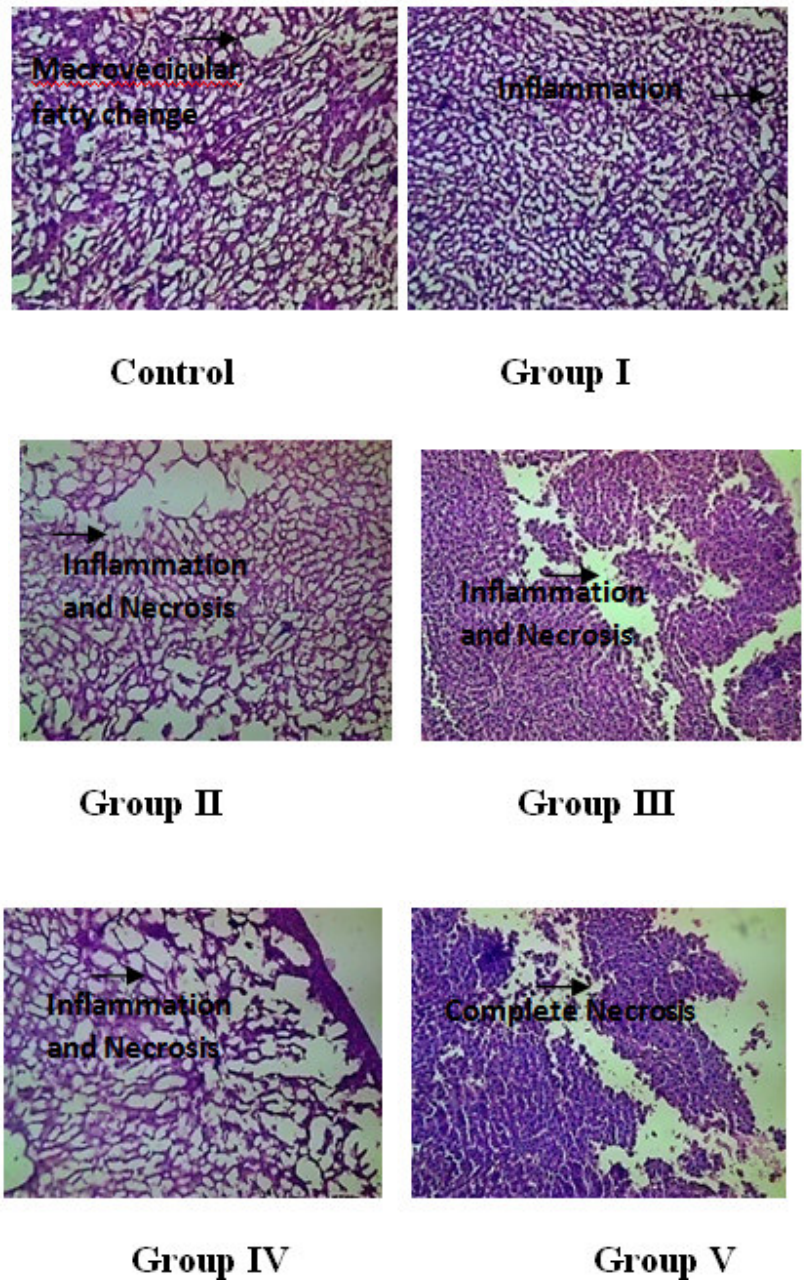
Graph 10
Comparative weight of rats during experimental period.



Histology

Liver sections were analyzed histologically. As the concentration of the extract increased more inflammation, necrosis and lobular disarray was observed in the hepatocytes indicating the deterioration of the liver. The damage to the liver cells was more prominently observed from group III onwards.

Figure 2
Histology of liver of Experimental Animals administered Cold Macerated Brassica oleracea 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

Brassica oleracea contains glucosinolates, which are secondary metabolites. These compounds are reported to have antibacterial, antioxidant, anticancer and antifungisidic activity[5]. The above results show that the liver parameters i.e. SGOT and ALP have significantly increased enzymatic activity as

compared to normal. The graph revealed the different enzymatic level alterations according to the concentration and frequency of the dose thereby giving us the idea of its therapeutic and toxic concentration. Amino transferases SGPT and SGOT catalyze the interconversion of amino acids and α-keto acids by the transfer of an amino group. These enzymes are very sensitive and are reliable indices for liver damage or curative effects of various compounds. Alkaline phosphatase (ALP) is produced by bone, liver, intestine placenta and is also excreted by bile. In the absence of bone disease and pregnancy, there is an elevated serum ALP levels due to increased production of ALP by hepatic parenchymal or duct cells. The degradation products of glucosinolates, like isothiocyanates which have beneficial effects for example, anticancer antifungal antibacterial activity, are also responsible for thyroid toxicity. They act as goitrogens, and increase the risk for goiter. These substances seem to interfere with iodine absorption, particularly where iodine is limited in the diet, and have been linked to the formation of goiters[10]. When the extracts of *Brassica* were administered to Wistar rats for 14 days was found to be toxic > 300 mg/kg of BW in case of both the extracts (hot soxhlet and cold macerated). In case of soxhlet extract, the SGPT, SGOT and ALP enzyme levels increased, indicative of liver damage and liver toxicity (2). 14th day enzyme levels were higher than the 7th day except in case of SGPT. This is because 80% SGPT is found in mitochondria where as SGOT is pure cytosolic enzyme and considered as an appropriate liver marker (1). The enzyme levels increase during liver injury or damage. These results corroborate the histology results, indicating that as the concentration of extract increased liver damage increased. Similar results were observed for cold macerated extract. The weight of liver down the group (I to V) also witnessed alterations due to administered doses. The increased liver weight signifies fatty acid generation of hepatocytes, inflammation and central lobular disaaray.

CONCLUSION

When *Brassica oleracea* extracts were administered in dose dependent fashion to wistar albino rats for 14 days, it caused toxicity at a concentration of ≥ 300 mg/kg BW. The cabbage crude extracts showed different enzymatic level alterations according to the concentration and frequency of the dose. As the concentration of extract increases above 300 mg/kg BW the liver damage increases, which causes inflammation, necrosis and lobular disarray in hepatocytes. The results suggest the administration of *Brassica oleracea L.var capitata* at a concentration of 300 mg/kg BW, a potential inhibitor of intracellular stress; thereby revealing that dose below 300 mg/kg BW can be used as preventional therapeutic concentration. Lastly there is still tremendous and varied scope for further research in establishing relationship between glucosinolates and diseases like cancer (pancreatic cancer, prostate cancer, stomach ulcers and breast cancer), pneumonia, appendicitis, alcohol toxicity, arthritis and diabetes in which cabbage can be used as a preventive and therapeutic supplement.

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