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THE PROTECTIVE EFFECTS OF BEETROOT AGAINST DEXAMETHASONE INDUCED DAMAGE IN LIVER, KIDNEY AND HYPERLIPIDEMIC EFFECTS IN MICE

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ABSTRACT

Background: beetroot is grown in many countries over the world, is frequently consumed as part of the normal diet, and usually used in manufacturing as a food colouring agent. Beetroot is also one of the many vegetables that contain a group of highly bioactive pigments such as betalains and a number of research have mention betalains to have to have a powerful antioxidant and anti-inflammatory effects in vitro and in vivo animal models. Aims and **Objectives:** To explore possible protective effects of beetroot extract against damage induced by dexamethasone on liver, kidney and possible hypolipidemic effects in mice. Materials and **Methods:** Thirty male Swiss albino mice aged nine to10 weeks and weighing 25 to 30 g were used throughout the study. After tow-weeks acclimatization period, the selected animals of nearly a similar weight were divided into three experimental groups so as to keep more or less the same mean body weight within the individual groups. Damage induced by dexamethasone at the dose of 4mg/kg body weight per day. **Results:** it was found that dexamethasone increase all lipid profile (except HDL concentration) significantly with a marked liver and kidney injury and on the other hand the present study confirm the protection of beetroot juice against oxidative damage on liver, kidney and a significant decrease of lipid profile in mice. **Conclusion:** Beetroot have shown to have a significant medical values and positive health effects, its significantly decrease a damge effects on liver kidney and shown a significant decrease of lipid profile in mice subjected to dexamethasone

KEYWORD: Dexamethasone, beetroot, SGOPT, SGOT, Cholesterol, triglyceride, LDL.

INTRODUCTION

Beetroot is grown in many countries over the world, is frequently consumed as part of the normal diet, and usually used in manufacturing as a food colouring agent. The recent attention in beetroot has been primarily focused by the discovery that sources of dietary nitrate that could be important implications for controlling cardiovascular health.[1]

Hypertension in itself has been the goal of many therapeutic research and there are frequent studies that show beetroot significantly decrease systolic and diastolic blood pressure. Beetroot's effect on the blood vessels is highly related to its high inorganic content, it also contain a high amount of phytochemical compounds, that includes ascorbic acid, carotenoids, flavonoids and phenolic acids.[2]

Beetroot is also one of the many vegetables that contain a group of highly bioactive pigments such as betalains and a number of research have mention betalains to have a powerful antioxidant and anti-inflammatory effects in vitro and in vivo animal models, This has increase the interest of a probable role of beetroot in clinical condition that characterised by oxidative stress such as liver disease, arthritis and also cancer. Beetroot supplement could increase the endogenous antioxidant defences and to protect cellular components from oxidative damage.[3]

Beetroot have been shown to decrease the radical formation and a number of studies report that beetroot, in the form of a juice supplement, defence against oxidative damage to chromosomes, lipid and protein structures in vitro. A study done by Wootton-Beard highlight that the main mechanism by which beetroot have its antioxidant effects is by scavenging radical species.[4]

Glucocorticoids are one of the most prescribed drugs, and they are potent medications for a huge number of inflammatory and immunological disease. in spite of its therapeutic activity, high doses with long-term consumption of these medications are associated with serious side effects, such as diabetes mellitus, liver disorder, cardiovascular disorders, hypertension, dyslipidemia, and osteoarthritis.[5]

Dexamethasone is one of the potent synthetic glucocorticoid drugs for innumerable inflammatory and immunological disorders. also it was found that high doses with long-term utilization of dexamethasone are associated with negative side effects.[6]

AIM OF THE STUDY

To explore possible protective effects of beetroot extract aganst damage induced by dexamethasone on liver, kidney and possible hypolipidemic effects in mice

MATERIALS AND METHODS

Beetroot was collected from local market of Iraq in 2018. It was then identify and selected depending on Choosing the highest degree of purity and removing the infected beet Extraction of beet were washed sliced and shade dried under room temperature for a period of 2 weeks. The dried plant material was powdered and 100 g of powder was subjected to hot percolation in a soxhlet apparatus using ethanol. Extract was then dried and stored at 4°C till further use.[7]

Experimental animals

Thirty male Swiss albino mice aged nine to10 weeks and weighing 25 to 30 g were used throughout the study in the (Biotechnology Research Center) Animals were feed by a similar diet ingredient and had free access to tap water. All mice were reserved under the same experimental condition, fed standard diet, and water was available ad libitum.

After tow-weeks acclimatization period, the selected animals of nearly a similar weight were divided into three experimental groups so as to keep more or less the same mean body weight within the individual groups. The selected animal groups (ten animals per each group) were treated as follows:

Positive control group (dexamethasone at the dose of 4mg/kg body weight per day), negative control group, beetroot extract-treated group plus dexamethasone at the dose of 4mg/kg body weight per day.

This study is conducted after approval from institutional animal ethics committee.

Statistical analysis

Data are presented as mean ± standard error mean and analyzed by using one-way analysis of variance followed by Bonferroni's multiple comparison test (post-test); $P \leq 0.05$ was considered as statistically signifi cant in all analyses.

RESULT

In related to cholesterol results There is a significant differences between positive and negative control group, cholesterol level shown a decrease in Beetroot with a highly significant deference in compare with the positive group. (Fig -1) on the other hand triglyceride level has no statistical significant differences in compare between positive and negative group while beetroot treated group show a highly significant differences in compare to positive treated group. (Fig -2)

HDL level shown a non significant differences in compare between negative to positive group and very high significant differences between Beetroot treated group and positive control group.(Fig -3)

LDL There is a highly significant differences between positive and negative control group, and Beetroot treated group decrease in compare with positive control group with statistical deference's. (Fig -4),VLDL There is a significant differences between positive and negative control group, and Beetroot treated group decrease in compare with positive control group with statistical deference's.

Urea levels has been increased significantly from 39.1±4 mg/dL (Negative control) to 48 ± 5.2 mg/dL in positive control group and all Beetroot extract show a significant differences in compare to positive control also on the other hand urea level show a significant differences in compare between Beetroot treated group to negative group.(Fig -5,6)

SGPT levels increased from 61.6 IU/L ± 7.6 to 78.6± 1.7 IU/L with a significant differences in compare between positive and negative group. Beet treated group has also shown a significant differences in compare to positive control group (the SGPT concentration has been decrease to 64.3 ± 3.7 IU/L) these results also found in SGOT levels while alkaline phosphatase concentrating shown a highly significant differences in compare between positive and Beet treated group and a Significant differences in compare to negative control group. (Fig -7,8)

Table-1.

	Cholesterol level mg/dL	Triglyceride level mg/dL	HDL level mg/dL	LDL level mg/dL	VLDL level mg/dL
Positive control	121 ± 11.5	122.1 ±10.7	46 ± 3.4	71 ± 5.1	31.66 ± 1.15
Negative control	87± 1.7^{x}	95 ± 18	37 ± 2.5^{x}	38 ± 6.4^{xx}	20 ± 2.0^{xx}
Beetroot treated group	92 ± 10.7**	74 ± 7**	41 ± 1**	59.6 ± 6.4*	17.3 ± 0.5**

Table 1- represent the effects of beetroot extract, lipid profile at the end of the experiment * represent Significant differences in compare to positive control group ** represent Highly Significant differences in compare to positive control group, [x] represent Significant differences in compare between positive and negative control group [xx] represent highly Significant differences in compare between positive and negative control group ▪ represent Significant differences in compare to negative control group ▪▪ represent Highly Significant differences in compare to negative control group. All values are mean +- SD data were analyzed by using spss 22 statistical test.

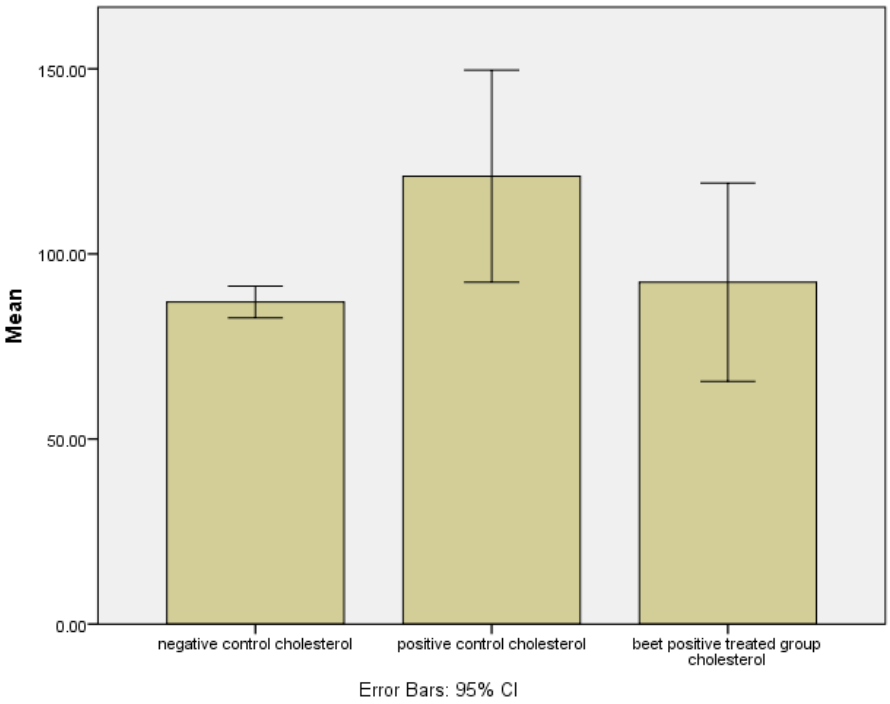

Fig. 1: Represent the mean difference of cholesterol levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

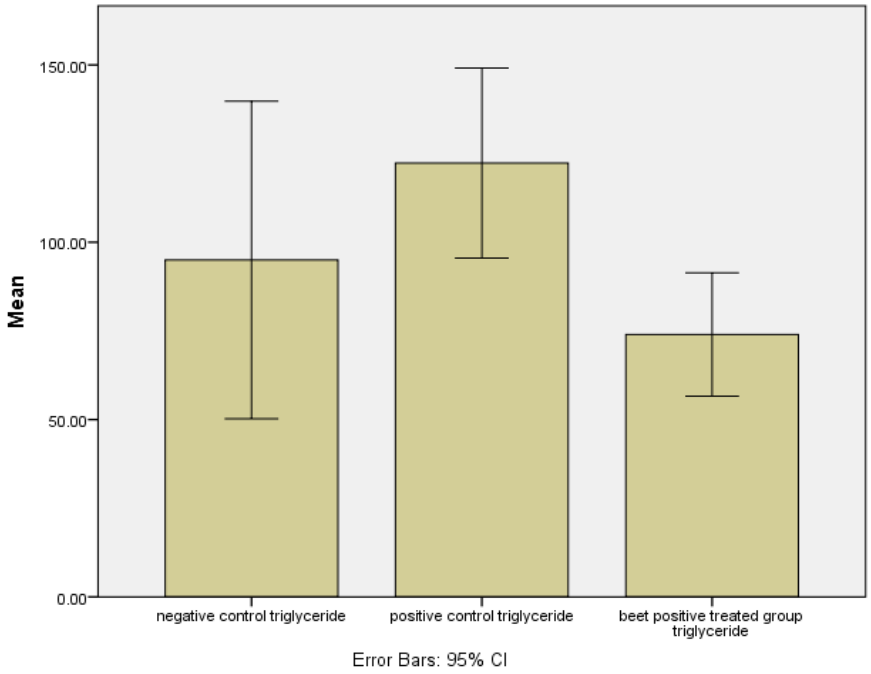

Fig. 2: Represent the mean difference of triglyceride levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

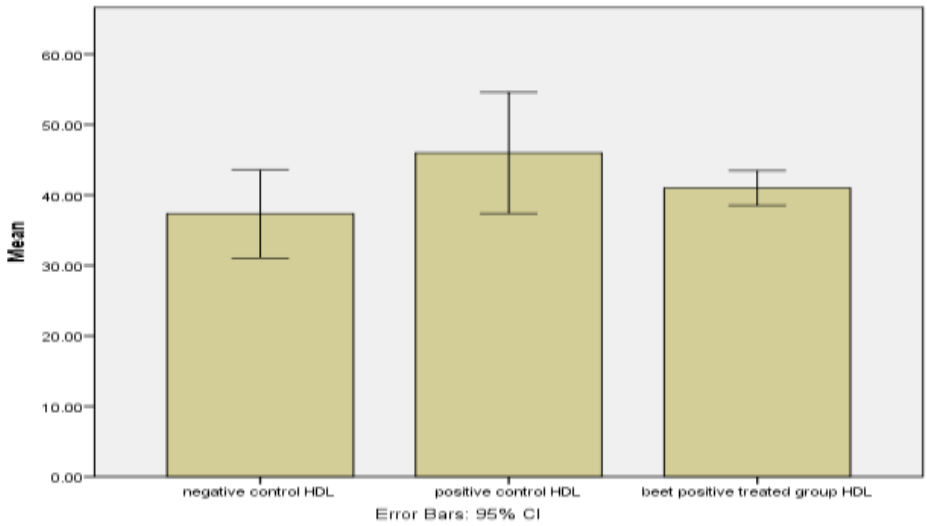

Fig. 3: Represent the mean difference of HDL levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

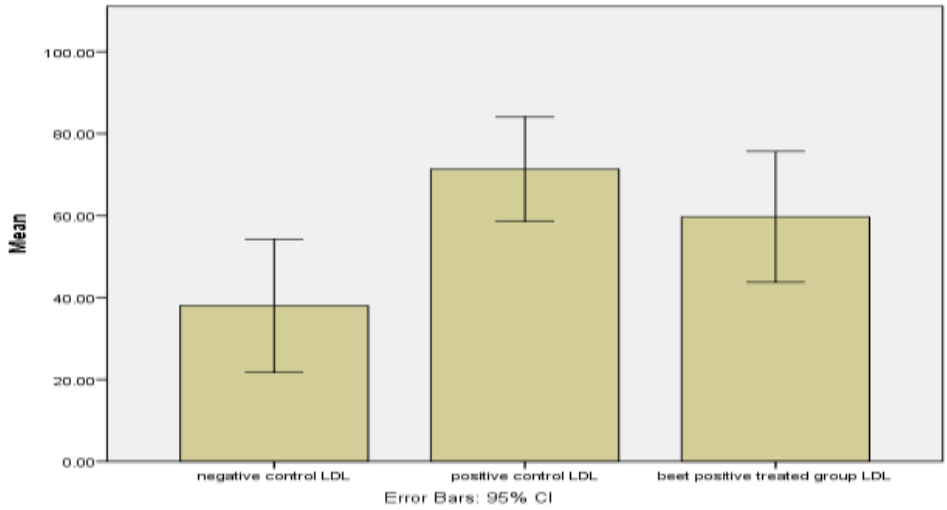

Fig. 4: Represent the mean difference of LDL levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

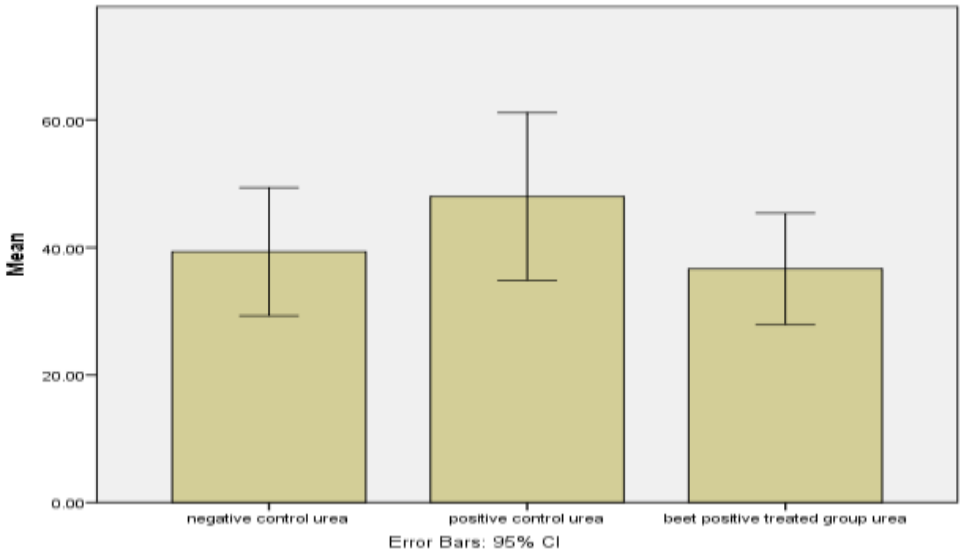

Fig. 5: Represent the mean difference of urea levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

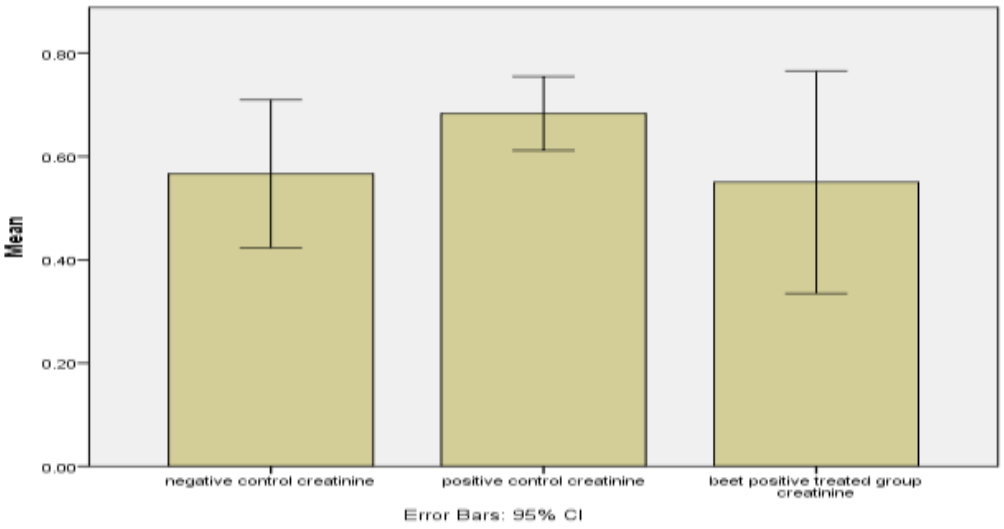

Fig. 6: Represent the mean difference of creatinine levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

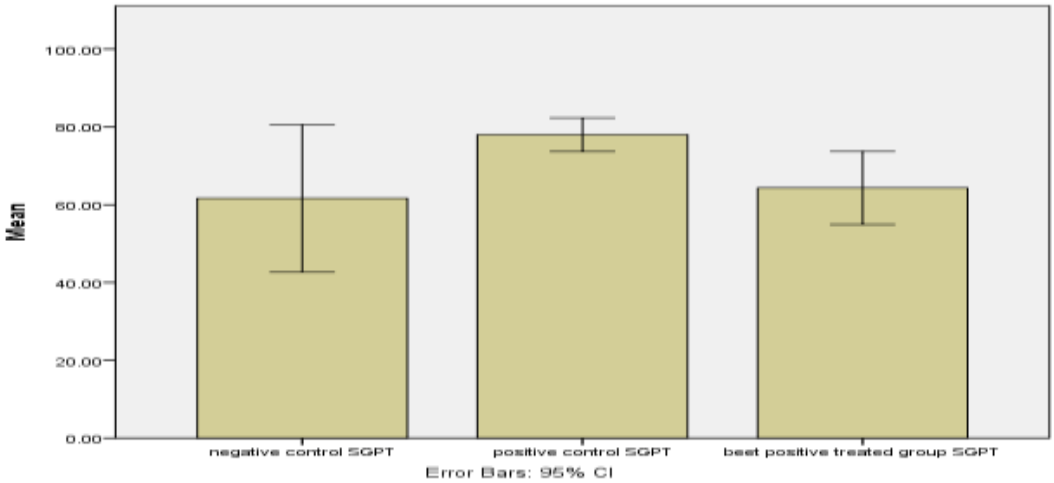

Fig. 7: Represent the mean difference of SGPT levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

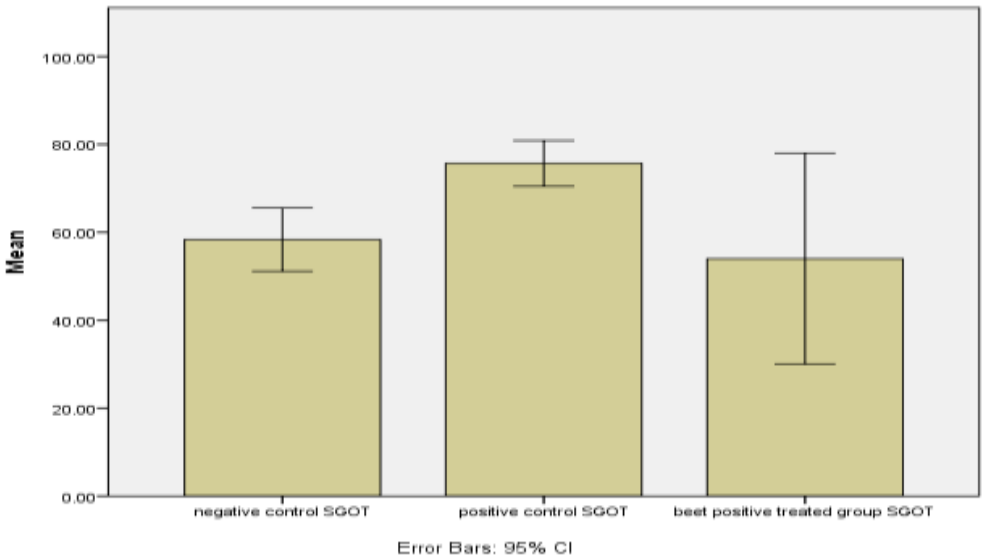

Fig. 8: Represent the mean difference of SGOT levels between positive (positive treated group, negative, beetroot extract treated group, on the end of the experiment.

Table-2.

	Urea (mg/dL)	Creatinine (mg/dL)
Positive control	48 ± 5.2	0.68± 0.02
Negative control	39.1±4	0.566± 0.05
Beetroot treated group	36.0± 3.5***▪**	0.55 ± 0.08*****

Table (2) Represent the effects of beetroot extract on renal function test at the end of the experiment * represent Significant differences in compare to positive control group ** represent Highly Significant differences in compare to positive control group, ˣrepresent Significant differences in compare between positive and negative control group ˣˣ represent highly Significant differences in compare between positive and negative control group▪ represent Significant differences in compare to negative control group ▪▪ represent Highly Significant differences in compare to negative control group. All values are mean +- SD data were analyzed by using spss 22 statistical test.

Table-3.

	SGPT IU/L	SGOT IU/L	ALP IU/L
Positive control	78.6± 1.7	75.66 ± 2	143.6 ± 3.2
Negative control	61.6± 7.6 ˣ	58.33 ± 2.8 ˣ	125 ± 5 ˣ
Beet treated group	64.3 ± 3.7 *****	54 ± 9.6*****	111.66 ± 1.5****▪**

Table (3) Represent the effects of beetroot extract on liver function at the end of the experiment * represent Significant differences in compare to positive control group ** represent Highly Significant differences in compare to positive control group, ˣ represent Significant differences in compare between positive and negative control group ˣˣ represent highly Significant differences in compare between positive and negative control group ▪ represent Significant differences in compare to negative control group ▪▪ represent Highly Significant differences in compare to negative control group. All values are mean +- SD data were analyzed by using spss 22 statistical test.

DISCUSSION

dexamethasone is a synthetic glucocorticoid that is indicated for treating and prevention of many inflammatory and autoimmune diseases. also it could be associated with a wide range of side effects, including liver and kidney dysfunction. The current study high light the protection and the dose–response effect obtained from beetroot against dexamethasone-induced oxidative stress damage in experimental male mice.The administration of dexamethasone significantly causes hepatic and renal injury at the end of the experimental period. These results were in accordance with the findings of other experiments.[8,9]

The side efects of glucocorticoid administration are familiar as well as the insulin resistance, metabolic disorders (hyperleptinemia, loss of appetite, and weight loss) with a renal and liver disorder.[10,11]

liver is structurally heterogeneous, and therefore have so important functions as detoxification, metabolism..etc. Liver enzymes such as AST and ALT are among the indicator enzymes for hepatic function which are usually increased in pathological condition such as acute hepatotoxicity and mild hepatocellular injury. In our study, administration of dexamethasone led to a significant elevation in AST ALT as well as alkaline phosphtase concentration, These results are in compatible with other experiments. on the other hand it was observed that rats treated with dexamethasone showed a significant elevation in serum uric acid and creatinine levels. Creatinine is a dependable indicator of how the kidneys are filtering out toxins and reflect the normal renal function.[12,13]

Kujawska M et al in his research "Protective effect of red beetroot against carbon tetrachloride- and N-nitrosodiethylamine-induced oxidative stress in rats" concluded that pretreatment with beetroot juice can counteract, to some extent, xenobiotic-induced oxidative stress in rats.[14]

Also Violetta Krajka-Kuz´niak in the study "Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats" has found that metabolic alterations induced by beetroot feeding may protect against liver damage.[15]

Krajka-Kuźniak et al also in his study on betanin which is the main component of beetroot has also found to have hepatoprotective effects as in the research "Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines." Which shown that beetroot juice protects against N-nitrosodimethylamine (NDEA)-induced liver injury and increases the activity of phase II enzymes.[16]

betanin also has shown to have a significant protective effect on glycoprotein components in plasma and tissue of diabetic rats in a research by Indumathi D et al".[17]

The potential protective role of betanin also found against organophosphate-induced hepatotoxicity in which the mechanism appears to be inhibition of Reactive oxygen species formation and mitochondrial protection as in Ahmadian E et al research.[18]

Betanin had a protective effect against paraquat-induced liver damage in rats. The mechanism of the protection appears to be the inhibition of CYP 3A2 expression and protection of mitochondria as found by JunyanHan et al in his research "Betanin attenuates paraquat-induced liver toxicity through a mitochondrial pathway".[18]

Present study confirmed the protection of beetroot juice against oxidative damage shown in previous studies and SGPT levels increased from 61.6 IU/L ± 7.6 to 78.6± 1.7 IU/L with a significant differences in compare between positive and negative group. Beet treated group has shown a significant differences in compare to positive control group (the SGPT concentration has been decrease to 64.3 ± 3.7 IU/L) these results also found in SGOT levels while alkaline phosphatase concentrating shown a highly significant differences in compare between positive and Beet treated group and a Significant differences in compare to negative control group. (Fig -7,8).

Beetroot extract has been studied in many research to explore its renal protective effects one of these research done by El Gamal eta al. in his research "Beetroot (Beta vulgaris L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model.**"** That beetroot treatment decrease renal dysfunction and structural damage through the reduction of oxidative stress, inflammation, and apoptosis in the kidney.[19]

Other scientists as (tan D.et al) has been found that betanin (the main ingredient in beetroot) have a protective effect against paraquat-induced acute kidney damage. The mechanisms of the protection appear to be the inhibition of oxidative stress and inflammation.[20]

This is compatible with our study that shows that a significant decrease of both urea and Creatinine (urea from 48 ± 5.2 mg/dL to 36.0± 3.5 and Creatinine from 0.68± 0.02 mg/dL to 0.55 ± 0.08 mg/dL).(Fig -5).(Fig -6) injection of dexamethasone results in the increase of cholesterol, low-density lipoprotein (LDL), triglyceride and reduction of high-density lipoprotein (HDL) of blood serum. And, as low-density lipoprotein (LDL) of plasma is the means by which cholesterol and ester cholesterol are carried to different tissues. Lipid-lowering drugs are used for primary and secondary prevention of cardiovascular diseases.[21]

The use of plant extracts such as betalains (beetroot ingredient) to treat or regulate the conditions of lipidemia are recently encouraged as they are side effects or toxicity free as compared to synthetic drugs. Betalains are found to inhibit the myeloperoxidase/nitrate-induced oxidation of human low-density lipoproteins (LDL) by scavenging the lipoperoxyl radicals.[22]

An experiment carried out on volunteers who consumed prickly pear fruit detected the incorporation of betalains in their LDL suggesting the involvement of betalains in the protection of LDL against oxidative modification.[23]

Feeding betalains enriched beet crisps significantly decreased the serum glucose level, atherogenic index, isovaleric acid concentration in the caecal and body weight in rats.[24]

feeding of 300 mg/kg (body weight) of red pitahaya extracts containing betalains in hypercholesterolemic rats reduced their total cholesterol level by 43.45%.[25]

In vivo experiments on obese individuals showed that betalains from red beet juice and chips inhibited neutrophil oxidative metabolism suggesting that betalains may have potential application in the management of hyperlipidemia.[26]

As Jeung Hee Lee, et al who found that volunteers who consumed freeze-dried beetroot leaves had significant decrease on biochemical levels of: total cholesterol, LDL cholesterol.[27]

These results are compatible with our study in which it was found that dexamethasone increase all lipid profile (except HDL concentration) significantly and this increment has been decrease significantly by administered beetroot extract as cholesterol level decrease from 121 ± 11.5 mg/dl to 92 ± 10.7 mg/dL (Fig-1) and triglyceride level from122.1 ±10.7mg/dl to 74 ± 7 mg/dl (Fig-2) and these results high light a possible strong hypolipidemic effect..

dexamethasone-induced oxidative stress damage leading to liver, kidney injury and hpyerlipidemia which consider as one of the most serious side effects, pretreatment with beetroot extract reduced the parameters of liver and kidney function damage as well as reduce the hyperlipidemic effects of dexamethasone, this powerful protective effects can be due to antioxidant effect of betalains that is found in high concentration and this is compatible with other researcher.

CONCLUSION

Beetroot have shown to appear a significant medical values and positive health effects, its significantly decrease a damage effects on liver kidney and shown a significant decrease of lipid profile in mice subjected to dexamethasone, its unlike synthetic colorants which may start an a adverse effects in humans, The pharmacological properties of betalains-rich foods as red beetroot show their great potential as functional foods.

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