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**STUDY ON THE EFFECT OF EUGENOL AND *Ocimum sanctum* Linn. LEAF
EXTRACT ON THE LIVER AND SERUM BIOCHEMICAL ALTERATIONS IN
FEMALE ALBINO RATS**

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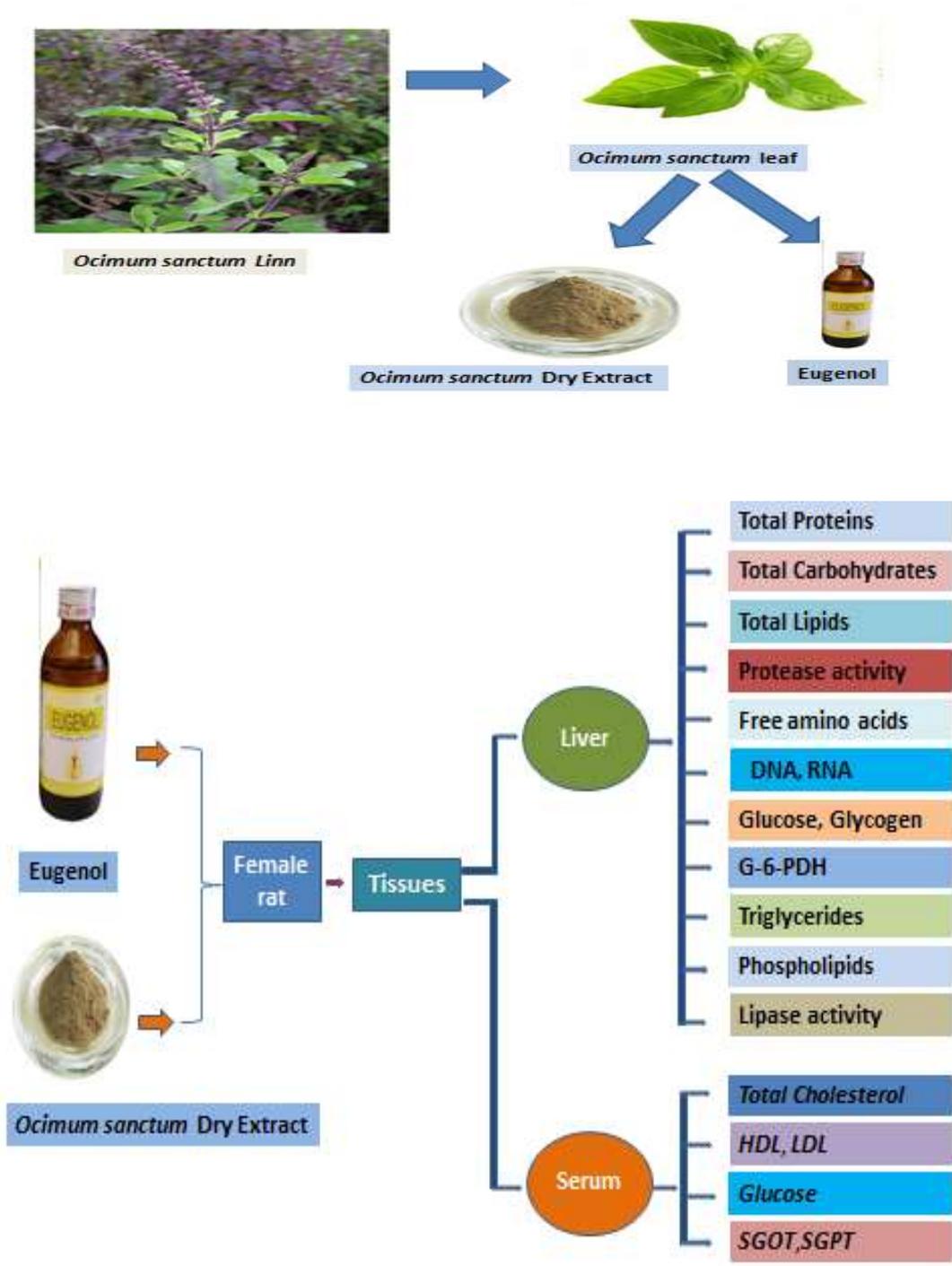
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ABSTRACT

This study was conducted to determine thereported the present study is aimed to evaluate the antifertility activity of the effect of Eugenol and *Ocimum sanctum* Linn. leaf extract on Liver and Serum biochemical alterations. The analysis was carried out the experiments were conducted to investigate the healthy female albino rats were administered with EUG (99% pure) at a dose of 0.4 ml/day/rat and *OS* Linn. (Tulsi) leaf extract at a dose of 500 mg/kg body weight/day/rat orally for 15 days. One-way ANOVA analysis with Dunnett's multiple comparison test is used for analyzing data. The biological assay results showed that the Total Lipids were significantly increased in both administrations ($P < 0.001$). The DNA and RNA levels were significantly increased in liver by both administrations ($P < 0.001$). SGOT levels were significantly elevated in serum by Eugenol administration ($P < 0.001$), in *OS* administration where it was reduced ($P < 0.001$). Consequently, according to our results, the present investigation suggest that the triglycerides were significantly increased in liver by both administrations. Liver cells can synthesize, store triglycerides and into cholesterol. The activity of SGOT, SGPT levels was significantly increased in Serum by *OS* administration. These we may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells.

Graphical abstract



Keywords: Eugenol, *Ocimum sanctum*, SGOT, SGPT, Cholesterol, HDL, LDL, Liver and Serum

INTRODUCTION

From the dawn of the civilization, humans have relied on plants and their products as a source of drugs for their primary health care. In recent years, their use as a popular alternative to modern medicine has increased considerably even in developed countries [1]. The importance of plants as a source of antifertility drugs has been emphasized by many researchers. Antifertility agents obtained from indigenous medicinal plants would be of immense benefit, especially to inhabitants of developing countries, since the cost of these drugs would be within their means [2]. The antifertility plants with estrogenic property can directly influence pituitary action through peripheral modulation of luteinizing (LH) and follicle-stimulating hormones (FSH) by decreasing the secretion of these hormones and blocking ovulation [3]. In addition, the plant may also intercept the synchronized development of the ovum and endometrium while others may have abortifacient or antiprogesterational effects [4].

Several studies have shown that steam distilled essential oils extracted from the fresh leaves of *Ocimum sanctum* L. Have therapeutic importance. The therapeutic potential of the essential oils extracted from the fresh leaves of *Ocimum sanctum* L. Has

been found to be largely due to eugenol (a major constituent of the essential oil) which is a phenolic compound (1-hydroxy-2-methoxy-4-allylbenzene). [5, 6, 7]. In order to understand the mode of action of *Ocimum sanctum* L., to explain its therapeutic potentials in management of various disease conditions and to establish its use in modern medicine, several investigations have been carried out to study the pharmacological actions of the eugenol, essential oils (extracted from Tulsi leaves) & extracts of Tulsi on immune system, central nervous system, gastric system, reproductive system, blood biochemistry etc. in experimental animals [5, 6, 8-13].

Tulsi leaves contain bright, yellow coloured and pleasant volatile oil (0.1 to 0.9%). The oil content of the drug varies depending upon the type, the place of cultivation and season of its collection. The oil is collected by steam distillation method from the leaves and flowering tops. It contains approximately 7.0% eugenol, carvacrol (3%) and eugenol-methylether (20%). The leaves of *Ocimum sanctum* L. are said to have an abortifacient effect in women [14, 15, 16]. *Ocimum sanctum* L. Has also got antifertility effect [16, 17]. In Kerala the local women as well as the Ayurvedic

physicians have been reported to use the leaves of Tulsi for antifertility effect. The benzene and petroleum ether extracts of leaves of *Ocimum sanctum* L. Has been reported to produce 80% and 60% antifertility activity, respectively in female rats [16, 12].

Known in English as Holy Basil and botanically called *Ocimum sanctum*, Tulsi belongs to plant family Lamiaceae. It has made an important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. Tulsi has been described as of two types-vanya (wild) and gramya (grown in homes). Although having identical usage, the former has darker leaves. Tulsi is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, otalgia, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders [18, 19]. It has also aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge and febrifuge properties [9].

METHODS OF PREPARATION

Animals and study design

This study was carried out during November-January 2018. In the present study healthy

adult (4 months old, weight 170±20g) female Wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean, air conditioned room, with a photo period of 12 hours light and 12 hours dark cycle, at 25 ± 2°C with a relative humidity of 50 ± 5%. The rats were fed with standard laboratory feed (Hindustan lever Ltd, Mumbai) and water *ad libitum*. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (CPCSEA, 2003). This study was also carried out in accordance with the guidelines for the care and use of laboratory animals (NRC, 1996). The use of animals was approved by the Institutional Animal Ethics Committee (IAEC) (Regd. No. 10(i)a/CPCSEA/IAEC/SVU/ZOOL/CC/ Dt.08-07-2012) at S V University, Tirupati, India.

Preparation of *Ocimum sanctum* leaves extract

The leaf extract was prepared according to WHO 1983 [20] protocol CG-04. Leaf was sliced, shed-dried, grounded into a fine powder and extracted with 95% D/W (v/v) at

55-60°C for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24° C until use.

Test chemical

Pure compound eugenol (99%) was purchased from Sigma Aldrich (St Louis).

Dosage of Animals

The female albino rats were divided into three groups, each group contains 6 rats. The initial body weight of each animal was recorded.

Experimental design

Group I: First group is controlled rats administered with 1 ml of saline (vehicle).

Group II: Second group is experimental, administered with pure compound Eugenol (99%) at dose 0.4 ml /day for 15 days by intramuscular injection.

Group III: Third group is experimental, administered with *Ocimum sanctum* leaf extract at dose 500 mg /Kg body weight/ day for 15 days administered orally using the gastric gavaging technique [21, 22].

Sacrification schedule

Twenty-four hours after their last dose, the rats were weighed and sacrificed under anesthesia. The following steps were taken to minimize the suffering of the rats. First, the rats were handled gently to reduce their discomfort and distress. Second, anesthesia

was administered prior to blood sample collection, body weight measurements and before animal sacrifice. Additionally, anesthesia, examinations and animal sacrifice were undertaken in separate rooms to avoid instilling fear in other rats.

Body and Organ weight measurements

The body weight has been recorded on the initial day of the experiment and also on the day of sacrifice (15 day), both the control and experimental groups, by using an automatic balance. Similarly the weight of different organs (ovary, uterus, vagina and liver) was also recorded.

Collection of Tissues

Both control and experimental animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room. Twenty four hours after the last dose, the animals were autopsied and the reproductive tissues like liver were excised at 4°C and used for biochemical analysis. The blood was collected by puncturing heart.

Tissue homogenate preparation

Rats were sacrificed under ether anesthesia after 15 days treatment. Liver was quickly removed, trimmed of extraneous tissue, washed with ice-cold physiological saline solution. Liver tissues were homogenized

solution for studying the biochemical parameters.

Biochemical analysis

Liver biochemical Analysis

The following biochemical parameters were analysed. Biochemical studies freshly removed liver tissues were weighed to required milligram for biochemical analysis, such as Total Proteins, Total Carbohydrates, Total Lipids, Protease activity, Free Amino acids, DNA, RNA, Glucose, Glycogen, G-6-PDH, Triglycerides, Phospholipids, Lipase activity and Total Cholesterol. The net weight of the tissues was estimated gravimetrically.

The biochemical estimation like Total Proteins [23], Total Carbohydrates [24], Total Lipids [25], Protease activity [26], Free Amino acids [27], DNA & RNA [27], Glucose and glycogen [28], G-6-PDH [29], Triglycerides [30], Phospholipids [31] and Lipase activity [32] were estimated using standard methods.

Serum biochemical profile

Collection of serum

Rats were sacrificed by cervical dislocation after 15 days of Eugenol and *Ocimum sanctum* leaf extract administered. The blood was collected by heart puncture and serum was separated by centrifugation (3000 rpm at 40C for 10 min).

Measuring of Total Cholesterol Level

Total cholesterol was determined by the enzymatic calorimetric method of Allain *et al.*, (1974) [33]. Auto Analyzer (Express plus, Ciba corning USA) and Elitech kit were used.

$$\text{Total Cholesterol (mg/dl)} = \frac{\text{Abs. Of Tc}}{\text{Abs. of S}} \times 200$$

Measuring of HDL Level

Chylomicrons, VLDL (very low-density lipoproteins), and LDL (low-density lipoproteins) were precipitated by adding phosphotungstic acid and magnesium ions to the Sample. Centrifugation left only the HDL (high-density lipoproteins) in the supernatant; their cholesterol content was determined [34].

$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Abs. of TH}}{\text{Abs. of S}} \times 50$$

Measuring of LDL Level

LDL cholesterol was calculated by the following formulae:

$$\text{LDL cholesterol (mg/dL)} = \frac{\text{TGL}}{5} - \text{HDL cholesterol}$$

The calculations were done automatically.

Serum glucose

Estimation of glucose was done by enzymatic glucose oxidase- peroxidase (GOD-POD) method with the help of Span Diagnostic Kit at 505 nm wavelength against blank reagent 35. The concentration of serum glucose was expressed in mg/dl.

Serum glutamate oxaloacetate transaminase (SGOT)

Serum SGOT concentration was estimated by 2,4-dinitrophenylhydrazine (DNPH) colorimetric method with the help of Span Diagnostic Kit at 505 nm wavelength 36. Concentration of SGOT was expressed in IU/L.

Serum glutamate pyruvate transaminase (SGPT)

Serum SGPT concentration was estimated by the DNPH colorimetric method with the help of Span Diagnostic Kit at 505 nm wavelength [37]. Concentration of SGOT was expressed in IU/L.

Statistical analysis

The data were expressed as a Mean value with their SD. Reading of the six different groups was compared using one-way ANOVA analysis with a DUNNETTS MULTIPLE COMPARISON TEST. Statistical analysis was performed using SPSS (Version 11.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office - 2007, Excel Software, the data has been analysed for the significance of the main effects (factors) and treatments along with their interaction 38. Differences were considered statistically significant a- $p < 0.001$, b- $p < 0.01$, c- $p < 0.05$ and d- non significance levels.

RESULTS

Effect on liver biochemical parameters

Organ weight, Tissue Somatic Index, Total proteins, Total Carbohydrates and Total Lipids

The results of this study are shown in **Table 1**, indicates the Organ weight was significantly increased in the liver by eugenol administration ($P < 0.01$), except in OS administration where it was reduced ($P < 0.001$). The Tissue Somatic Index was significantly increased in the liver by eugenol administration ($P < 0.001$), except in OS administration where it was reduced ($P < 0.001$). In liver, the Total proteins were significantly increased in both administrations ($P < 0.001$). The Total Carbohydrates were significantly increased in the liver by both administrations ($P < 0.001$). In liver, the Total Lipids were significantly increased in both administrations ($P < 0.001$).

Protease activity, Free Amino Acids, DNA and RNA

As shown in **Table 2**, the Protease activity was significantly increased in liver by both administrations ($P < 0.001$). The Free Amino acid levels were significantly increased in liver by both administrations ($P < 0.001$). The DNA and RNA levels were significantly increased in liver by both administrations ($P < 0.001$).

Glucose, Glycogen and G-6-PDH

The data represented in **Table 3**, the Glucose levels were significantly reduced in liver by both administrations ($P < 0.001$). In the liver, The Glycogen levels were significantly elevated in both administrations ($P < 0.001$). The G-6-PDH levels were significantly elevated in liver by both administrations ($P < 0.001$).

Triglycerides, Phospholipids, Lipase activity and Total Cholesterol

The data represented in **Table 4**, the liver, The Triglyceride levels were significantly elevated in both administrations ($P < 0.001$). The Phospholipids levels were significantly elevated in liver by both administrations ($P < 0.001$). The Lipase activity was significantly elevated in liver by both administrations ($P < 0.001$). The Total Cholesterol levels were significantly elevated in liver by both administrations ($P < 0.001$).

Effect on serum biochemical parameters**Total Cholesterol, HDL, LDL and Glucose**

As shown in **Table 5**, the serum biochemical parameters like Levels of Total Cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL) and Glucose of control

and treated rats. The total cholesterol levels were significantly elevated in serum by both administrations ($P < 0.001$). HDL, in serum were significantly elevated in both administrations ($P < 0.001$). LDL, in serum were significantly elevated in both administrations ($P < 0.001$). The Glucose levels were significantly elevated in serum by Eugenol administration ($P < 0.01$), in OS administration where it was reduced ($P < 0.001$).

Triglycerides and Phospholipids

From the **Table 6**, we can observe The Triglyceride levels were significantly elevated in serum by both administrations ($P < 0.001$). The Phospholipids levels were significantly elevated in serum by both administrations ($P < 0.001$).

SGOT and SGPT

The data represented in **Table 7**, SGOT levels were significantly elevated in serum by Eugenol administration ($P < 0.001$), in OS administration where it was reduced ($P < 0.001$). SGPT levels were significantly elevated in serum by Eugenol administration ($P < 0.001$), in OS administration where it was reduced ($P < 0.001$).

Table 1: Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract in the Liver.

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Organweight (grams)	7.80 ± 0.53	8.33 ± 0.63 + 6.79 ^b	6.22 ± 0.42 - 20.25 ^a
2	Tissue Somatic Index (w/w)	4.025 ± 0.212	4.786 ± 0.305 + 18.90 ^a	3.381 ± 0.201 - 16.00 ^a
3	Total proteins (mg/g)	264.93 ± 19.64	418.14 ± 35.43 + 57.83 ^a	338.83 ± 28.63 + 27.72 ^a
4	Total Carbohydrates (mg/g)	4.80 ± 0.27	9.35 ± 0.54 + 94.58 ^a	7.58 ± 0.39 + 57.91 ^a
5	Total Lipids (mg/g)	29.32 ± 2.18	56.91 ± 4.81 + 94.09 ^a	41.64 ± 3.65 + 42.01 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a-p<0.001, b- p<0.01 indicates the level of significance.

Table 2: Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract in the Liver

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Protease activity (µmoles of tyrosine equivalents /mg of protein / hr)	0.96±0.07	1.88±0.11 +95.83 ^a	1.52±0.09 +58.33 ^a
2	Free Amino Acids (mg/g wet wt)	32.46±2.35	49.74±3.86 +53.23 ^a	53.78±4.54 +65.68 ^a
3	DNA (mg/g)	3.69±0.28	6.20±0.51 + 68.02 ^a	6.63±0.56 + 79.67 ^a
4	RNA (mg/g)	6.05±0.48	9.50±0.83 + 57.02 ^a	8.39±0.75 + 38.67 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a- p<0.001 indicates the level of significance

Table 3: Effect of Eugenol and *Ocimum sanctum* Linn. leaf extract in the Liver

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Glucose (mg/g)	6.40±0.42	4.86±0.27 -24.06 ^a	3.66±0.21 - 42.81 ^a
2	Glycogen (mg/g)	5.45±0.35	8.29±0.69 + 52.11 ^a	7.74±0.52 + 42.01 ^a
3	G-6-PDH (µmoles of formazan formed/mg protein /hr)	4.16±0.34	3.27±0.28 - 21.39 ^a	2.67±0.19 - 35.81 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a- p<0.001 indicates the level of significance.

Table 4: Effect of Eugenol and *Ocimum sanctum* Linn. leaf extract in the Liver.

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Triglycerides (mg/g)	17.62±1.08	24.62±1.36 +39.72 ^a	30.03±2.16 +70.43 ^a
2	Phospholipids (mg/g)	32.35±2.23	58.16±4.61 +79.78 ^a	47.42±3.38 +46.58 ^a
3	Lipase activity (µmoles of PNPA cleaved/mg protein/hr)	4.19±0.36	8.30±0.74 +98.09 ^a	7.89±0.69 +87.58 ^a
4	Total Cholesterol (mg/g)	4.17±0.31	6.49±0.48 +55.63 ^a	8.15±0.69 +95.44 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a- p<0.001 indicates the level of significance.

Table 5: Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract in a Serum.

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Total Cholesterol (mg/dL)	162.16±13.81	197.03±16.67 +21.50 ^a	183.58±14.35 +13.20 ^a
2	HDL (mg/dL)	38.26±2.20	53.45±4.16 +39.70 ^a	42.42±3.69 +10.87 ^b
3	LDL (mg/dL)	127.11±9.05	181.69±14.14 +42.93 ^a	153.20±12.11 +20.52 ^a
3	Glucose (mg/dL)	79.08±5.41	83.94±6.37 +6.14 ^b	61.87±3.78 -21.76 ^a

Table 6: Effect of eugenol and *Ocimum sanctum* Linn. leaf extract in a Serum.

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	Triglycerides (mg/dL)	68.84±5.08	92.38±7.67 +34.19 ^a	83.61±6.34 +21.45 ^a
2	Phospholipids (mg/dL)	57.46±3.83	81.69±6.98 +42.16 ^a	76.79±6.46 +33.64 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a- p<0.001, b- p<0.01 indicates the level of significance.

Table 7: Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract in a Serum

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	SGOT (µmoles of sodium pyruvate formed/mg protein/hr)	72.39±5.32	85.02±6.78 +17.44 ^a	58.50±4.13 -19.18 ^a
2	SGPT (µmoles of sodium pyruvate formed/mg protein/hr)	59.37±4.36	67.41±3.94 +13.54 ^a	47.58±2.90 -19.85 ^a

Mean± SD of six individual observations

+ and – percent increase and decrease respectively, over control.

a- p<0.001 indicates the level of significance.

DISCUSSION

In this study, the organ weight was significantly elevated in the liver, suggests some alterations in liver metabolic activities due to the eugenol administrations. The weight of the liver was significantly increased due to the accumulation of fat around the organ weight [39]. The organ weight was significantly reduced in the liver by OS administrations. It could be attributed to increased triglyceride accumulation leading to enlarged liver which could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from the liver resulting from a deficiency of apolipoprotein B synthesis [40]. The proteins were significantly increased in liver by both administrations over control. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [41]. The liver is expected not only to perform physiological functions, but also to protect against the hazards of harmful drugs and chemicals [42].

In the present study, the Carbohydrates were significantly increased in both administrations over control. These results

clearly indicate, as duration of both administrations increases, the accumulation of carbohydrates also increases.

The lipid was significantly increased in liver by both administrations over control. The liver plays a key role in lipid metabolism. Depending on species it is, more or less, the hub of fatty acid synthesis and lipid circulation through lipoprotein synthesis. Eventually the accumulation of lipid droplets into the hepatocytes results in hepatic steatosis, which may develop as a consequence of multiple dysfunctions such as alterations in beta-oxidation, very low density lipoprotein secretion and pathways involved in the synthesis of fatty acids [43]. Hence, due to the fat metabolism is disrupted, the fat can accumulate in the liver, and accumulation of fat may also be accompanied by a progressive inflammation of the liver (hepatitis). Defects in fatty acid metabolism are responsible for pathogenesis, which may be due to imbalance in energy consumption and its combustion, resulting in lipid storage [44].

The Protease activity was significantly increased in liver by both administrations. The elevated protease activity, in general, indicates a profound loss of proteins causing structural disorganization and disassembly of structural proteins [45]. The Protease activity

was significantly increased in the liver should be resulted from tissue autolysis in the presence both administrations. Autolysis phenomena cause to protease elevation to broke lysis proteins [46]. In this study, the free amino acids were significantly increased in liver content may be due to the breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis [47]. The elevated levels of DNA and RNA in the liver. The levels of DNA and RNA progressively increased in fibrosarcoma female rat by both administrations [48]. The DNA and RNA levels were significantly increased in liver by both administrations. Therefore, increased demand of folate is postulated to be an increased hepatic level of DNA and RNA [49].

In this study, the Glucose levels were significantly reduced in liver the liver plays a central role in this process by balancing the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. The several substrate cycles in the major metabolic pathways of the liver play key roles in the regulation of glucose production [50]. The elevated levels of glycogen in the liver. This may be due to an increase in glucose uptake by the tissues following both

administrations, increased glycogen synthesis or a combination of both, probably mediated through the action of insulin [51]. These results may indicate that the increase liver glycogen of both administrations rats might be dependent upon enhanced gluconeogenesis. Then such a relatively elevated liver glycogen must be derived from other ways than glucokinase. In this present study, there was a significant reduction in Glucose-6-phosphate dehydrogenase (G6PDH) activity in the liver by both administrations. The reduction witnessed in G6PDH activity may probably be due to insulin deficiency as this enzyme activity depends on insulin. G6PDH is the key enzyme in the pentose phosphate pathway which plays a pivotal role in maintaining normal blood glucose levels. Reduction in G6PDH activity in liver is associated with obstruction in glucose utilization which results in hyperglycemia [52]. Our result shows that both administrations could improve the glycemic control by direct activation of glucose.

In the present study the triglycerides were significantly increased in liver by both administrations. Liver cells can synthesize, store triglycerides and into cholesterol. The liver is extremely active in oxidizing triglycerides to produce energy [53]. The significantly elevated of triglycerides in the

liver may be due to a number of factors such as the increased availability of fatty acids for esterification by both administration [54]. The phospholipids were significantly elevated in liver due to the enhanced cholesterogenesis or due to the decreased excretion of fecal sterols by both administrations [55]. In the present study the Lipase activity was significantly increased in liver by both administrations. This increase may be attributed to the enhanced release of fatty acids to meet the metabolic energy demand during the protection of the liver [56]. The total cholesterol levels were significantly increased in liver by both administrations. The liver is central to the regulation of cholesterol levels in the body. Not only does it synthesize cholesterol for export to other cells, but it also removes cholesterol from the body by converting it to bile salts and putting it into the bile where it can be eliminated in the feces [57]. So, in the present study some alterations in cholesterol metabolism were observed in liver. The significantly elevated of total cholesterol in the liver by both administrations, Suggest that the contains ingredients capable of enhancing the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthesis, glucose 6-phosphate dehydrogenase and HMG-CoA

reductase which are all required for cholesterol synthesis [54].

The total cholesterol level was significantly increased in serum by both administrations. Suggested that the occurrence of atherosclerosis, one of the factors that trigger cardiovascular disease, as hypertension; coronary heart and stroke [58]. A significant increase serum level of HDL in hyperlipidemic rats could play a role in prevention of coronary heart disease by both administrations [59]. A significant increase serum level of HDL in the results of this study and are controversial of cholesterol in both administrations [60]. A significant increase of LDL levels in serum was considered the best indicator of the risk of atherosclerosis, but atherosclerosis also reflects by both administrations [61]. A significant increased level of LDL this is a large and important indicator of the development of atherosclerosis in both administrations [62]. A significantly elevated in both administrations by serum glucose levels of impairment of activities glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and glucokinase [63]. A significant increases serum in glucose levels was associated with a gradual decrease in the secretion of insulin, which reached its lowest level around by the eugenol administration

[64]. A significant increases serum in glucose levels. Suggesting carbohydrate dependency of amelioration of fasting hyperbilirubinemia in eugenol administration [65]. A significantly reduced in serum glucose concentration by the OS administration may be due to these effects involving serotonin receptors, an increase in pancreatic secretion of insulin from beta cells or release of bound insulin [66]. The serum level of glucose was significantly decreased in both administrations. Although we did not show any antilipidemic effect [67]. The serum level of glucose was significantly decreased in both administrations, and this information could be recognised to the potentiation of the insulin effect on blood glucose by increasing the pancreatic secretion of insulin from β -cells or its release from bound insulin Results of the insulin release from pancreas directly indicate that the antidiabetic activity of may be through the release of insulin from the pancreas [68]. The serum level of glucose was significantly decreased in both administrations, due to rapid absorption and utilization of soluble carbohydrate and lipids by the administrations and also impaired absorption of glucose from the gut [69].

The elevated level of serum triglycerides in both administrations. The present study may be as results of decreased clearance and

increased production of the major transporters of endogenously synthesized triglycerides [70]. The Phospholipids level was significantly increased in serum by both administrations. Suggested that the Phospholipids have metabolic and structural function in mammals and are the main precursors of lipoproteins, the carriers for triglyceride transport [71].

The enzyme activity of SGOT, SGPT levels was significantly increased in Serum by eugenol administration. The above findings for SGPT and SGOT are in accordance with the earlier findings recorded by which might be due to alteration in the cell membrane permeability which may permit these enzymes to leak from the cells with intact membrane, when there is stress or any damage to the liver cells [72].

The enzyme activity of SGOT, SGPT levels was significantly increased in Serum by OS administration. This may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells [73].

CONCLUSION

The weight of the liver was significantly increased due to the accumulation of fat around the organ weight. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical

pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. The elevated levels of DNA and RNA in the liver. The levels of DNA and RNA progressively increased in fibro sarcoma female rat by both administrations. The serum level of glucose was significantly decreased in both administrations, due to rapid absorption and utilization of soluble carbohydrate and lipids by the administrations and also impaired absorption of glucose from the gut. The enzyme activity of SGOT, SGPT levels was significantly increased in Serum by OS administration. This may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells.

Highlights

1. Eugenol and *Ocimum sanctum* Linn. leaf extract on Liver and Serum biochemical alterations.
2. The DNA and RNA levels were significantly increased in liver by both administrations
3. The elevated levels of DNA and RNA in the liver. The levels of DNA and RNA progressively increased in fibrosarcoma female rat by both administrations
4. The enzyme activity of SGOT, SGPT levels was significantly increased in

Serum by OS administration. Which may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

- [1] Ahirwar D, Ahirwar B, Kharya MD (2008). Reversible Antifertility Activity of Hydro alcoholic Extract of *Ficus Racemosa* L. in male Mice. *J of Reproduct & Contracep.* 22(1): 37-44.
- [2] Musa TY, Bimbo BB (2009). Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Contracep.* 80: 308-313.
- [3] Andey M, Debnath M, Gupta M, Chikara SK (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *J of Pharm and Phyto.* 3 (3): 27-37.
- [4] Veerapura VP, Thippeswamy BS, Prabhakar KR, Nagakannana P, Shivasharana BD, Bansal P, Sneha SD, Mishra B, Priyadarsini KI,

- Unnikrishnanb MK (2011). Antioxidant and renoprotective activities of *Ficus racemosa* Linn. Stem bark: Bioactivity guided fractionation study. *Bio & Pre Nut.*1: 273–281.
- [5] Sen P (1993). Therapeutic potentials of Tulsi : from experience to facts. *Drugs News & Views.* 1(2):15–21
- [6] Rajeshwari S (1992). *Ocimum sanctum*. The Indian home remedy. In: *Current Medical Scene*, March-April (Edited and published by S. Rajeshwari, Cipla Ltd., Bombay Central, Bombay).
- [7] Mukherji S.P (1995). *Ocimum* - a cheap source of Eugenol. *Science Reporter* 1987, p. 599. 31. Eugenol-rich *Ocimum* variety released. In: *CSIR NEWS*(Published by PID CSIR, New Delhi).45:256.
- [8] Khanna N and Bhatia J (2003). Action of *Ocimum sanctum*(Tulsi) in mice: possible mechanism involved. *J Ethnopharmacology.* 88(2–3): 293–296.
- [9] Gupta SK, Prakash J, Srivastava S (2002). Validation of claim of Tulsi, *Ocimum sanctum* Linn as a medicinal plant. *Indian J Experimental Biology.* 40(7): 765–773.
- [10] Reghunandana R, Sood S, Reghunandana V, Mehta RM, Singh GP (1995). Effect of *Ocimum sanctum* Linn (Tulsi) extract on testicular function. *Indian J Medical Research.* 49(4): 83–87.
- [11] Bhargava KP, Singh N (1981). Antistress activity of *Ocimum sanctum* Linn. *Indian J Medical Research.*73: 443–451.
- [12] Nagarajun S, Jain HC, Aulakh GS (1989). Indigenous plants used in the control of Diabetes. In: *Cultivation and utilization of medicinal plants.* Editors: Atal CK and Kapoor BM (Published by PID CSJR). 584
- [13] Sarkar A, Pandey DN, Pant MC (1994). Changes in the blood lipid profile level after administration of *Ocimum sanctum* (Tulsi) leaves in the normal albino rabbits. *Indian J Physiology Pharmacology.*38(4): 311–312.
- [14] Chopra RN, Chopra IC, Handa KL, Kapoor LD (1993). *Indigenous drugs of India* (Published by UN Dhar, Pvt. Ltd., Calcutta).
- [15] Kirtikar KR and Basu BD (1965). In: *Ocimum sanctum* in Indian Medicinal Plants (Published by LB Basu, Allahabad).
- [16] Batta SK and Santhakumari G (1971). The antifertility effect of *Ocimum sanctum* and *Hibiscus Rosa Sinensis*. *Indian J Medical Research.* 59: 777–781.
- [17] Khanna S, Gupta SR, Grover SK (1986). Effect of long term feeding of Tulsi. *Indian J Experimental Biology.* 24: 302–304.

- [18] Das SK and Vasudevan DM (2006). Tulsi: The Indian holy power plant. *Natural Product Radiance*.5:279-83.
- [19] Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003). *A Hand Book of Medicinal Plant*, 1st Ed. Agrobios, India. 367.
- [20] WHO (1983) Protocol CG-40. Preparation of Alcoholic Extract for Bioassay and Phytochemical Studies. (APJF/IP, 1001 A). Geneva. World Health Organization.
- [21] Shankar Mondal, Bijay R, Mirdha and Suhil C, Mahapatra (2009). The science behind sacredness of tulsi (*Ocimum sanctum* Linn), *Indian J Physiol Pharmacol*. 53 (4): 291–306.
- [22] Kulkarni D (2011). Eugenol induced changes in reproductive cycle of female albino rats. *Biosci Biotech Res Comm*. 4(1): 98-101.
- [23] Lowry OH, Rosenberg NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin–phenol reagent. *J Biol Chem*. 193:265–71.
- [24] Carrol NV, Longley HM, Roe JH (1956). Glycogen determination in the liver and muscle by use of anthrone reagent. *J Biol Chem*. 220: 583–95.
- [25] Folch JM, Lees MP, Stana-stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*.226:497–505.
- [26] Moore S and Stein WH (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J BioL Chem*. 211: 907-913.
- [27] Munro HN, Fleck A (1966). The determination of nucleic acids, *Meth Biochem Anal*. 14: 113-176.
- [28] Mendel BA, Kemp and DK Mayes (1954). A colorimetric micro method for the determination of glucose. *Biochem J*. 56: 639-645.
- [29] Lohr GW, Waller HD (1965). Glucose-6-Phosphate dehydrogenase. In: Bergmeyer HU (ed) *Methods of enzymatic analysis*, Verlag Chemie Weinheim. 744-752
- [30] Natelson S (1971b). Triglycerides procedure in: *techniques of clinical chemistry*, Charless C Thomas Publishers Springfield Illinois USA 3rd edn. 273-280.
- [31] Zilversmidth DB and Davis BS (1950). Micro determination of plasma phospholipids by trichloroacetic acid precipitation. *J Lab Clin Med*. 35: 155-160.
- [32] Huggins C and Lapidés J (1955). Lipase; in *Methods in Eric ymology y I eds S P Colowick and N O Kaplan* (New York: Academic Press). 627-642
- [33] Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu (1974).

- Enzymatic determination of total serum cholesterol. Clin. Chem. 20: 470-475.
- [34] Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem. 23:882-4.
- [35] Sacks DB (1998). Carbohydrate. In: Tietz Textbook of Clinical Chemistry. 3rd ed. W. B. Saunders Company, U.S.A.
- [36] Bergmayer HU, Bernt E (1974). Methods of enzymatic analysis. Verlag Chemie, Weinheim, Academic Press, London.
- [37] Tietz NW (2011). Fundamentals of Clinical Chemistry. W. B. Saunders Company. Amah C. Ifeanyi, Yama O. Eboetse, Duru F. Ikechukwu, Osinubi A. Adewale, Noronha C. Carmel, and Okanlawon A. Olugbenga. effect of momordica charantia on estrous cycle of sprague-dawley rats. Pacific Journal of Medical Sciences. 8(1):37-48.
- [38] Fischer, R.A (1950). Statistical methods for research workers Oliver Boyd. London.
- [39] Vishnu Priya Korukanti, Himabindu Ponnamp, Butchi Raju Akondi (2013). Evaluation of antiobesity activity of Fucus vesiculosus. Indian Journal of Research in Homoeopathy. 7 (3):126-132.
- [40] Muhammad Zafar & Syed Naeem-ul-Hassan Naqvi (2010). Effects of STZ-Induced Diabetes on the Relative Weights of Kidney, Liver and Pancreas in Albino Rats: A Comparative Study. Int. J. Morphol. 28(1):135-142.
- [41] Ward FM and Daly MJ (1999). Hepatic disease In. Clinical Pharmacy and Therapeutics Walker R Edward C Eds. Sci Pharm. 195-212.
- [42] Muthu Gounder Palanivel, Balasubramanian Rajkapoor, Raju Senthil Kumar, John Wilking Einstein, Ekambaram Premkumar, Mani Rupes Kumar, Kunchu Kavitha, Mohan Raj Pradeep Kumar, Balasundaram Jayakar (2008). Hepatoprotective and Antioxidant effect of Pisonia aculeate L against CCl4-Induced Hepatic Damage in Rats. Sci pharm. 76: 203-215.
- [43] Nguyen P, Leray V, Diez M, Serisier S, Le Bloc'h J, Siliart B, Dumon H (2008). Liver lipid metabolism. J Anim Physiol Anim Nutr (Berl). 92(3): 272-83.
- [44] Reddy JK and Rao MS (2006). Lipid metabolism and liver inflammation II Fatty liver disease and fatty acid oxidation. Am J Physiol Gastrointest Liver Physiol. 290 (5): 852-8.
- [45] Savithri Y, Ravi Sekhar P, Narasimha Rao C and Srineetha U (2016). Toxicity of chlorpyrifos on protease and glutamate dehydrogenase enzyme

- activities in albino rats. *International Journal of Bioassays*. 5(2): 4744-4747.
- [46] Narges Anasori, AliFarahnak, TaghiGolmohamadi, Mohamadreza Eshraghian, Mohamadbagher Molaei Rad (2008). Comparative assay of protease enzyme activity in protoscolices (Hydatid cyst) and liver tissues. *Journal of Paramedical Sciences*. 6 (1): 49-78.
- [47] Sharma Dilip K and Ansari Badre A (2011). Effect of Deltamethrin and a Neem Based Pesticide Ahook on Some Biochemical Parameters in Tissues Liver, Ovary and Muscle of Zebrafish *Danio rerio* (Cyprinidae). *Research Journal of Chemical Sciences*. 1 (4): 125-134.
- [48] Selva Kumar Sivagnanam, Mudiganti Ram Krishna Rao, and Maruthaiveeran Periyasamy Bala Subramanian (2012). Chemotherapeutic Efficacy of *Indigofera aspalathoides* on 20-Methylcholanthrene-Induced Fibrosarcoma in Rats. *International Scholarly Research Network*. 1-7.
- [49] Manokaran Kalaiselvi, Rajasekaran Narmadha, Paramasivam Ragavendran, Ganesan Ravikumar, Duraisamy Gomathi, Dominic Sophia, Chinthamony arul Raj, Chandrasekar uma and Kalaivani K (2012). In vivo and in vitro antitumor activity of *jasminum sambac* (linn) ait oleaceae flower against dalton's ascites lymphoma induced swiss albino mice. *Int J Pharm Pharm Sci*. 4(1): 144-147.
- [50] Govardhan Naik A, Vengaiah V, Lalithamma A, Changamma C (2014). Effect of Betel leaf stalk Extract on Liver and Serum Profiles with Reference to Carbohydrate Metabolism in Male Albino Rats. *International Journal of Innovative Research in Science, Engineering and Technology*. 3(3): 9971-9976.
- [51] Jasmine R (2014). Restoration of Liver and Muscle Glycogen by *Eugenia jambolana* in Diabetic Rats. *International Journal of Research in Pharmacy and Biosciences*. 1(2):16-21.
- [52] Sarah Onyenibe Nwozo, Ayodeji Babatope Afolabi, Babatunji Emmanuel Oyinloye (2016). Antioxidant, lipid modulating and hypoglycemic effects of the aqueous extract of *Anacardium occidentale* leave in streptozotocin-induced diabetic rats. *J Mol Pathophysiol*. 5(4):59-65.
- [53] Bowen R (2001). Secretion of Bile and the role of Bile acids in Digestion, Colorado State Hypertext book article on Bile, 2001, Achieved from the original on.29.
- [54] Oyewole OI, Adanlawo IG and Arise RO (2013). Serum and tissue lipid profile in wistar rats

- administered leaf extract of *Ficus exasperate*. *Annals of Biological Research*. 4(2):288-291.
- [55] Kottaimuthu A, Sethupathy S, Manavalan & Karar PK (2005). Hypolipidemic effect of methanolic extract of *Dolichos biflorus* Linn In high fat diet rats. *Indian J Exp Biol*. 43(6):522-25.
- [56] Pratibha devarshi, Aruna kanase, Ravindra kanase, Sadashiv mane, Subhash patil and Varute (1986). Effect of mandur bhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCl₄ induced hepatic injury. *J Biosci*. 10(2): 227-234.
- [57] Rao DS (1970). Effect of curcumin on serum and liver cholesterol levels in the rat. *J Nutr*. 100:1307-1315.
- [58] Noor Nailis Saadah A, Kristanti Indah Purwani, Awik Puji Dyah Nurhayati, Nova Maulidina Ashuri (2016). Analysis of Lipid Profile and Atherogenic Index in Hyperlipidemic Rat (*Rattus norvegicus* Berkenhout, 1769) that Given The Methanolic Extract of Parijoto (*Medinilla speciosa*). *Proceeding of International Biology Conference*. 1-8.
- [59] Vijayaraj P, Kannan M, Jayaraja S, Vasanthi N (2011). Antihyperlipidemic activity of *Cassia auriculata* flowers in triton WR 1339 induced hyperlipidemic rats. *Experimental and Toxicologic Pathology*. 65:135-41.
- [60] Kokab Namakin, Fatemeh Tavakoli1, Mahmoud Zardast (2015). Effect of Vitamin D supplementation on Lipid Profile in Children Aged 10-14 Years Old. *Int J Pediatr*. 3(5.2): 987-994.
- [61] Adams LB (2005). Guidelines for Adolescent Nutrition Services. Division of Epidemiology and Community Health, School of Public Health, University of Minnesota..
- [62] Fatma Mustafa Mohammad, Nihad AbdulJabbar Jalal and Chateen I Ali Pambuk (2017). A Comparative Study to Evaluate the Serum Lipid Profile in Pre and Postmenopausal Woman in Sulaymaniyah City Iraq. Chateen I Ali Pambuk. *Biomed J Sci & Tech Res*. 1(3):1-6.
- [63] Mohamed Z Gada, Noha a Ehssanb, Mansour H Ghieta and Lobna F Wahmanb (2010). Pioglitazone versus metformin in two rat models of glucose intolerance and diabetes. *Pak. J. Pharm. Sci*. 23 (3): 305-312.
- [64] Shahram Javadi, Siamak Asri-Rezaei, Maryam Allahverdizadeh (2014). Interrelationship of beta-2 microglobulin, blood urea nitrogen and creatinine in streptozotocin-induced diabetes mellitus in rabbits. *Veterinary Research Forum*. 5(1): 7 – 11.

- [65] Azizi F and Rasouli HA (1987).Serum glucose, bilirubin, calcium, phosphorus, protein and albumin concentrations during ramadan. Medical Journal of The Islamic Republic of Iran. 1:38-41.
- [66] Sanjeev Kumar, Ashok Kumar1 and Astha Chandra (2013). Alteration in serum biochemical parameters due to garlic (*Allium sativum*) supplementation in broilers' diets. African Journal of Biotechnology. 12(29): 4691-4698.
- [67] Mohammad Reza Shahraki, Mohammad Reza Arab Ebrahim Mirimokaddam and Mony Jey Palan (2007). The Effect of *Teucrium polium* (Calpoureh) on Liver function, Serum Lipids and Glucose in Diabetic Male Rats. Iranian Biomedical Journal. 11 (1): 65-68.
- [68] Aravind Patchimatla, Sainath Reddy Kankanala, Sharavanabhava Sheshagiri Bandaru, Umasankar Kulindaivelu, Venkateshwar Rao Jupally, Venkateshwarlu Eggadi (2014). Investigation of lipid profile and ocular oxidative stress of *Chloroxylon swietenia* on Streptozotocin nicotinamide induced diabetic rats. International Journal of Green Pharmacy. 90-96.
- [69] Zeinab K and Ebrahim (2018). Effect of Gastrointestinal Parasites Infestation on Some Hematological and Biochemical Parameters in Sheep. Alexandria Journal of Veterinary Sciences. 59 (2): 44-47.
- [70] Eman GE, Helal and Mohamed MA (2006). Shahat. Hypolipidimic effect of some medicinal plants on diabetic rats. The Egyptian Journal of Hospital Medicine. 23: 200 – 211.
- [71] Brijender Bhushan, Sunita Pande, Nishi Saxena, Prabhu Narain Saxena (2013). Serum biochemical responses under stress of cypermethrin in albino rat. Environmental and Experimental Biology. 11: 81–89.
- [72] Farheen A. Fani, S.P. Mehesare, D.B. Pawshe, K.M. Khan and N. D. Jadhav (2008). Hematological and Biochemical changes during Epidural Xylazine hydrochloride anaesthesia in Dogs. Veterinary World.1(6): 175-177,.
- [73] Sanjeev Kumar, Ashok Kumar1 and Astha Chandra (2013). Alteration in serum biochemical parameters due to garlic (*Allium sativum*) supplementation in broilers' diets. African Journal of Biotechnology. 12(29): 4691-4698.