IJPSR (2017), Volume 8, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 March, 2017; received in revised form, 17 May, 2017; accepted, 27 May, 2017; published 01 November, 2017

THE EFFECTIVE ROLE OF URSOLIC ACID AND *OCIMUM SANCTUM* (LINN.) LEAF EXTRACT ON SOME MARKER CARBOHYDRATE METABOLITES IN MALE ALBINO RATS

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Keywords:

Carbohydrates, Glucose, G-6 PDH, LDH, Proteins, SDH

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ABSTRACT: Ursolic acid is a pentacyclic triterpinoid compound, the major constituents of the Tulsi leaves, has been suggested to possess anti-fertility effect in rats of both sexes. This study has attempted to investigate the anti-fertility potentials of *Ocimum sanctum* leaf extract and ursolic acid in male albino rats. Three groups of male albino rats were taken, first group as control, second group were administered with *Ocimum sanctum* leaf extract and third group with ursolic acid. The administrations of OS and UA bring the alterations in carbohydrate metabolizing enzymes and lipid composition which leads to changes in functional quality of spermatozoa. The reduced lipid in seminal vesicle and prostate represents the lowered lipid metabolism and lipogenesis, suggesting some alterations in the chemical composition of the seminal plasma and prostatic fluids. The elevated glucose levels in testes and epididymis due to the decreased uptake by the spermatogenic cells in OS and UA administered rats. The administrations may mediate the up regulation of the G6PDH with increased production of ribose-5-phosphate which enhances DNA synthesis and thus increased cell proliferation of the hepatocytes. Besides the up regulation of G6PDH that was observed in the liver in this experiment, it can be suggested that in the administration of *O. sanctum*, carbohydrates are facilitated more along the hexose monophosphate pathway.

INTRODUCTION: In recent years, there has been a concern about the use of plant products in affecting fertility of humans. Many plants have been identified and tested for their antifertility effect in both rats and mice [1]. Literature reports state *Ocimum sanctum* have, anti-hyperlipidemic, anti-lipid peroxidative, anti-oxidant, anti-ulcer, cardio protective, anti-fertility [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(11).4624-36

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (11).4624-36

Ocimum sanctum (Linn.), also known as Tulsi, is an aromatic plant belongs to the family Lamiaceae has a number of medicinal properties, these medicinal properties are due to the presence of phytochemical constituents such as tannins, saponin, flavonoids, steroid, and terpenoids. Several plants have been confirmed as antifertility according to the literature flavonoids and plumbagin (napthaquinone) are known to exhibit antifertility activity [3 - 4]. The abortifacient activity of several plant extract may be due to the presence of flavonoids. Supplementation with phytochemical compounds has received significant attention for its potential applications. The phytochemical constituents of *O. sanctum* are ursolic acid, α-triterpenoid and rosmarinic acid (phenyipropanoid).

It contains volatile oil comprising mainly of eugenol and β-caryophyllene with minor terpenes like bornyl acetate, β-elemene, methyl eugenol, neral, β-pinene etc. The stem and leaf of *O. sanctum* contains a variety of chemical compounds which include saponins, flavonoids, terpinoids and tannins [5-6]. Ursolic acid is a pentacyclic triterpinoid compound that naturally occurs in fruits, leaves and flowers of medicinal herbs. Recently, UA has been demonstrated to have important biological roles including anti-fat accumulation, anti-insulin resistance *via* IGF-1, anti-muscle atrophy, anti-cancer, anti-oxidation, anti-inflammatory effects [7], and antifertility effect [2, 8-9]. Recent studies show that ursolic acid suppresses cell proliferation *via* the induction of apoptosis in various prostate cells, including PC-3 cells, DU145 cells, and primary human prostate cancer cells [10-13]. Ursolic acid, one of the major constituents of the Tulsi leaves, has been suggested to possess antifertility effect in rats of both sexes and in male mice. Ursolic acid because of its anti-estrogenic effect reduces spermatogenesis and causes a decrease in sperm counts [2]. UA has been shown to have the potential of inhibiting sperm motility, and also possess antifertility activity in rats and mice [14]. Hence, this study was aimed to evaluate whether ursolic acid present in *Ocimum sanctum* leaf extract showing effect on fertility potentials. Thus the present study was focused on the effect of *Ocimum sanctum* leaf extract and Ursolic acid on reproductive tissues of male albino rats.

MATERIALS AND METHODS:

Preparation of Extract: The leaf extract of *Ocimum sanctum* (Linn.) was prepared by following the standard protocol (WHO, 1983) [15]. The leaves of *Ocimum sanctum* L. were collected and extract was prepared by adding 500g of dried, crushed and powdered leaves of *Ocimum sanctum* in 1500 ml of 95 % ethanol in a round bottom flask and was kept at room temperature for 3 days in dark place. The mixture was filtered through muslin cloth and Whatman filter paper. The filters were aliquots by using rotary evaporator; the remnants of ethanol content were removed by drying at 40-50 °C. The extract was subjected to further concentration by keeping in water bath for complete dryness. The final yield of leaf extract weighed 30g (6%), the extract obtained in this method were stored for future use at 4 °C.

Experimental Design: In the present study healthy adult (4 months old, weight 160 ± 10g) male wistar strain albino rats were obtained from an authorized vendor (Sri Venkateswara Traders, Bangalore, India). Upon arrival, rats were housed in polypropylene cages containing sterilized paddy husk as the bedding material, and provided with filtered tap water and standard rat pellet diet *ad libitum*. Animals were maintained in a well-controlled laboratory facility (temperature 26+2 °C; 12: 12hr light: dark cycle, humidity 50 ± 5%). The usage of animals was approved by the Institutional Animal Ethics Committee (IAEC) (Regd. No. 438/01/a/ CPCSEA/ dt.17/07/2001) at S. V. University, Tirupati, India.

Study Protocol: All the animals were grouped into three groups in which the first group as control group (Group-I), the second and third groups are experimental groups in which the second group (Group-II) was given *Ocimum sanctum* leaf extract and third group (Group-III) was given ursolic acid an bioactive compound obtained from the Sigma Aldrich was given. The group I animals were administered 1ml distilled water /rat/day orally for 20 days and the test group II received ethanol extract of *Ocimum sanctum* L. leaves of 500mg/kg/day orally for 20 days [16], while group III receives ursolic acid 10mg/kg/day for 20 days [17]. Six animals from each group were used for test. 24 hours after the last dose, the control and treated animals were sacrificed by cervical dislocation and the tissues like testes, epididymis, seminal vesicle prostate gland and liver were isolated, chilled immediately and blood was collected, serum was separated and used for biochemical analysis. The Proteins[18], Carbohydrates [19], Lipids [20], Glucose [21], lactic acid [22], pyruvic acid [23], Glucose-6-phosphatedehydrogenase [24], Succinate dehydrogenase [25], and Lactatedehydrogenase [26] were estimated biochemically both in control and experimental rat tissues such as testis, epididymis, seminal vesicle, prostate gland, liver and serum.

Statistical Analysis: The data are expressed as Mean values with their SD. Reading of the six different groups were compared using one-way ANOVA analysis with Dunnetts Multiple Comprarision Test. Statistical analysis was performed using SPSS (Version 16.5; SPSS Inc., Chicogo, IL, USA). Using M. S. Office, Excel

Software the data has been analyzed for the significance of the main effects (factors) and treatments along with their interaction. The results were presented with F-value was found to the significant with 'P' value less than a-indicates P < 0.001, b-indicates P < 0.01, c-indicates P < 0.05 the level of significance and d-indicates Non significant changes.

RESULTS: The results in **Fig. 1 - 6** represents the effect of *Ocimum sanctum* leaf extract and ursolic acid administration on total proteins, total carbohydrates and total lipids in reproductive tissues like testes, epididymis, seminal vesicle and prostate gland and non reproductive tissue like liver and serum of male albino rats. The total proteins were significantly reduced in all tissues in both administrations. In ursolic acid administration these were elevated in seminal vesicle (+23.64%, P<0.001). The testicular carbohydrates were decreased in both administrations (-21.86%, P<0.001; - 14.74%, P<0.05) while in sex accessory tissues these were elevated.

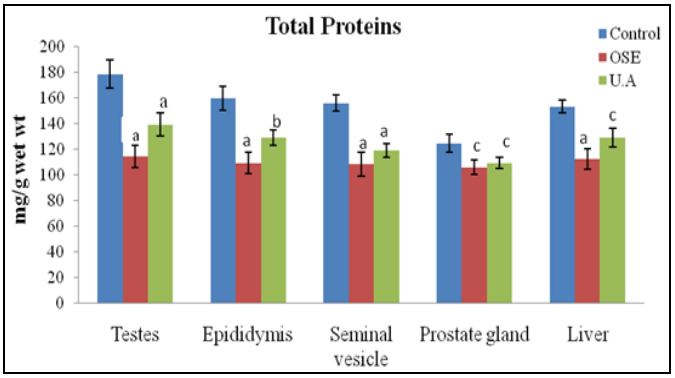

FIG. 1A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL PROTEINS

Each bar represents Mean ± SD of six individual observations a- P < 0.001, b- P < 0.01 and c- P < 0.05 indicates the level of significance.

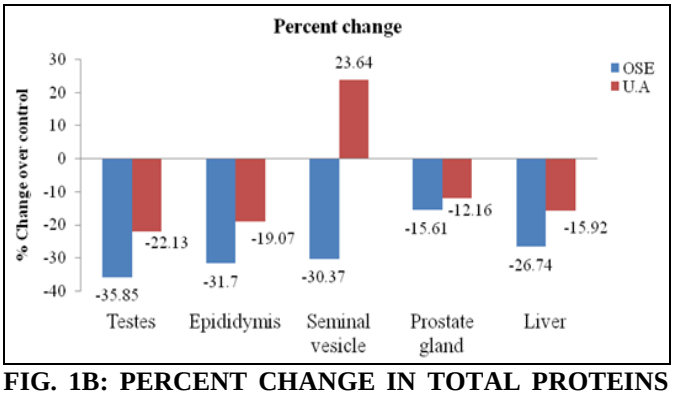

FIG. 1B: PERCENT CHANGE IN TOTAL PROTEINS OVER CONTROL AND URSOLIC ACID

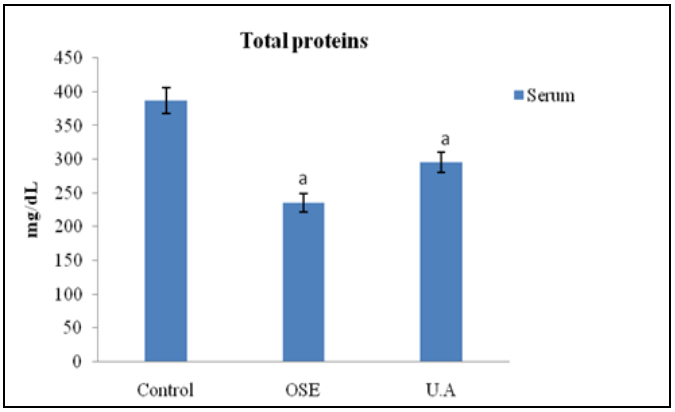

FIG. 2A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL PROTEINS IN SERUM

Each bar represents Mean ± SD of six individual observations a –P < 0.001 indicates the level of significance.

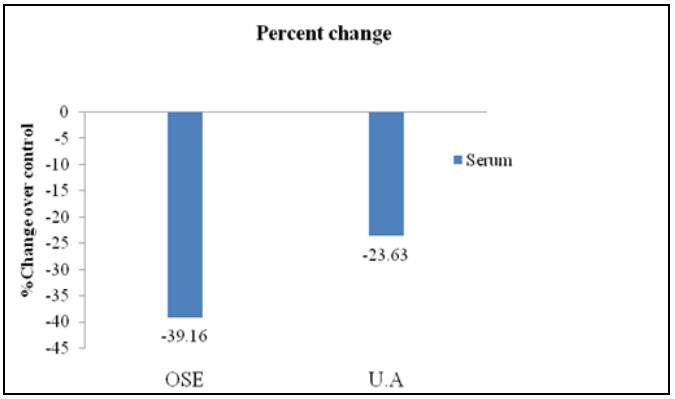

FIG. 2B: PERCENT CHANGE IN TOTAL PROTEINS IN SERUM OVER CONTROL AND URSOLIC ACID

The hepatic carbohydrates (-13.71%, P<0.01) were significantly lowered by OS administration and elevated by UA administration. The testicular lipids were increased in both administrations, but less in UA administration.

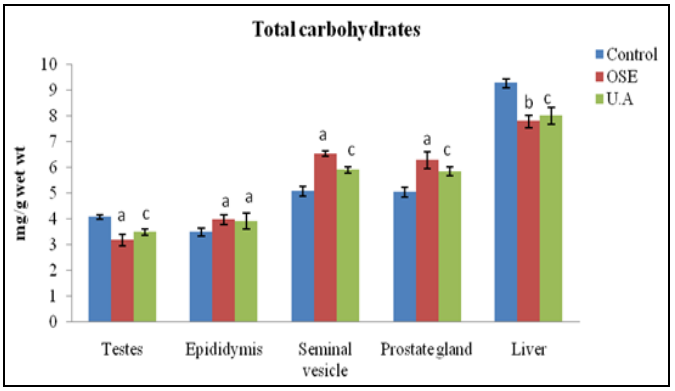

FIG. 3A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL CARBOHYDRATES

Each bar represents Mean± SD of six individual observations a- P < 0.001, b- P < 0.01 and c- P < 0.05 indicates the level of significance.

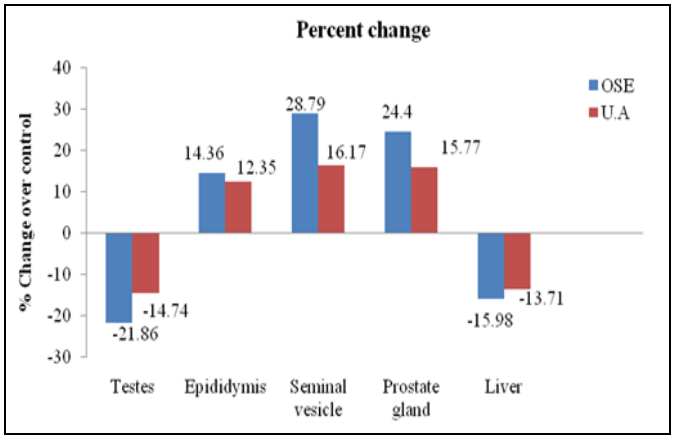

FIG. 3B: PERCENT CHANGE IN TOTAL CARBOHYDRATES OVER CONTROL AND URSOLIC ACID

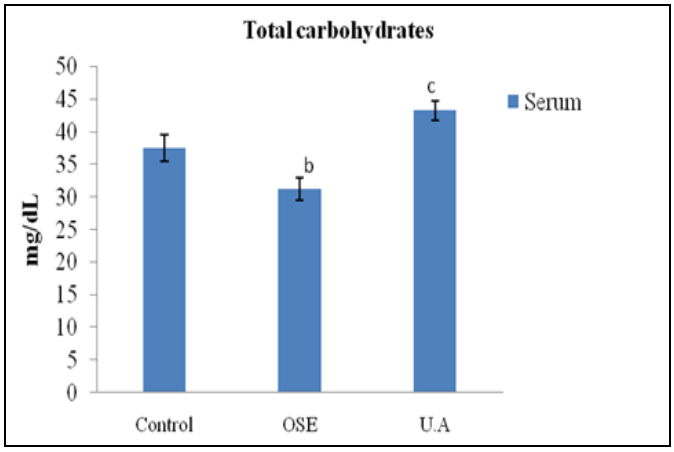

FIG. 4A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL CARBOHYDRATES IN SERUM

Each bar represents Mean ± SD of six individual observations b- P < 0.01 and c- P < 0.05 indicates the level of significance.

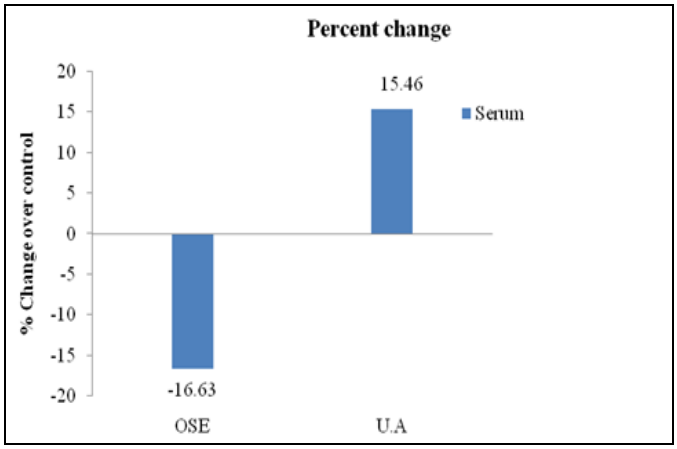

FIG. 4B: PERCENT CHANGE IN TOTAL CARBOHYDRATES IN SERUM OVER CONTROL AND URSOLIC ACID

In sex accessories these were reduced except in epididymis (+15.21%, P<0.05) in ursolic acid administration. However, the serum total lipids were enhanced by both administrations.

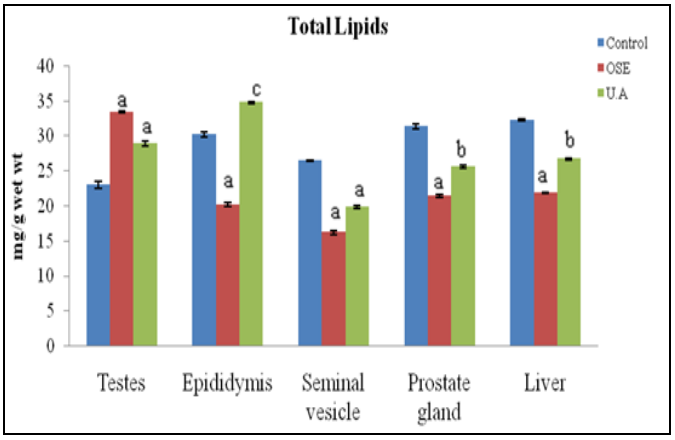

FIG. 5A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL LIPIDS

Each bar represents Mean ± SD of six individual observations a- P < 0.001, b- P < 0.01 and c- P<0.05 indicates the level of significance.

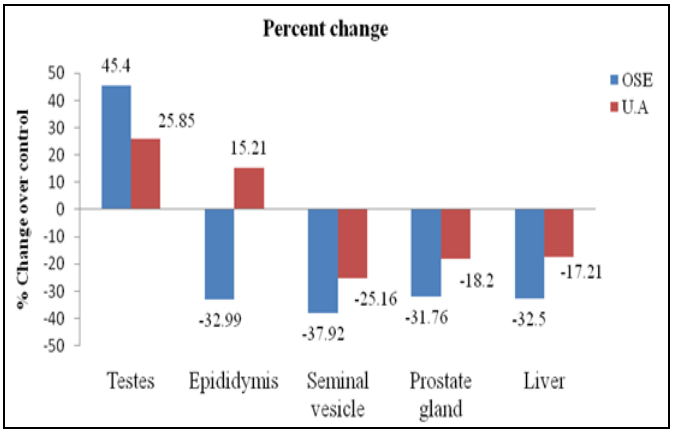

FIG. 5B: PERCENT CHANGE IN TOTAL LIPIDS OVER CONTROL AND URSOLIC ACID

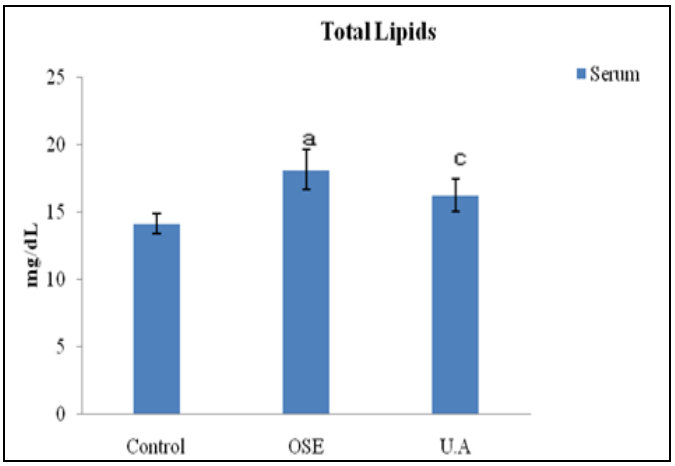

FIG. 6A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL LIPIDS IN SERUM

Each bar represents Mean ± SD of six individual observations a- P < 0.001, and c- P < 0.05 indicates the level of significance

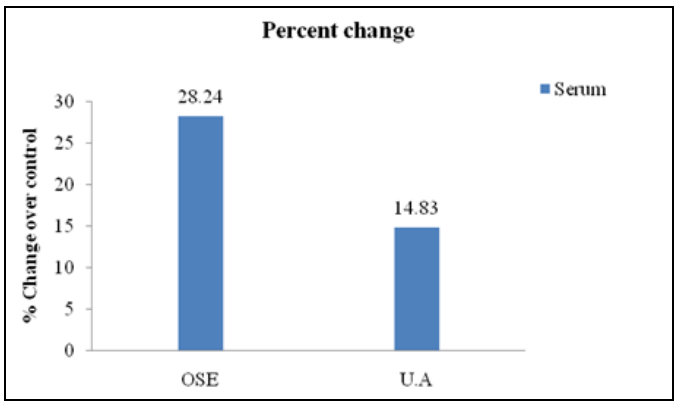

FIG. 6B: PERCENT CHANGE IN TOTAL LIPIDS IN SERUM OVER CONTROL AND URSOLIC ACID

The data represented in **Fig. 7 - 12** showed the effect of *Ocimum sanctum* leaf extract and ursolic acid administration on Glucose, Lactic acid and Pyruvic acid. The glucose levels were significantly increased in testes and epididymis and decreased in seminal vesicle and no significant changes in prostate gland.

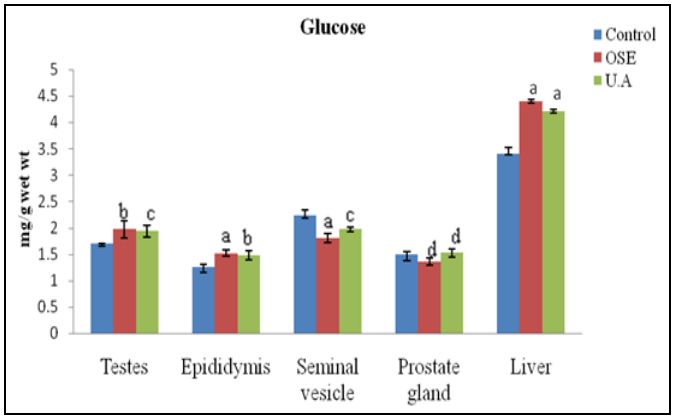

FIG. 7A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON GLUCOSE

Each bar represents Mean ± SD of six individual observations a- P < 0.001, b- P < 0.01 and c- P < 0.05 indicates the level of significance, d- Indicates Non significant changes.

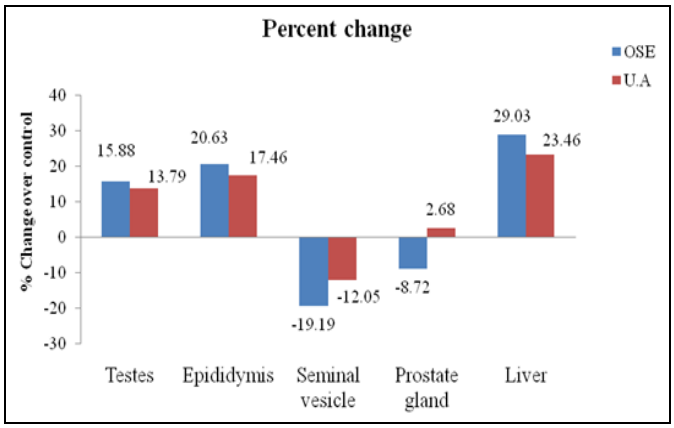

FIG. 7B: PERCENT CHANGE IN GLUCOSE OVER CONTROL AND URSOLIC ACID

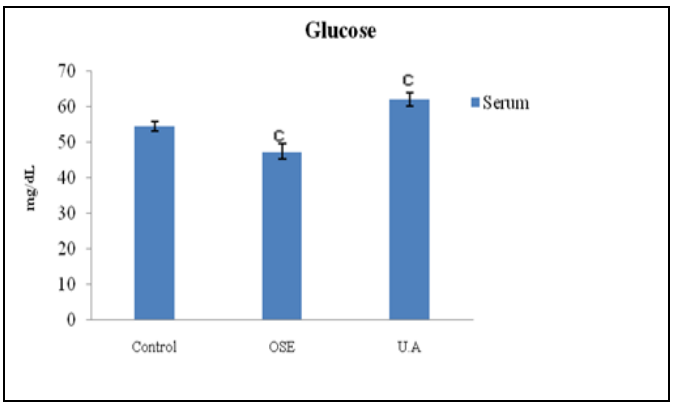

FIG. 8A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON GLUCOSE IN SERUM

Each bar represents Mean ± SD of six individual observations c- P < 0.05 indicates the level of significance

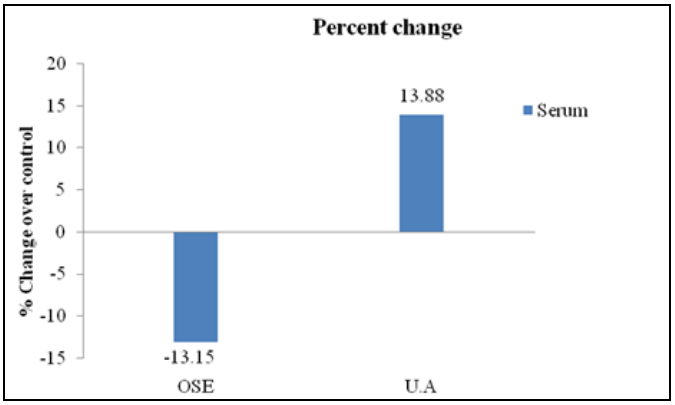

FIG. 8B: PERCENT CHANGE IN GLUCOSE IN SERUM OVER CONTROL AND URSOLIC ACID

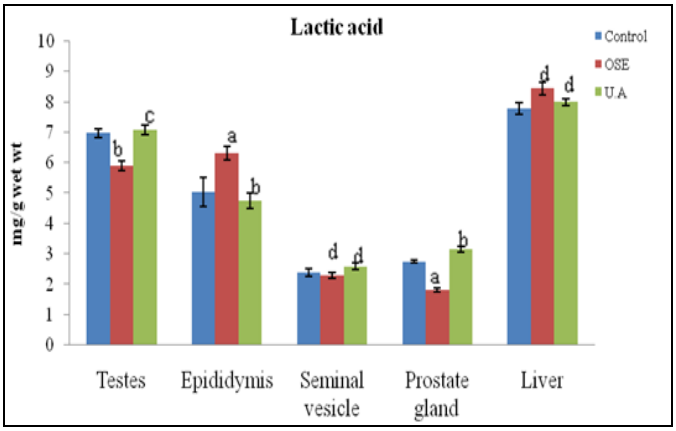

FIG. 9A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON LACTIC ACID

Each bar represents Mean ± SD of six individual observations a- P < 0.001, a- P < 0.01 and c- P < 0.05 indicates the level of significance, d- Indicates Non significant changes.

The glucose content in liver increased (+29.03, P < 0.001) and in serum decreased (-13.15%, P < 0.05) significantly in OS administration. A significant reduction and elevation was noticed in testicular

lactic acid and pyruvic acid by OS administration, but in US administration both were elevated.

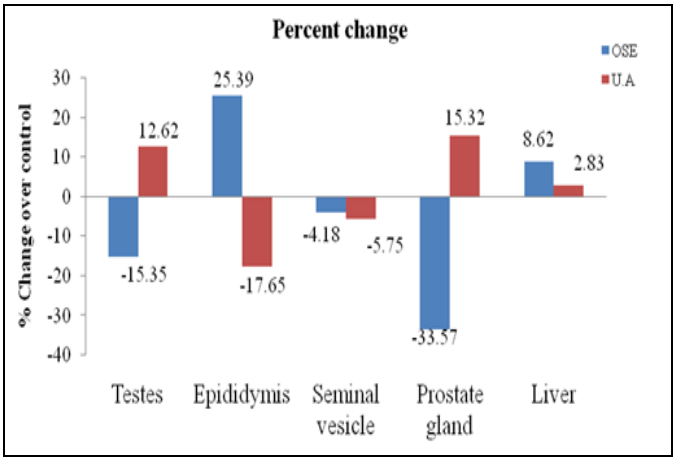

FIG. 9B: PERCENT CHANGE IN LACTIC ACID OVER CONTROL AND URSOLIC ACID

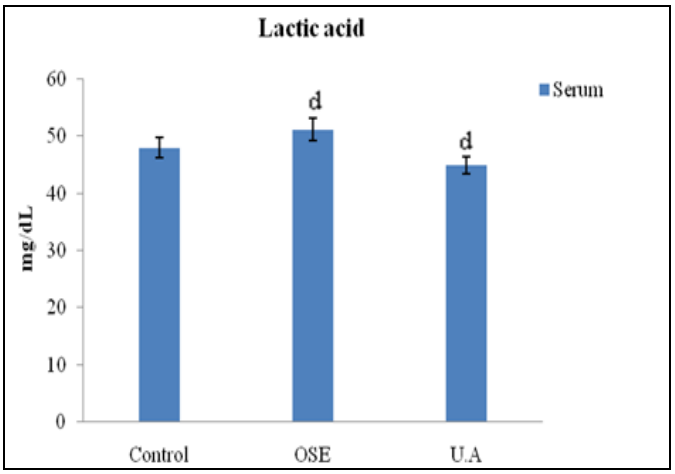

FIG. 10A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON LACTIC ACID IN SERUM

Each bar represents Mean ± SD of six individual observations
d- Indicates Non significant changes.

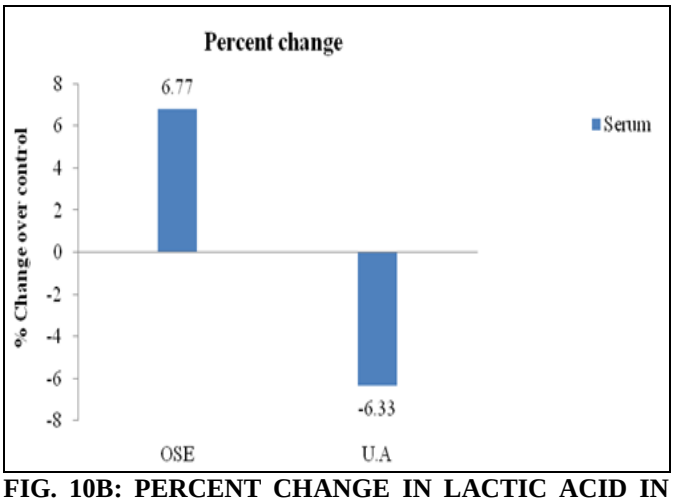

FIG. 10B: PERCENT CHANGE IN LACTIC ACID IN SERUM OVER CONTROL AND URSOLIC ACID

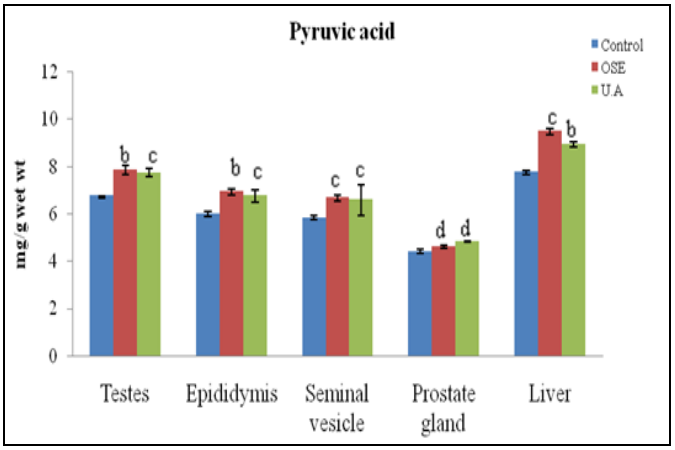

FIG. 11A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON PYRUVIC ACID

Each bar represents Mean ± SD of six individual observations
a- P < 0.001, a- P < 0.01 and c- P < 0.05 indicates the level of significance; d- Indicates Non significant changes.

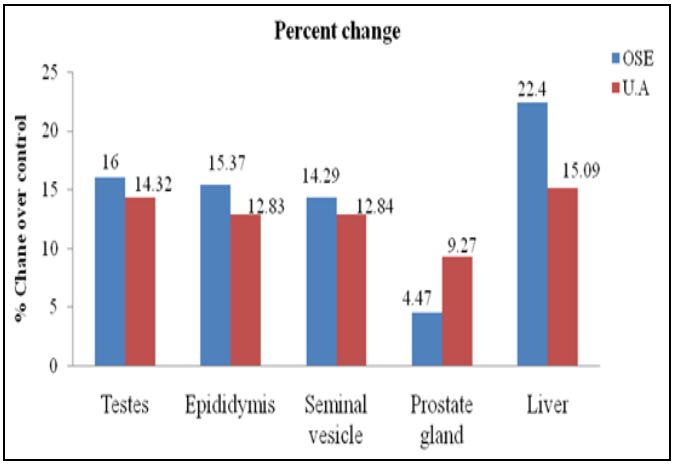

FIG. 11B: PERCENT CHANGE IN PYRUVIC ACID OVER CONTROL AND URSOLIC ACID

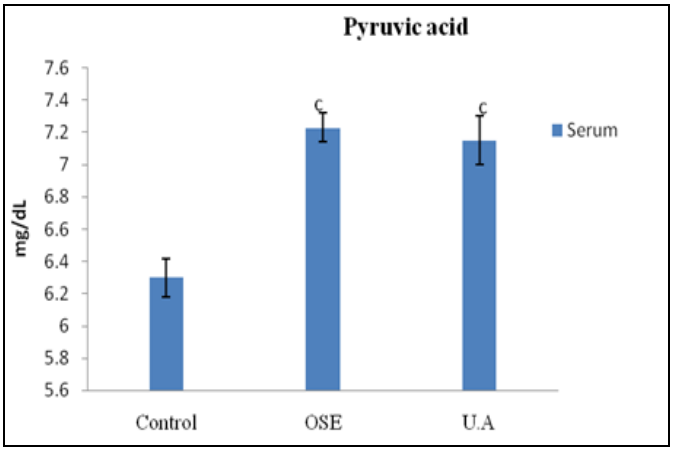

FIG. 12A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON PYRUVIC ACID IN SERUM

Each bar represents Mean± SD of six individual observations
c- P < 0.05 indicates the level of significance.

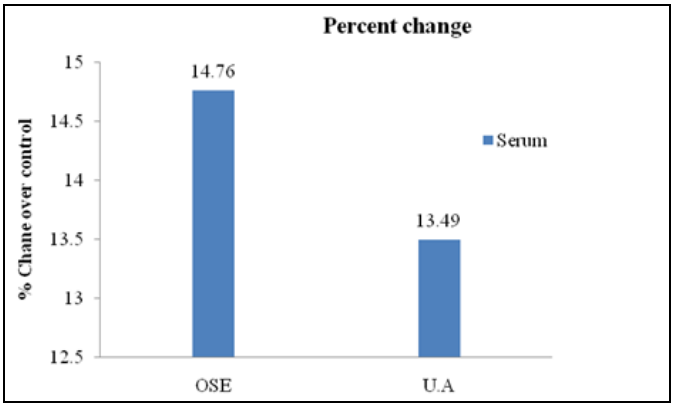

FIG. 12B: PERCENT CHANGE IN PYRUVIC ACID IN SERUM OVER CONTROL AND URSOLIC ACID

The data in **Fig. 13 - 15** represents the effect of *Ocimum sanctum* leaf extract and ursolic acid administration on cytosolic enzymes G-6-PDH, SDH and LDH activity levels in Testes, Epididymis, Seminal vesicle, Prostate gland and liver over control.

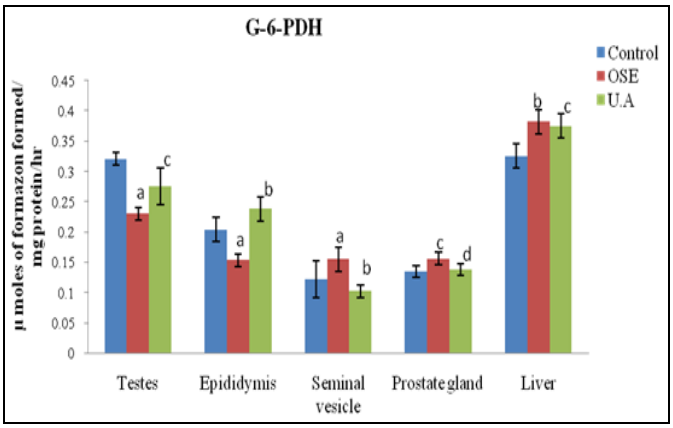

FIG. 13A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON G-6-PDH

Each bar represents Mean± SD of six individual observations a- P < 0.001, a- P < 0.01 and c- P < 0.05 indicates the level of significance; d- Indicates Non significant changes.

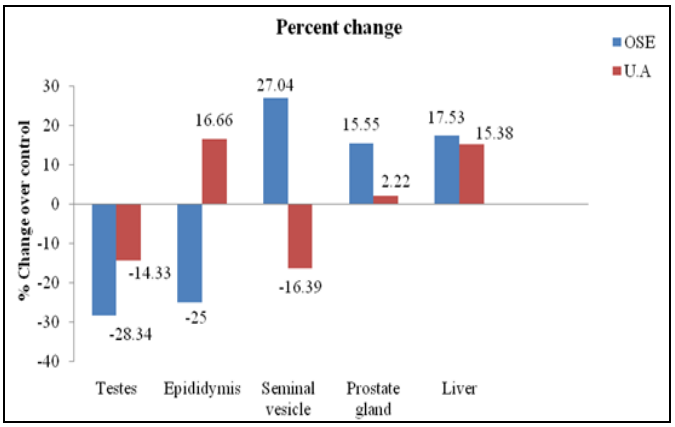

FIG. 13B: PERCENT CHANGE IN G-6-PDH OVER CONTROL AND URSOLIC ACID

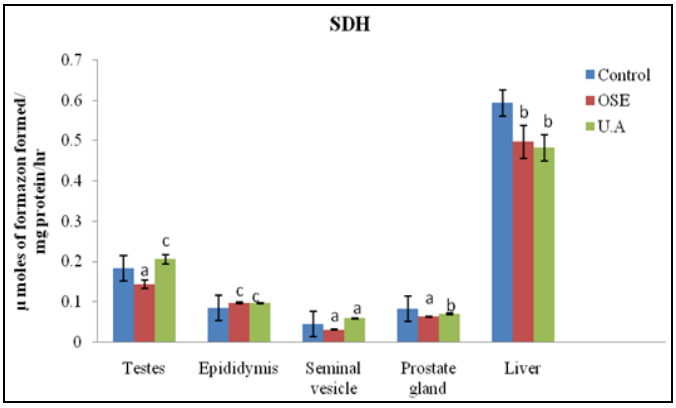

FIG. 14A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON SDH

Each bar represents Mean ± SD of six individual observations a- P < 0.001, a- P < 0.01 and c- P < 0.05 indicates the level of significance.

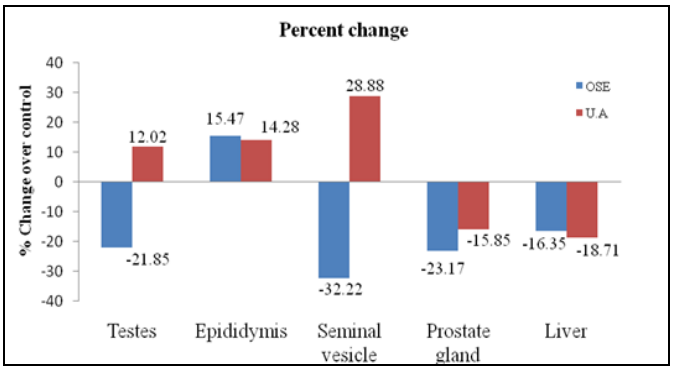

FIG. 14B: PERCENT CHANGE IN SDH OVER CONTROL AND URSOLIC ACID

OS administration reduces G-6-PDH levels in testes and epididymis but in glandular tissue and in liver these were elevated. In UA administration these were lowered in testes and seminal vesicle and elevated in other tissues.

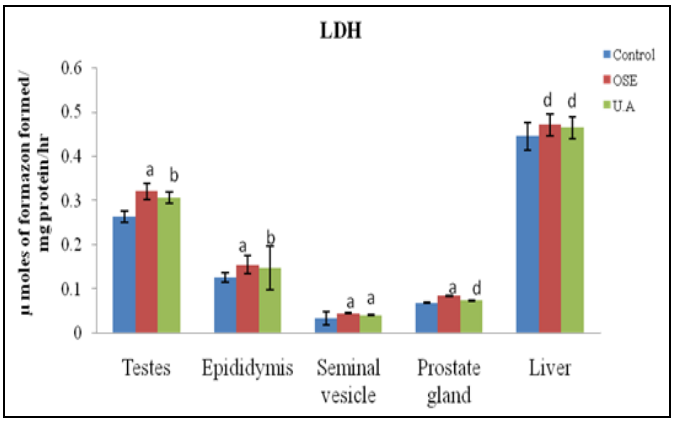

FIG. 15A: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON LDH

Each bar represents Mean ± SD of six individual observations a- P < 0.001 and b- P < 0.01 indicates the level of significance d- Indicates Non significant changes.

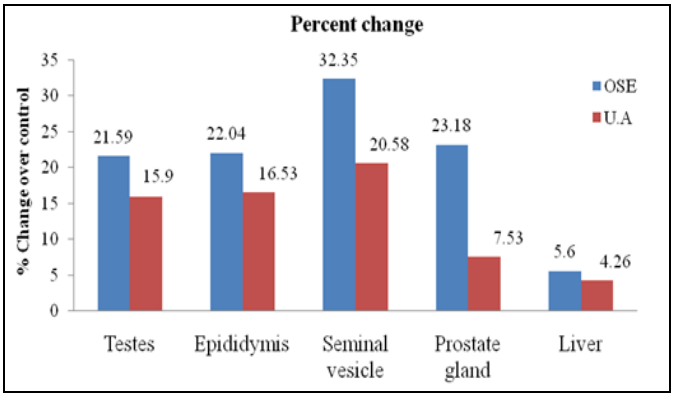

FIG. 15B: PERCENT CHANGE IN LDH OVER CONTROL AND URSOLIC ACID

The testicular SDH and LDH levels were enhanced by UA administration. In accessories the LDH levels were increased in both administrations.

DISCUSSION: The study on carbohydrate metabolism was undertaken to understand any impairment in the carbohydrate metabolism. Testis energy is provided mostly by glucose, which is the preferred substrate in mammals [27], and most germ cell protein synthesis is under control of glucose metabolism [28-27]. Testicular proteins are one of the constituents that ensure the maturation of spermatozoa [29].

In the present study (**Fig. 1-2)** the total protein content was depleted in all tissues, but the extent of depletion is more in testes and serum. Alterations in the secretion and functions of these proteins which are maintained by androgens may impair sperm maturation. The observed decrease in testicular protein may be due to the anti androgenic property of OS leaf extract [30-31]. The testicular fluid contains both stimulatory factors as well as inhibitory factors that selectivity alters the protein secretions. Thus, the changes in protein suggested that there is a reduction in the synthetic activity in testes [32]. The reduced testicular carbohydrate (**Fig. 3-4**) represents reduced carbohydrate metabolizing enzymes [33]. The mature germ cell population depends on testicular carbohydrate reserves.

TABLE 1: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON TOTAL PROTEINS, TOTAL CARBOHYDRATES AND TOTAL LIPIDS IN TESTES, EPIDIDYMIS, SEMINAL VESICLE, PROSTATE GLAND, LIVER AND SERUM

Name of the tissue	Total Proteins			Total Carbohydrates			Total Lipids		
	Control	OS	UA	Control	OS	UA	Control	OS	UA
Testis (mg/g)	178.85 ±11.29	114.79 ± 8.65 -35.85[a]	139.27 ± 9.08 - 22.13[a]	4.07 ± 0.10	3.18 ± 0.23 - 21.86[a]	3.47 ± 0.12 - 14.74[c]	22.97 ± 0.49	33.40 ± 0.17 +45.40[a]	28.91 ± 0.34 +25.85[a]
Epididymis (mg/g)	159.96 ± 9.16	109.24 ± 8.28 - 31.70[a]	129.44 ± 6.02 - 19.07[b]	3.48 ± 0.17	3.98 ± 0.19 +14.36[c]	3.91 ± 0.32 + 12.35[c]	30.16 ± 0.37	20.21 ± 0.23 - 32.99[a]	34.75 ± 0.15 +15.21[c]
Seminal vesicle (mg/g)	156.17 ± 6.09	108.73 ± 9.28 - 30.37[a]	119.24 ± 5.38 +23.64[a]	5.07 ± 0.18	6.53 ± 0.30 +28.79[a]	5.89 ±0.12 +16.17[b]	26.50 ± 0.10	16.20 ± 0.26 - 37.92[a]	19.83 ± 0.16 - 25.16[a]
Prostate gland (mg/g)	124.65 ±7.12	105.85 ± 5.66 - 15.61[c]	109.49 ± 4.52 -12.16[c]	5.04 ± 0.19	6.27 ± 0.34 +24.40[a]	5.84 ± 0.17 + 15.77[c]	31.36 ± 0.32	21.40 ± 0.21 - 31.76[a]	25.65 ± 0.23 - 18.20[b]
Liver (mg/g)	153.64 ± 5.04	112.55 ± 8.12 - 26.74[a]	129.17 ± 7.31 - 15.92[c]	9.26 ± 0.17	7.78 ± 0.25 -15.98[c]	7.99 ± 0.32 - 13.71[c]	32.30 ± 0.17	21.80 ± 0.08 - 32.50[a]	26.74 ± 0.14 - 17.21[b]
Serum (mg/dl)	387.14 ±19.39	235.65 ± 13.53 - 39.13[a]	295.64± 15.23 -23.63[a]	37.44 ±2.03	31.21 ± 1.70 - 16.63[b]	43.23 ± 1.44 +15.46c	14.16 ± 0.75	18.16 ± 1.47 +28.24[a]	16.26 ± 1.23 +14.83[c]

Mean± SD of six individual observations.
+ and – indicates percent increase and decrease respectively.
a- P < 0.001, b- P < 0.01 and c- P < 0.05 indicates the level of significance, d- Non significance changes.

The impaired germ cell structure and function of spermatocytes, spermatids and spermatozoa [34- 35], may be the reason as they were not utilized for spermatogenesis. In epididymis, as duct tissue the elevated carbohydrate indicates sperm anomalies due to both administrations. The carbohydrates in glandular tissues like seminal vesicle and prostate gland were accumulated due to some alterations in the chemical composition of their secretions by the administrations [36]. The accumulated testicular

lipids (**Fig. 5-6**) were observed indicating impaired lipid metabolism in the testis as suggested by earlier reports in both administrations [37]. It was reported that the lipid composition of the sperm membrane exert a significant effect upon the functional quality of spermatozoa [38-39], Spermatozoa are rich in polyunsaturated fatty acids, which are more liable for lipid peroxidation by reactive oxygen species (ROS) [40]. Hence, the administrations of OS and UA bring the alterations in lipid composition which leads to changes in functional quality of spermatozoa. The reduced lipid in seminal vesicle and prostate represents the lowered lipid metabolism and lipogenesis, suggesting some alterations in the chemical composition of the seminal plasma and prostatic fluids.

TABLE 2: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON GLUCOSE, LACTIC ACID AND PYRUVIC ACID IN TESTES, EPIDIDYMIS, SEMINAL VESICLE, PROSTATE GLAND, LIVER AND SERUM

Name of the Tissue	Glucose			Lactic acid			Pyruvic acid		
	Control	OS	UA	Control	OS	UA	Control	OS	UA
Testis (mg/g)	1.70 ±0.05	1.97 ± 0.17 +15.88[b]	1.94 ± 0.11 +13.79[c]	6.97 ± 0.14	5.90 ± 0.15 - 15.35[b]	7.85 ±0.17 +12.62[c]	6.77 ± 0.09	7.85 ±0.18 +16.00[b]	7.74 ±0.12 +14.32[c]
Epididymis (mg/g)	1.26 ± 0.10	1.52 ± 0.06 +20.63[a]	1.48 ± 0.09 +17.46[b]	5.04 ± 0.48	6.32 ±0.23 +25.39[a]	4.15 ±0.25 - 17.65[b]	6.00 ± 0.10	6.93 ±0.12 +15.37[b]	6.77 ±0.14 +12.83[c]
Seminal vesicle (mg/g)	2.24 ± 0.06	1.81 ± 0.09 - 19.19[a]	1.97 ± 0.04 - 12.05[c]	2.39 ± 0.12	2.29 ±0.09 - 4.18[d]	2.58 ±0.11 - 5.75[d]	5.84 ± 0.08	6.67 ±0.14 +14.29[c]	6.59 ±0.12 +12.84[c]
Prostate gland (mg/g)	1.49 ± 0.11	1.36 ± 0.07 - 8.72[d]	1.53 ± 0.08 +2.68[d]	2.74 ± 0.05	1.82 ±0.07 - 33.57[a]	3.16 ±0.09 +15.32[b]	4.42 ± 0.09	4.62 ±0.06 + 4.47[d]	4.83 ±0.07 + 9.27[d]
Liver (mg/g)	3.41 ± 0.03	4.40 ± 0.03 +29.03[a]	4.21 ±0.04 +23.46[a]	7.77 ± 0.19	8.44 ±0.21 + 8.62[d]	7.99 ±0.12 + 2.83[d]	7.75 ± 0.10	9.49 ±0.13 +22.40[a]	8.92 ±0.12 +15.09[b]
Serum (mg/dl)	54.50 ±1.37	47.33 ±2.16 - 13.15[c]	62.07 ±1.96 +13.88[c]	47.99 ±1.81	51.24 ±2.04 + 6.77[d]	44.95 ±1.44 - 6.33[d]	6.30 ± 0.31	7.23 ±0.34 +14.76[c]	7.15 ±0.36 +13.49[c]

Mean ± SD of six individual observations.
+ and – indicates percent increase and decrease respectively.
a- $P < 0.001$, b- $P < 0.01$ and c- $P < 0.05$ indicates the level of significance, d- Non significance changes.

Fig. 7 - 12 showed the effect of OS and UA on glucose, lactic acid and pyruvic acid. D-Glucose is the major fuel for most cells and its homeostasis requires an integrated control by the whole organism. UA stimulates glucose uptake through a cross talk between different signaling pathways, linking the PI3K and MAPK pathways with Ca^{2+}-CaMKII network in intracellular translocation [41]. Testis energy is provided mostly by glucose, which is the preferred substrate in mammals and most germ cell protein synthesis is under control of glucose metabolism [42].

Glucose acts both as a source of energy and as a source of starting material for nearly all types of biosynthetic reactions [43]. In the present study the glucose levels (**Fig. 7 - 8**) were significantly elevated in testes and epidydimis due to the decreased uptake by the spermatogenic cells in OS and UA administered rats. With reference to our other findings OS extract and Ursolic acid because of its anti-estrogenic effect reduces spermato-genesis and causes a decrease in sperm counts.

Thus these findings support our results. The glucose levels were significantly increased in liver (+29.03% $P<0.001$) and reduced in serum (-13.15% $P < 0.05$) over control. Liver is the primary organ for glucose metabolism. Apart from expressing the enzymes involved in glucose metabolism and regulation, liver possesses numerous enzymes involved in detoxification and toxicity enhancement. Accumulated glucose content could be due to accelerated glycogenolysis and or due to active uptake of glucose from the blood by the administrations. The Lactate which is an end

product of the glycolytic pathway is an index of the relative aerobic or anaerobic nature of the tissue under study. It is converted into pyruvic acid before it can be metabolized and the conversion of lactate into pyruvic acid is depending upon NAD concentrations and thereby it showed enter into TCA cycle [44]. The lactate content (**Fig. 9-10**) in testis, epididymis, seminal vesicle, prostate and liver of control rats was in the order of liver > testis > epididymis > prostate > seminal vesicle. It was observed the lactate content was decreased in testis, seminal vesicle and prostate due to increased conversion to pyruvate under aerobic conditions.

The reduction in lactate content might be attributed to defect in catabolising glycogen *in vitro* [45] but in epididymis it was elevated significantly by the administration of OS leaf extract. Accumulated lactates in the seminal plasma adversely affect the sperm metabolism in the epididymis [46]. Pyruvate represents an important junction point in carbohydrate catabolism. In aerobic glycolysis, the pyruvic acid results as an end product and it forms acetyl Co-A by oxidative decarboxylation, which plays an important role in intermediate metabolism as a main source of carbon input for Kreb's cycle, where as in anaerobic condition it will be converted to lactate by the action of LDH enzyme [47]. The rate of production of pyruvate by glycolysis exceeds the rate of oxidation of pyruvate by the citric acid cycle [48].

The pyruvic acid content (**Fig. 11-12**) in testis, epididymis, seminal vesicle, prostate and liver of control rats was in the order of liver > testis > epididymis > seminal vesicle > prostate. It was observed that the pyruvate contents were increased in these tissues of OS and UA treated rats as compared to control rats. This further supported the lactic acid deployment. Increased in pyruvate level might be because of reduced uptake of the metabolite by the mitochondrial membranes as reported [49]. This might be because of the pyruvate contents slightly increase under the toxic conditions, pyruvate gets accumulated which indicates the administration interference with the normal metabolic activities [50].

TABLE 3: EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT AND URSOLIC ACID ADMINISTRATION ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH), SUCCINATE DEHYDROGENASE (SDH) AND LACTATE DEHYDROGENASE (LDH) IN TESTES, EPIDIDYMIS, SEMINAL VESICLE, PROSTATE GLAND AND LIVER

Name of the Tissue	G-6-PDH (µ moles of formazan formed / mg protein /hr)			SDH (µ moles of formazan formed/mg protein /hr)			LDH (µ moles of formazan formed / mg protein /hr)		
	Control	**OS**	**UA**	**Control**	**OS**	**UA**	**Control**	**OS**	**UA**
Testis	0.321 ± 0.01	0.230 ± 0.01 - 28.34[a]	0.275 ± 0.03 - 14.33[c]	0.183 ± 0.012	0.143 ± 0.011 - 21.85[a]	0.205 ± 0.012 +12.02[c]	0.264 ± 0.012	0.321 ± 0.019 +21.59[a]	0.306 ± 0.013 +15.90[b]
Epididymis	0.204 ± 0.02	0.153 ± 0.01 - 25.00[a]	0.238 ± 0.02 +16.66[b]	0.084 ±0.001	0.097 ± 0.002 +15.47[c]	0.096 ± 0.002 +14.28[c]	0.127 ± 0.011	0.155 ± 0.021 +22.04[a]	0.148 ± 0.015 +16.53[b]
Seminal vesicle	0.122 ± 0.03	0.155 ± 0.02 +27.04[a]	0.102 ± 0.01 - 16.39[b]	0.045 ± 0.002	0.030 ± 0.001 - 32.22[a]	0.058 ± 0.001 +28.88[a]	0.034 ± 0.015	0.045 ± 0.001 +32.35[a]	0.041 ± 0.001 +20.58[a]
Prostate gland	0.135 ± 0.01	0.156 ± 0.01 + 15.55[c]	0.138 ± 0.01 + 2.22[d]	0.082 ± 0.002	0.063 ± 0.001 - 23.17[a]	0.069 ± 0.002 - 15.85[b]	0.069 ± 0.001	0.085 ± 0.001 +23.18[a]	0.074 ± 0.001 +7.53[d]
Liver	0.325 ± 0.02	0.382 ± 0.02 +17.53[b]	0.375 ± 0.02 +15.38[c]	0.593 ± 0.051	0.496 ± 0.041 - 16.35[b]	0.482 ± 0.032 - 18.71[b]	0.446 ± 0.031	0.471 ± 0.024 + 5.60[d]	0.465 ± 0.025 + 4.26[d]

Mean ± SD of six individual observations.
+ and – indicates percent increase and decrease respectively.
a- P<0.001, b- P<0.01 and c- P<0.05 indicates the level of significance, d- Non significance changes.

In the present study **Fig. 13-15** represents the effect of OS and UA on G6PDH, SDH, and LDH enzymes. Glucose-6-Phosphate Dehydrogenase (G6PDH), this enzyme is related to the balance between anabolism and catabolism of carbohydrates and the conversion between pyruvic and lactic

acids [51]. Thus, in this study we investigated the effect of the extract of *Ocimum sanctum* and ursolic acid on G6PDH and Lactate Dehydrogenase (LDH) levels. These are very important enzymes in the metabolism of glucose [51]. LDH on the other hand, catalyzes the inter conversion of pyruvate and lactate with the concominant inter conversion of NADH and NAD^+. SDH is one of the most important marker enzymes for mitochondria. G6PDH levels (**Fig. 13**) were significantly lowered in testes and epididimis, elevated in seminal vesicle, prostate and liver with the administration of OS extract.

In UA administration G6PDH levels were significantly lowered in testes and seminal vesicle, elevated in epididimis, prostate and liver. NADPH is essentional for the conversion of cholesterol to testosterone. But the supply of this coenzyme was also reduced due to the decreased activities of glucose 6-Phosphate dehydrogenase in testis [52]. The deficiency of testosterone causes a decline in the number of spermatozoa. In our earlier findings reduction in the level of testosterone and also in the sperm count was observed, these supports decreased testicular glucose 6-Phosphate dehydrogenase.

The specific activities of a number of enzymes in the epididymis were dependent on the androgen status of the animal. These includes G-6-PDH, the glycolytic enzyme, the first enzyme of the pentose phosphate cycle, is quite active in epididymal tissue. Mammalian spermatozoa have a high glycolytic capacity and this is reflected in the greater activities of the glycolytic enzymes [53]. Tulsi offered liver protection against various experimentally induced damages. In liver G6PDH levels were increased significantly in OS extract and UA administration.

Thus these administrations may mediate the up regulation of this G6PDH with increased production of ribose-5-phosphate which enhances DNA synthesis and thus increased cell proliferation of the hepatocytes [54]. Besides the up regulation of G6PDH that was observed in the liver in this experiment, it can be suggested that the administration of *O. sanctum*, carbohydrates are facilitated more along the hexose monophosphate pathway.

In the present observations LDH (**Fig. 14**) levels were significantly elevated. The activity of LDH is closely associated with spermatogenesis and male testicular development. LDH is often used as a marker of tissue breakdown. The increased activity of lactate dehydrogenase suggests a shift in the tissue respiration from anaerobic to which would be adverse to the metabolism of stored spermatozoa in the epididymis. It is further substantiated by the reduced sperm count and motility in the rats treated with both OS leaf extract and ursolic acid [52].

SDH (**Fig. 15**) is one of the most important marker enzymes for mitochondria. Succinate dehydrogenase (SDH) is an important enzyme of TCA cycle which catalyzes the reversible oxidation of succinate to fumarate and it is also associated with the electron transport chain due to its ability to transfer electrons to respiratory chain. The mitochondrial respiratory enzyme SDH is a primary enzyme in the oxidative catabolism of sugars [55], and as such is used effectively as a marker of mitochondrial abundance and activity. The administration of OS extract reduces the SDH levels significantly in all tissues except in epididymis in which these were elevated. In UA administration SDH levels were elevated in testes, epididymis and seminal vesicle, but these were lowered in prostate and liver.

CONCLUSION: These findings suggest that carbohydrates are facilitated more along the hexose monophosphate pathway for G6PDH and the generation of lactate may serve as substrate for gluconeogenesis.

ACKNOWLEDGEMENT: K. Srinivasulu is grateful to University Grant Commission, New Delhi for financial support in the form of UGC-Basic Scientific Research Fellowship.

CONFLICT OF INTEREST: The authors report no conflict of interest.

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How to cite this article:
Srinivasulu K, Sivakumar E and Changamma C: The effective role of ursolic acid and *Ocimum sanctum* (linn.) Leaf extract on some marker carbohydrate metabolites in male albino rats. Int J Pharm Sci Res 2017; 8(11): 4624-36.doi: 10.13040/IJPSR.0975-8232.8(11).4624-36.