Research Article

Protective role of *Gymnema sylvestre* leaf extract on high sucrose diet-induced diabetic like phenotype, oxidative stress, reproductive fitness and longevity in *Drosophila melanogaster*

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Abstract

Objective: Dietary components play important role in physiology, development and longevity of organisms. In the present study, *D.melanogaster* (fruit fly) was used to evaluate toxic effects resulted due to high sucrose diet such as phenotypic response, circulating metabolites, oxidative stress, reproductive traits and longevity. **Material and methods:** Flies of *Drosophila melanogaster* (w^{118}) was raised in control diet,30% high sugar diet and treatment diet were subjected for lifespan (longevity) assay, body weight analysis, metabolites level, biochemical assays (ROS,SOD and CAT) and male accessory gland and sperm traits measurements. **Results and conclusion:** Adult fly fed with 30% of high sucrose diet showed increased levels of glucose, trehalose, triacylglycerol and hydrogen peroxide. In addition to this, the level of antioxidant enzymes SOD and CAT were significantly increased. The *Gymnema sylvestre* (GS) leaf extract treatment along with 30% high sucrose diet elicited reversal of above changes leading to normal phenotype, biochemical markers and reproductive traits in *D.melanogaster*. Thus the treatment of *Gymnema sylvestre* leaf extract counteracted the phenotypic abnormalities along with reduction in circulating metabolites to normal levels and over expressions of stress marker enzymes are blunted. Reproductive fitness parameter and longevity were positively reverted in the GS extract treated flies in *D. melanogaster.*

***Keywords*:** *Drosophila melanogaster*, *Gymnema sylvestre*, longevity, oxidative stress, sucrose

Introduction

Diabetes mellitus is one of the management disease spread worldwide. Since, last 30 years of efforts which were made to cure this disease have so far proved unsuccessful. However, management of this disease by the use of oral anti-diabetic drug and dietary therapy using murine model has shown some promise (Karthikeyan et al., 2017; King et al., 2012; Eddouks et al., 2012; Frode et al., 2008). The murine model used for understanding metabolic diseases suffers from high maintenance cost and lengthy developmental time; therefore, scientists were looking for a good model organism which is having low maintenance cost and its developmental time should be shorter (Beckingham et al., 2005; Hales et al., 2015; Wolf et al., 2008).

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There is a high degree of conservation among the neuroendocrine and metabolic architecture among members of the animal kingdom. The insulin signaling pathways and storage of excessive glucose in the form of glycogen and triglycerides are some of these evolutionary ancient and highly conserved pathways (Musselman et al., 2011; Zhou et al., 2014). *D. melanogaster* and humans have similar insulin signaling machineries and homologies between important transcription regulators of carbohydrates, proteins and lipid metabolism, such as FOXO TOR and PGC –K (Baker and Thummel, 2007) in recent years growing evidence suggests that *Drosophila* has been extensively utilized to understand carbohydrate metabolism because carbohydrates are the main component of a fruit fly natural food. Although the influence of under and over nutrition on fly physiological parameters have also been studied (Coogan, 2013; Morris et al., 2012). Despite that, many physiological, biochemical and molecular aspects of carbohydrates overfeeding remain obscure.

Further, the mechanisms underlying the long-term

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complication of type 2 diabetes are still not completely understood. However, strong evidence points out hyperglycemia as a potent inducer of the damage or induction of oxidative stress, this is because the biochemical pathway that is activated during hyperglycemia shows glucose auto-oxidation, protein glycation and advanced glycation end products (AGEs) formation which can trigger oxidative damage via free radicals overproduction and antioxidant defenses repairment (Hunt et al., 1991; Yim et al., 2001).

Oxidative stress can damage several biomolecules like proteins, lipids, and DNA thereby leading to the inactivation of enzymes affecting DNA integrity and cellular membrane composition (Therond et al., 2005; Shokolenko et al., 2009). Reproductive efforts during mating, courtship and copulation also produces the oxidative stress (Merkling, 2017; Romero et al., 2016; Salmon et al., 2001) therefore oxidative stress resulted due to intake of dietary components such as carbohydrate and proteins along with oxidative stress may lead to changes in metabolic phenotype and affects survival of an organism. Studies have shown that compounds with strong antioxidant property can potentially be effective in delaying diabetes-related complications, one such class of compound is plant-derived polyphenols, which can be divided into catechin, flavones (e.g. Luteolin) and isoflavones (e.g. Genistein) etc., (Kumar et al., 2013; Mooza et al., 2014). Different plant extract have different flavones, therefore there is an option for usage of ayurvedic medicinal plants which are a very rich source of the bioactive component having the potential of counteracting the ill effects of diet-related disorder which are popularized today (Vinayagam et al., 2015). Studies have also shown that phytochemicals derived from plants have managed oxidative damage caused by free radicals and its effect on survival of the organism (Lobo et al., 2015).

Gymnema sylvestre (GS) has been used as an ayurvedic medicine from almost 2000 years for treating diabetes (Thakur et al., 2012) GS also known as madhunashini (Sanskrit) or sugar destroyer is a valuable herb belonging to the family Asclepiadaceae which is widely distributed in India, Malaysia, Srilanka, Tropical Africa (Saneja et al., 2010; Stoecklin et al., 1869). *Gymnema* extract earlier was used as a decoction or the leaves are chewed directly for the treatment of type 2 DM (Kanetkar et al., 2007). *Gymnema* contains a bio-active molecule called Gymnemic acid belonging to the class oleanane Saponins (Yoshikawa et al., 1992) which is the active component present in GS extract. Gymnemic acid though being hypoglycemic compound but also shows anti-oxidant properties (Kang et al., 2012).

Recent pharmacological studies have shown that Gymnemic acid is the principal component in the leaf extract of *Gymnema sylvestre* and it is made of molecules similar to that of glucose which shows hypoglycemic activity. First it promotes the cells necessary for insulin production, secondly, it inhibits the absorption of glucose in the intestine and lastly it increases the utilization of glucose by increasing the activity of enzymes responsible for glucose metabolism by insulin-dependent pathways and phosphorylase activity (Kanetkar et al., 2007; Saneja et al., 2010).

In recent studies Ecker et al., (2017) who while working on *D. melanogaster* have shown that *S. cumini* and *B. forficata* as a protective agents against Hyperglycemia and oxidative stress, in the similar way Studies have also been carried out to know the anti-diabetic and anti-oxidant role of *Gymnema* in diabetic induced mice (Ibrahim et al., 2017). However no work till now has been carried out in establishing interrelationship between hyperglycemia induced by high sucrose diet, oxidative stress and reproductive traits (Accessory gland and sperm traits) and longevity of an organism on one hand and the protective role of *Gymnema sylvestre* in counteracting these traits on the other hand. Therefore present study has been undertaken in *D. melanogaster* to understand the protective role of *Gymnema sylvestre* leaf extract on high sucrose diet-induced diabetic like phenotype, oxidative stress, reproductive traits and longevity.

Material and methods

Collection of Plant Material

Gymnema sylvestre leaves were collected from Government Ayurvedic plant collection centers, Mysuru, India and authenticated by the Institute personnel. The plant leaves were dried (5% humidity, room temperature) in shade and powdered for further extraction.

Extraction of plant material

The shade dried leaf powder of GS was subjected to the extraction process as follows. Exactly 50 g of the plant material was extracted with various organic solvents successively in the ascending order of polarity (hexane, dichloromethane, ethyl acetate, and methanol) in Soxhlet apparatus. In brief, 50 g of the plant material was initially extracted with 1L hexane at 60°C for 24 h, the residue obtained was completely dried and extracted with 1 L of dichloromethane for 24 h, and subsequently, the residue obtained was extracted with 1 L of ethyl acetate and followed by 1 L of methanol. Extracts were concentrated by Rotar evaporator under reduced pressure at room temperature, and 5mg of dried extract was used for the treatment.

Fly strain and culture conditions

Experimental stock used in the present experiment was W^{118}

strain flies, expressing t-GPH proteins which were obtained from *Drosophila* stock center Bloomington, Indiana University. This strain was earlier used for understanding insulin resistance in many experiments (Coogan et al., 2013; Morris et al., 2012). These flies were used to collect the eggs using Delcours procedure (Delcour, 1969). Eggs (100) were transferred to each of the *Drosophila* culture bottle (250 ml) containing wheat cream agar medium and were maintained at 22± 1^{0}C and 70% RH with a 12:12 L: D photoperiod. Unmated males and virgin females were isolated within 3 hrs of their eclosion. Twenty flies (20 males and females together/and also separately) were transferred to quarter-pint bottles (250 ml) containing 5 ml of control diet (wheat cream agar media), 30% High sugar diet (HSD-wheat cream agar media+30% sucrose), treatment diet (30% HSD+GS extract, prepared by adding 5 mg of GS extract/litre of 30% HSD) for 10 days. Following this, flies were subjected for body weight measurement, whole body metabolite measurement, biochemical analysis, and reproductive traits analysis.

Lifespan assay

For lifespan study, flies were obtained from the culture bottles set up at a density of about 100 eggs per bottle. As with all our assays, females were exposed to males for 72 h following eclosion and then separated according to their sex. A total of 100 mated males and 100 mated females were used in this experiment. These flies were housed separately in vials at defined density supplemented with different diets (Control, 30% HSD, 30% HSD+GS extract) (5 vials with 20 flies each per sex per diet). All flies were transferred to new vials with fresh diet every 2–3 days depending on media condition to eliminate the emerging larval stages in the vials containing female flies and replenish nutrients which may have exhausted. Mortality was recorded every 24 h until all flies had died.

Body weight measurement

Ten males/females flies (control diet/30%HSD/30% HSD +GS extract) in a group were used to take their weight (in mg) using a microbalance. A total of five trials were made separately for control, 30% HSD and 30% HSD+GS extract diets.

Whole body metabolite measurements

Heads of twenty-five flies fed in different diets (control diet/30%HSD/30% HSD +GS extract) were decapitated using anesthetic ether and stereomicroscope and transferred them into chilled micro centrifuge tubes containing buffer A provided with assay kit and homogenized using a motorized micro pestle. After centrifugation, supernatants were used for determination of metabolite levels. Glucose was determined by using the glucose detection kit (SIGMA). Trehalose and glycogen were determined after converting them into glucose through the addition of trehalase (SIGMA, 0.2 U/ml) and amyloglucosidase (SIGMA, 0.1 U/ml) respectively. Assays were measured using BioTek Synergy 2 96- well plate reader. A total of three trials were performed separately for each of the control, 30% HSD and 30%HSD with GS leaf extract diets.

Biochemical assays

Fifty male and females obtained from flies fed under different diets (control diet/30%HSD/30% HSD +GS extract, ten flies per vial-cold anesthesia treated) were homogenized in 1ml of respective assay buffers and centrifuged at 2500 g for 10 mins at 4^{0}C. Stress enzymes namely ROS and antioxidant enzymes (SOD and CAT) were assayed from the supernatant. A total of three trials were run/diet/sex. Separate experiments were run for control, 30% HSD and 30% HSD with GS leaf extract.

Reactive oxygen species

Estimation of ROS was done using 2′7′-Dichlorofluorescin diacetate (DCFH-DA) (Sigma Chemical, St. Louis, MO, USA) in which reaction involving the conversion of nonfluorescent DCFH-DA into a highly fluorescent product, 2′7′-dichlorofluorescein (DCF), in the presence of ROS was measured in a spectrofluorometer with the excitation wavelength of 488 nm and emission at 525 nm. Quantification of ROS was done from a DCF standard curve and it was expressed as μmoles of DCF formed/min/mg protein (LeBel et al., 1992).

Antioxidant enzymes

Pyrogallol (2 mM) was used to measure SOD activity (Sigma Chemical, St. Louis, MO, USA) wherein autoxidation of pyrogallol in 0.1 M Tris buffer (pH 8.2) was monitored at 420 nm for 3 min and expressed as units of enzyme required to inhibit 50% pyrogallol autooxidation (Marklund and Marklund, 1974). 1% hydrogen peroxide in 0.05 M phosphate buffer (pH 7)was used as a substrate to measure (H_2O_2) catalase activity which is estimated by monitoring the change in absorbance at 240 nm for 3 min and expressed as mili moles of H_2O_2 decomposed/ min/mg protein (Aebi,1983).

Protein estimation

Lowry's method was used to estimate Protein content in the homogenate by using BSA (Sigma Chemical, St. Louis, MO, USA) as the standard (Lowry et al., 1951).

Measurement of accessory gland and sperm traits

Accessory gland of etherized unmated male/mated male (5mins after mating) [mating was observed by placing an unmated male and virgin female in an Elens-Wattiaux chamber along with a virgin female (5–6 days old). The pair was observed for 1 hr. Pairs that did not mate within 1 hr

were discarded. If mating occurred, the copulation duration was recorded] were dissected out using medium A (Ashburner, 1970) with the help of stereomicroscope and fine needle and fixed in 1N HCL for 5mins.Individually these glands were photographed using a digital camera. The area of the gland was calculated by dividing each gland into smaller areas consisting of triangles, trapeziums, and rectangles following the procedure of Raviram and Ramesh (2002). Following this accessory gland were transferred into 2% lactoacetoorcein stain for 20mins and squashed between a glass slide and a cover slip using 45% acetic acid. The total number of main cells in each accessory gland was counted, and main cell sizes were measured.

Quantification of accessory gland protein by Lowry's method

To quantify accessory glands of unmated males (10 days old) Approximately 50µL of Acps were obtained from unmated male (10 days old) obtained as described above were mixed with 5 ml Bradford reagent, which was generated by adding 100 mg Coomassie Brilliant Blue G-250 (in 50 ml 95% ethanol) to 100 ml 85% phosphoric acid and then diluting the mixture to1 L with distilled water. The solution was allowed to stand for 5 min to develop color. The quantity of proteins in each sample was determined by measuring optical density at 595 nm using a spectrophotometer. Bovine serum albumin was used as the standard. Fifty trials were run for each group. Separate experiments were run for control, 30% HSD and 30% HSD with *GS* leaf extract.

Measurement of sperm traits

Mated female obtained as above was dissected in 20µL Beadle-Ephrussi Saline (128.3mM NaCl, 4.7 mMKCl, 23 mM $CaCl_2$) (Ephrussi-Beadle, 1936), to dissociate sperm from its reproductive organ into the solution.20 ml of lacto-acetoorcein was added to the slide without draining the saline. The number of sperm was then counted using an Olympus CX21 microscope. Total sperm counts include sperms in the spermatheca, seminal vesicle, and sperms in the genital tract of the mated female. Fifty trials were run separately for each of the control, 30% HSD and 30% HSD with *Gymnema* leaf extract diets. The repeatability index was also calculated to understand the consistency of an individual (repeatability, often symbolized as r, ranges from 0 to 1, and expresses the proportion of variations in a trait that is due to differences among individuals not due to differences within the individual). In our experiment, three counts were made per male to calculate repeatability Index. In the result, it was noticed that r value was 0.528 (sperm in spermatheca), 0.513 (sperm in seminal vesicle) and 0.534 (total sperm). These variations are due to differences among individuals, which can only happen if individuals are consistent. Therefore the r-value of 0.5which is pretty consistent for our data.

Statistical analysis

Kaplan-meier survival curve analysis was carried out on life span assay (longevity) using IBM SPSS statistics 20 software version. One way ANOVA followed by Tukey's post hoc test was carried out on body weight, metabolites level (Glucose, Glycogen and Trehalose), oxidative stress enzymes, and accessory gland and sperm traits parameters.

Results

Analysis of *Gymnema sylvestre* extract

Figure-1 shows HPLC analysis of *Gymnema sylvestre* methanolic leaf extract showing the active component Gymnemagenin which is the aglycone central structure of the bioactive molecule in gymnemic acid at RT of 7.366.

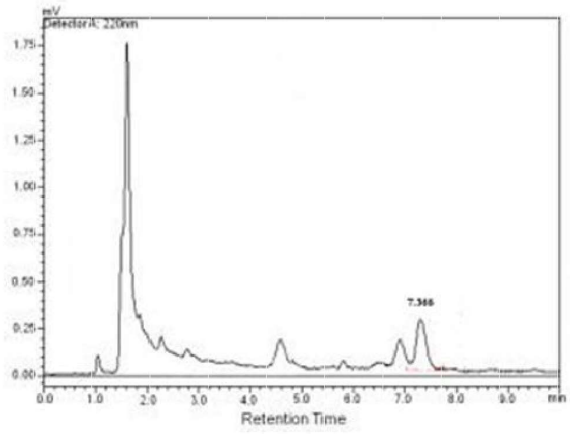

Figure 1. HPLC Chromatogram of *Gymnema sylvestre* leaf extract with retention time of 7.366 min corresponding to gymnemagenin

Survival

The lifespan study as seen in the survivability curve reveals that females live longer than males (Figure 2). The mean lifespan was 63.3 for females and 56.5 for males. Feeding of 30% HSD significantly reduced the development of flies compared to the flies fed on the control diet. Flies fed with 30% high sucrose diet exhibited an average lifespan reduction of approximately 10 days than those flies which fed with control diet. Further, 30% HSD induced developmental abnormality that is obesity which is seen in 5^{th} day onwards of feeding. Flies fed with treatment diet (30%HSD+*Gymnema sylvestre* leaf extract) reduced the mortality rate suggesting that treated flies live longer than those flies which is fed with 30%HSD alone but did not lived with that of flies fed on control diet.

Analysis of bodyweight

Mean values of body weight (Figure 3) obtained showed that diet rich in sucrose (30% HSD) induced a change in

body weight of both male and female flies when compared to the control group. In males, body weight increased significantly when flies fed with 30% high sucrose diet. The obese phenotype of the male flies was reduced by the treatment of GS extract given along with 30% HSD; however, it did not reach the level of control groups. Even in females, similar rise in weight was observed when fed with 30% HSD; however, GS extract treated along with 30% HSD had completely reversed the phenotypic abnormality of female flies.

One way ANOVA followed by Tuckey's post hoc test carried out on above data revealed that in both male and female flies significant increase in the body weight was noticed which was reversed though not completely but significantly by the treatment of GS extract along with 30% HSD.

Metabolite levels

Mean value and analysis of data for Whole-body glucose, glycogen and trehalose levels in the flies fed with control diet, 30% HSD and 30% HSD+GS extract diets were provided in figure 4. Mean values of the metabolite levels increased significantly in flies fed with 30%HSD when compared to the normal or control group of flies (Figure 4). It was observed that there was approximately two-fold increase in glucose concentration and one fold increase in glycogen level in flies fed with 30%HSD compared to control group which was counteracted by diet consisting of 30% HSD along with GS extract further trehalose levels also increased in a similar way to that of glycogen, however, this rise was less compared to glucose level.

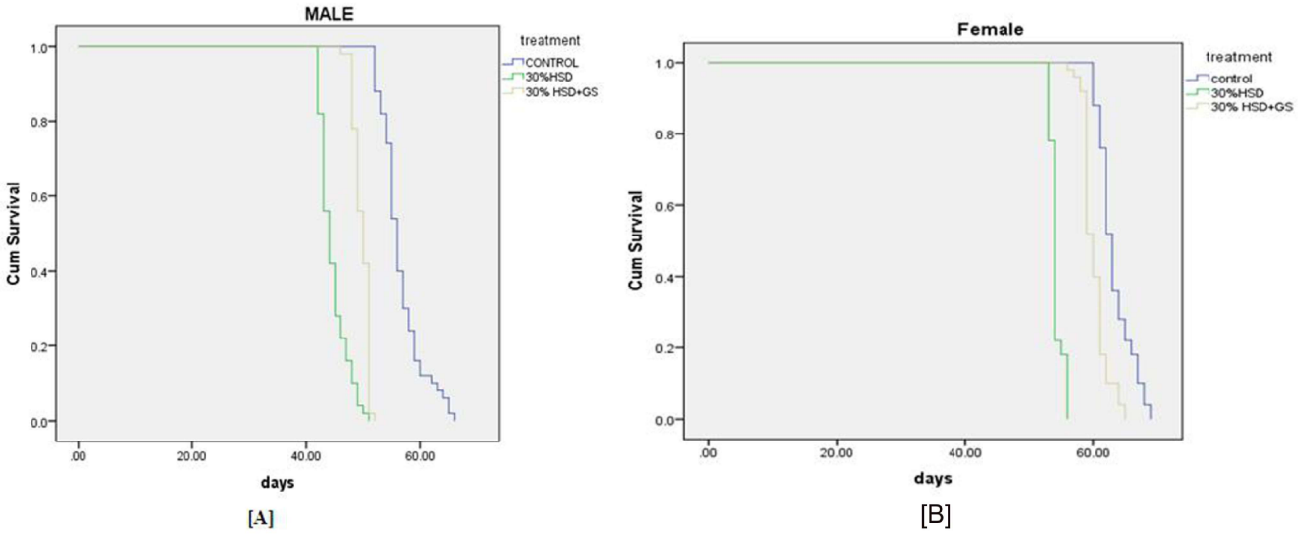

Figure 2. Sex difference in the survivability of *D. melanogaster* as determined by the Kaplan–Meier survival analysis [A] Male [B] Female. The graphs represent the survivorship of male and female flies housed separately in vials (n=150; 5 vials with 10 flies each per group per sex). The log-rank test revealed a statistically significant difference in the survivorship between males and females (Chi square value=410.070 and P<0.001)

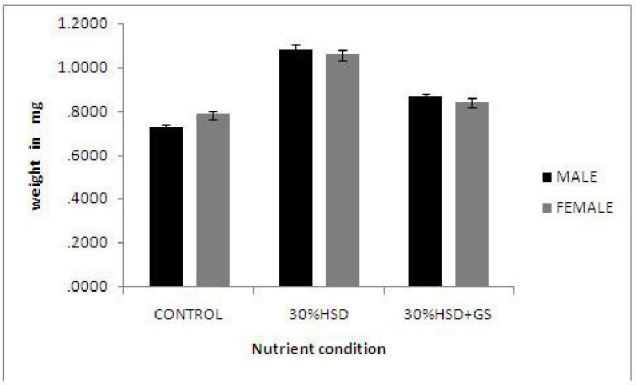

Figure 3. Effect of *Gymnema sylvestre* extracts on total body weight of male and female flies fed on 30%high sucrose diets [male F=97.353; df=2.87; Female F=48.522, df=2.87]

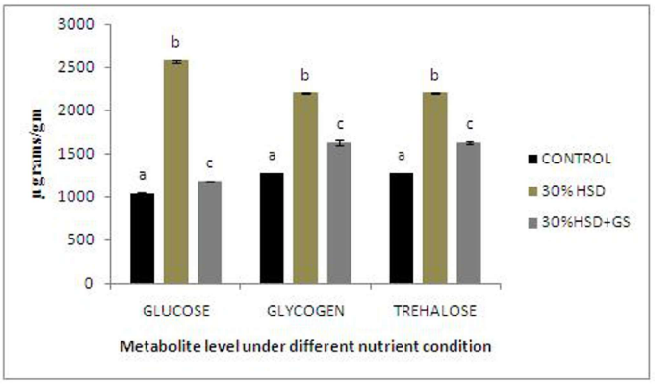

Figure 4. Effect of *Gymnema sylvestre* extracts on carbhohydrate content of flies fed on high sucrose diet [Glucose: F=6035.85, df=2.87; P<0.000 Glycogen: F=624.412, df=2.87; P<0.000; Trehalose: F=2547.273,df=2.87; P<0.000]

Oxidative stress

Figure 5 shows the mean value of ROS, SOD and CAT and analysis of data flies fed with different diets. It was noticed that endogenous ROS, SOD and CAT levels increased compared to the control group of flies when fed with 30% HSD. In the meanwhile flies treated with *Gymnema sylvestre* extract along with 30% HSD showed a reduction in ROS levels. One way ANOVA followed by Tukey's post hoc test applied on the above data revealed significant variation in the ROS levels between diets in both male and female.

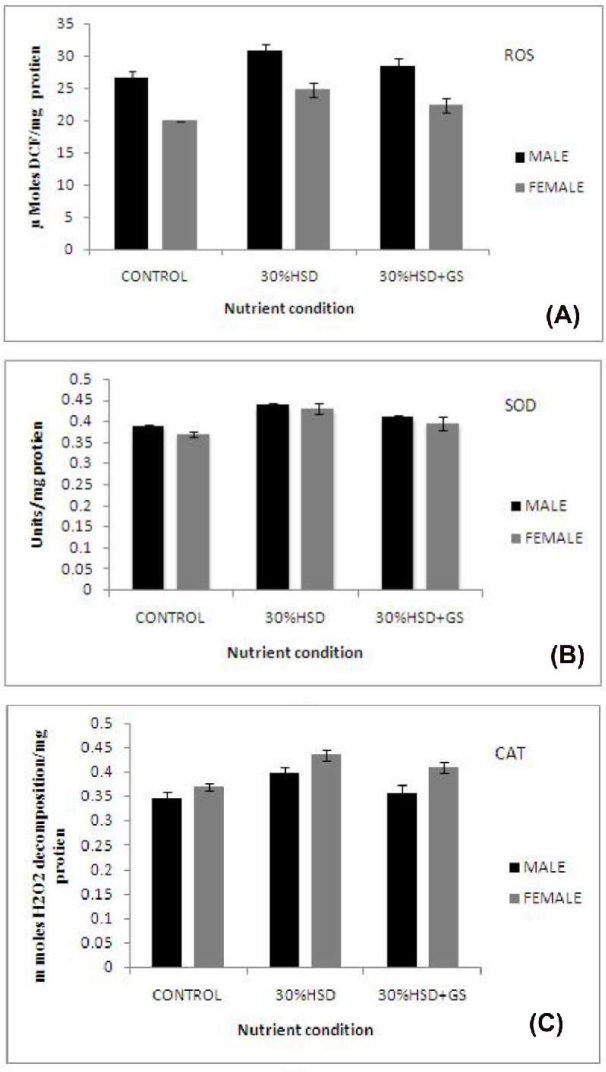

Figure 5. Effect of *Gymnema sylvestre* on enzyme activity on flies fed with 30% HSD [A-ROS, B-SOD, and C-CAT]

Antioxidant parameters

ROS, SOD and CAT enzyme activity

The concentration of ROS increased in flies fed with high sucrose diet whereas the activity of antioxidant enzymes SOD and CAT were disrupted in flies fed on 30% HSD. The sucrose intake, at this concentration, caused an increase in CAT activity and SOD activity (represented by the inhibition of quercetin oxidation) respectively, when compared to the values found in the control group. The changes caused by 30% HSD on the antioxidant enzymes were reversed by *G. sylvestre.*

Reproductive fitness

Table 2 shows the mean value of number and size of the main cells in accessory glands and size of the accessory gland in flies grown in different diets. According to the data number of cells in the accessory gland and main cell size in accessory gland and size of the accessory gland decreased in flies fed with 30% HSD. *Gymnema* extract along with 30% HSD was unable to increase the number of the main cell in accessory gland and size of the accessory gland whereas main cell size in accessory gland was brought to normal to that of the control group flies. One way ANOVA followed by Tukey's post hoc test on accessory gland parameters showed a significant difference in the values obtained between different diets.

The quantity of Acps in unmated /mated and transferred quantity decreased in flies fed with 30% HSD. *Gymnema sylvestre* extract treatment along with 30% HSD was successful in reverting the situation to normal levels (Table 2 A). One way ANOVA followed by Tuckey's post hoc test revealed significant variation between diets in quantity of Acps in unmated and mated flies and also transferred quantity of accessory gland proteins. Duration of copulation also varied significantly between different diets lowest copulation duration was noticed in flies fed with 30% HSD; however, it did not reverted back to normal duration when flies were treated with GS along with 30% HSD.

Sperm traits such as spermathecae, seminal receptacle, total sperm transferred number of eggs (fecundity),progeny production(fertility) showed a remarked decrease in flies

Table 1. Nutritional component and caloric content from diets

Diet	Rava		Yeast		Agar		Jaggery		Sucrose		Total	
	g/L	Kcal/L	g/L	Kcal/L	g/L	Kcal/L	g/L	Kcal/L	g/L	Kcal/L	g/L	Kcal/L
Control(Normal Diet	100	209	10	28	10	_	100	358	_	_	220	595
30%HSD	100	209	10	28	10	_	100	358	300	1200	520	1795

Table 2A. Effect of *Gymnema sylvestre* extract on reproductive fitness parameters in flies fed with 30% HSD (accessory gland traits/sperm traits): [A] Accessory gland parameters

Nutrient condition	Reproductive fitness parameters (mean±SE) (N=50; df=2,147)						
	Number of main cells in accessory glands	Main cell size in accessory gland (in mm)	Accessory gland size(in cm^2)	Quantity of Acps in µgm/pair of gland(unmated male)	Quantity of Acps in µgm/pair of gland(mated male)	Transferred quantity of Acps (in µgm/pair of gland)	Copulation duration(in mins)
Control	1546.6667±50.9435[a]	.0066877±.0000128[a]	.3395000±.0005788[a]	15.9750000±.0605411[a]	11.1181000±.0018688[a]	3.856667±.057771[a]	17.8506667±.05269939[a]
30%HSD	1462.0000±39.5881[b]	.0057867±.0000201[b]	.3362667±.0008761[b]	14.4130667±.0004744[b]	12.0350000±.0242295[b]	3.574000±.133607[b]	10.1480000±.00272915[a]
30%HSD+GS	1391.6667±2.71578[c]	.0066807±.0000120[c]	.3350667±.0006963[b]	15.8086667±.0459678[c]	11.1121000±.0002507[b]	4.079967±.075789[c]	10.6150000±.32186516[b]
F-value	4.334	1122.052	9.936	382.020	1432.793	525.955	7.162

Table 2B. Effect of *Gymnema sylvestre* extract on reproductive fitness parameters in flies fed with 30% HSD (accessory gland traits/sperm traits): [B] Sperm traits.

Nutrient condition	Sperm traits (N=50;df=2,147)				
	Spermathechae	Seminal receptacle	Total sperm transferred	Egg	Fertility
Control	34.66±.439[a]	177.80±.787[a]	6354.80±16.137[a]	382.62±1.831[a]	251.36±1.421[a]
30%HSD	18.24±.334[b]	95.50±.436[b]	3916.40±16.258[b]	164.04±1.231[b]	124.60±3.015[b]
30%HSD+GS	23.60±.316[c]	110.56±.612[c]	4690.40±13.040[c]	272.90±1.847[c]	155.32±1.441[c]
F-value	520.347	4868.742	6703.903	4328.906	994-802

fed with 30% HSD diet compared to flies fed with control diet however reversal of such reduction was noticed in the hyperglycemic flies which were treated with 30%HSD+GS extract (Table 2 B). One way ANOVA followed by Tukey's post hoc test revealed a significant difference in sperm traits between different diets.

Discussion

The growing evidence show high mortality rates which is associated with the metabolism-related diseases which made us to study the role of nutritional components in the etiology of the metabolic diseases. In the present study, *D. melanogaster* flies were used as a model organism for understanding potential toxic effects of consumption of diets rich in sugar on oxidative stress and reproductive fitness on one hand and the protective effects of extracts from medicinal plants *Gymnema sylvestre* which is popularly used to treat DM related problems on the other hand.

In *D. melanogaster* high sucrose diets consumption increased body weight and decreased their longevity and reproductive traits (Figure 3, Table 2), these effects were resulted due to elevated haemolymph glucose, trehalose and triglycerides levels triggering in inducing oxidative stress. Interestingly in the present study, most of these adverse effects were counteracted by concomitant ingestion of *Gymnema sylvestre* leaf extract.

In *D. melanogaster* the principle sugar in circulation are glucose and trehalose. In the present study, the consumption of high sucrose diet elevated the haemolymph and whole body glucose and trehalose content and haemolymph triacylglycerols levels (Figure 4). This suggests that excess dietary consumption of sucrose shows toxic effects on glucose metabolism which resulted in elevated body weight and induces phenotype similar to type 2 DM via insulin signaling dysregulation (Morris et al., 2012) characteristics of type 2 DM. Our results confirm harmful effects of high sucrose diets on metabolic homeostasis in *Drosophila* (Rufilson et al., 2002; Musselman et al., 2011; Morris et al., 2012; Pasco et al., 2012) .In the present study, fig-4 shows that *Gymnema* leaf extract along with 30% HSD was more efficient in normalizing the elevated level of glucose and triacylglycerols in *D. melanogaster* (Figure 4) suggesting that *Gymnema sylvestre* is one of the potent medicinal plants to treat the type 2 DM.

Lifespan analysis has been performed to show differences between males and females in laboratory species such as *C. elegans, D. melanogaster* and *Mus musculus* (Austad and Fischer 2016). As evident from the survival curves, *D. melanogaster* used in the study has proved to be good model to study the gender difference in aging since females live longer than males. Results on survivability of *Drosophila* showed that there are gender differences in mortality rates exhibited with age which could be attributed to aging pattern in the sexes. Age-related mortality was positively correlated with endogenous ROS which was consistent with earlier studies showing inverse relationship between oxidative stress and lifespan (Ku et al., 1993; Sohal et al., 1995).

Studies have suggested that consumption of high sucrose diets always associated with the developmental of hyperglycemic state in the fly and it is responsible for

triggering oxidative events (Ecker et al., 2017).Our study in *D. melanogaster* has also showed an increased level of oxidative stress marker enzymes (SOD and CAT) in the flies fed with 30% HSD thereby confirming hyperglycemic state which triggers oxidative stress in *D. melanogaster (*Fig-5). The toxic effects of high sucrose diets caused hydrogen peroxide overproduction and mitochondrial viability loss. Further, the activity of antioxidant enzymes SOD and CAT are also varied in these groups (Figure 5B, C). The variation in antioxidant enzymes SOD and CAT could be a compensatory response to the elevated levels of hydrogen peroxide in flies fed with 30% HSD. Our result confirms the work of Ecker et al. (2017) who while working in *D. melanogaster* have also found 30% HSD intake increased transcription of SOD and CAT.

It was also noticed in the present study that flies fed with 30% HSD along with *Gymnema sylvestre* leaf extract have counteracted these effects and bringing down the level of SOD and CAT these results suggest that *Gymnema sylvestre* reduced oxidative damage in flies and restoring the levels of SOD and CAT (Fig-5) it is possible that the constituents of *Gymnema sylvestre*-gymnemic acid acting individually, additively and /or synergistically are responsible for beneficial effects of *Gymnema sylvstre* leaf extract .

Studies have shown that males of *Drosophila* consists of two accessory glands in their reproductive system which develop from the set of cells in the genital imaginal disc (Nothiger et al., 1972) there are two morphologically distinct cell types in the accessory gland namely the main cells (96%) and secondary cells (4%). Main cells are known to involve in the production of accessory gland proteins. Chemically accessory gland proteins form a complex mixture of proteins that form a component of the seminal fluid (Chen, 1984). The number and size of the main cells were known to affect the quantity of Acps produced by males of *D. melanogaster*. Both biotic and abiotic factors are known to affect the quantity of Acps produced (Santhosh et al., 2013). These are transferred to females during copulation. In mated females; the secretion of accessory gland protein brings about physiological and behavioral changes in the female. This involves egg production increased ovulation rates and reduced sexual receptivity (Baldini et al., 2012; Heifetz et al., 2004; Ravi ram and Ramesh, 2007). It also assists in sperm storage (Ravi ram and Ramesh, 2007). Greater is the quantity of Acps greater will be the effect of these proteins on the above functions.

In the present study, an attempt has also been made to study whether or not *Gymnema sylvestre* leaf extract treatment along with HSD will attenuate the toxic effects of HSD on male reproductive traits in *D. melanogaster*. Careful observation of table 2 revealed that male consumed with 30% HSD had smaller and less number of main cells in their accessory glands and secreted significantly less quantity of Acps when compared to males which consumed the normal diet. Further, it was also noticed that *Gymnema sylvestre* leaf extract treatment along with 30% HSD had shown an increased number of main cells in the accessory glands and also the size of the main cells in the accessory glands thereby these males produced a significantly greater quantity of Acps than those males which consumed 30% HSD alone. This suggests that the treatment of GS leaf extract along with HSD had attenuated the toxic effects of HSD on male accessory glands traits in *D. melanogaster*. This is the first study of this kind to study toxic effects of HSD on the male reproductive trait in *D. melanogaster*.

In the present study males fed with HSD had copulated lesser time and transferred significantly lesser quantity of Acps and sperms into the mated female than those males consumed normal diet (Table 2). However, males fed with HSD along with GS extract had transferred significantly greater quantity of Acps and sperms to mated females. In species of *Drosophila* duration of copulation varies between different species (Singh et al., 2004; Hirai et al., 2004), between strains of the same species (Singh et al., 2004). Factors such as size of the fly and age (Santhosh et al., 2013) were known to affect the duration of copulation. Longer the duration of copulation greater will be the transfer of the quantity of accessory gland proteins and sperms to unmated females (Santhosh et al., 2013). Nutrition also plays important role on copulation duration (Socha et al., 2004). In the present study males fed with 30%, HSD had copulated lowest time than those flies fed with control diet this suggests that quantity of carbohydrate in the food had a significant influence on reproductive fitness of male fly. Further quantity of accessory gland proteins and sperm transferred to mated female was least when the female was mated. It was noticed that shorter the duration of copulation had transferred less quantity of accessory gland proteins and sperms to mated female compared to female mated with male fed with control diet. Therefore the present study confirms the effect of nutrition on copulation duration, fecundity, and fertility in sperms of *Drosophila*.

In the present study female mated with male fed with 30% HSD laid least number of eggs and less number of progenies obtained compared to female mated with male fed with the normal diet or GS extract along with 30% HSD fed males. This suggests that the toxic effect of HSD on fecundity and fertility had counteracted by male fly fed with GS leaf extract along with 30% HSD. Thus these studies in *D. melanogaster* suggests the protective role of GS leaf extract on type 2 DM phenotype, oxidative stress, and reproductive fitness.

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Conflicts of interest

Authors do not have any conflict of interest regarding the publication of this manuscript.

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