phenotype. This is true for males (Figure 1a) and females (Figure 1b) showing the phenomenon of the turning "*on*" and "*off*" genes. The genotypes in case of females are more bunched together than for males tested under blue light implying that the females are better buffered than the males under blue light. It is interesting, however, to note that in case of males (Figure 1a) the genotypes 1 and 2 have a *contact/meeting* point under white light, and later forms a small and large canal when tested under red and blue light, respectively. For females (Figure 1b), genotypes 4 and 5 show the *contact* point under white light and diverges out forming a canal when tested under blue and red light. The data on point of contact and canal formation shows the phenomenon of *canalization* in males and females.

Finally, the data presented show a variation in average weight for males and for females for a given genotype when tested under different colored lights. Such a variation in weight is attributed to the difference in wavelength among colored lights in this experiment (the wavelength for ***white*** light: unfiltered incandescent bulb; for ***red*** light: long pass filter, wavelength greater than 620 nm; and for ***blue*** light: short pass filter , wavelength smaller than 650 nm as measured by Spectrograph). These data suggest the likelihood of the allele(s) for the average male weight and the average female weight may very well become fixed for colored light in time and space under a given colored light spectrum and thereby leading towards the isolation of that allele(s) (that is, it will lead towards the isolating mechanism for the light dependent gene among populations maintained under different colored lights. This, in fact, is a part of selection and the evolutionary process) where selection depends upon the individual reaction norm of a genotype and not on the mean of genotypes tested under a given colored light.

The results are not only in accordance with those published by Gupta (2009a, b) and Gupta and Lewontin (1982) in strains of *D. pseudoobscura* using temperature for the development of a phenotypic trait, but also with those well documented and published data on skin cancer caused by the exposure, in time and space, to sun as an external environmental stimulus.

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Genotoxicity studies on the drug progynova on fitness of *Drosophila melanogaster*.

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Abstract

Steroids form a group of drugs in variety of therapeutics. Biologically active classes of steroids, such as estrogen, progesterone, androgen, and anabolic steroids, are among the major therapeutic drugs. In the present study, the effect of commercially available drug, namely Progynova, prescribed as a contraceptive in Hormone Replacement Therapy (HRT), such as in post menopausal treatment, containing the Estradiol hormone was used. *Drosophila melanogaster* flies were treated by adult feeding method. Three concentrations of the drug used were 50 µg, 100 µg,

and 150 µg along with one control group. The fitness parameters employed for analyzing the effect of drug were fertility, viability, and longevity. Comparisons were made between the control and the treatment groups. Statistical analysis showed significant values for viability and female longevity. The variations in the sex ratio were also seen in the control and the treatment group. (Key words: Steroids, Estradiol hormone, *Drosophila melanogaster*, fertility, viability, and longevity)

Introduction

Human populations are continuously exposed to mutagenic/carcinogeneic chemicals either environmentally, occupationally, or through therapeutics. The human survival rate and reproductive potential, which is greater in recent years, have posed many problems. As a consequence the use of oral contraceptives (OCS) to control the births is one aspect. These drugs are tremendously used by millions of women throughout the world to prevent births (Madhuri *et al*., 2007). It is also one of the interesting topics in toxicology, which is a subject concerned with addressing the potential harmful effects of chemicals in order to assess the hazards and risks to human and lower animals (Gunzel *et al*, 1989; Guzel *et al*., 2006). This study was made using *Drosophila* as the model system because of its advantages. Moreover, the genome sequence projects have shown about 61% similarity between human and *Drosophila* genomes. Recent reports also revealed that human genes implicated in disease were found to have strong homologs or orthologs. All this evidence made the *Drosophila* a super model for toxicity study.

Use of hormones for therapeutic purposes is becoming more common in recent years. But exogenous administration of these hormones like progesterone, estrogen, and androgen cause two levels of adverse effects, namely, short-term effects and long term effects in mammals (Gunzel *et al*., 1989). Short term effects including liver tumor, jaundice, water retention, high cholesterol, kidney disease, stunted growth, and heart damage are likely to occur. Estradiol (E2), a steroid hormone derived from cholesterol, targets a variety of tissues. As a drug therapy, it is widely used in the treatment of ovarian failure in young women and for menopausal syndrome, post menopausal osteoporosis, as well as certain kinds of breast cancer (Bachmann, 2007). Long and adverse effects include changes in sex drive, fluid retention, weight gain or loss, depression, dizziness, skin irritation, darkening of the skin, and most importantly increased risk of cancer of the uterus. Exogenous administrated dose may interfere with endogenous dose, may affect cellular function and growth. Work on rodents depicts that primarily the over doses have an effect on reproductive organs (Doyle, 2000), and increased incidence of bone, pituitary, and lymphoid tumors are also accompanied with high doses of estradiol (Lone, 1997; Forbes, 1999). Further, reports on rodents have revealed that the exogenous overdoses of these hormones either interfere with the endogenous dose or affect the different tissues including reproductive tissues. There is no report of genetic effects of such drugs. Furthermore, estradiol is a hormone involved in growth and development of the uterus at puberty. Whether the hormone has any effect on fitness also is not known. Hence the present study is an attempt to understand the effect of such therapeutic drugs frequently consumed by women worldwide for many reasons.

Materials and Methods

Fly stock used is *D. melanogaster* Oregon-K strain obtained from Drosophila Stock Centre. Experimental stocks were maintained on the normal wheat cream agar media seeded with live yeast at 25ºC with relative humidity of 75%.

Estradiol: Drug Progynova (5 mg) obtained commercially contains estradiol-valerate as a prodrug of the natural human estradiol. This drug is prescribed for many therapeutics like menopause and breast cancer, the sub-lethal concentration of 50 µg, 100 µg, and 150 µg with one control group were maintained. As the drug was administered through 5% sucrose solution, the same was given as control. For convenience, treatment groups are abbreviated as E1, E2, and E3, respectively.

Methods: The virgin male and female flies were collected from uncrowded culture bottles to get equal aged flies and aged for five days. These adult flies of five days old were collected in a bottle and starved for five hours. Starved flies were fed with different concentration of estradiol as well the control. For each group separate bottles were used. Thus, flies fed overnight were used to analyze fitness parameters.

Parameters: The fitness parameters such as fertility, longevity, and viability were assessed following the methods of Ramachandra and Ranganath (1986) and Hegde and Krishna (1999).

Statistical analysis: Statistical analysis was performed with SPSS 11.5 version. Fertility and viability data were subjected to ANOVA. Percentage of emergence has been calculated from different groups. For pair-wise comparison, Duncan's Multiple Range Test is used.

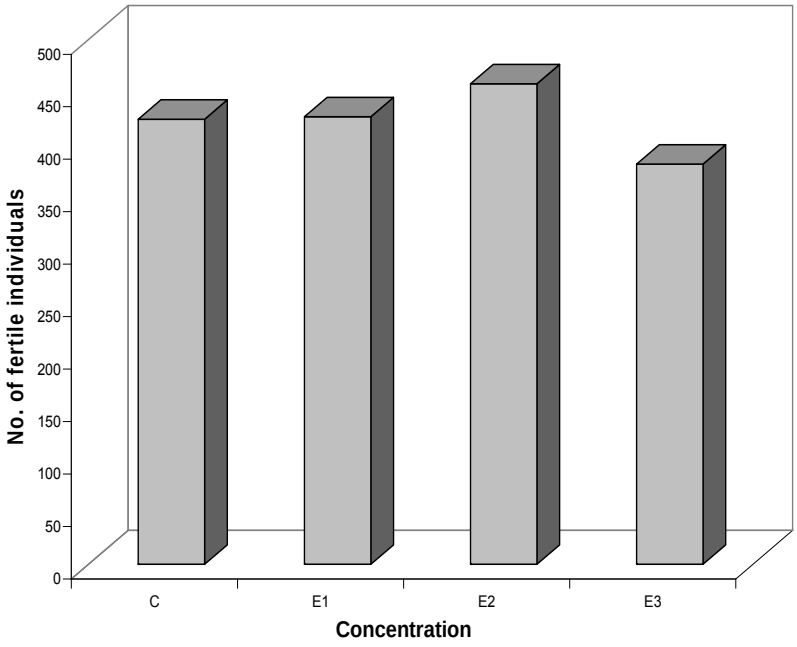

Figure 1. Effect of estradiol on fertility of *D. melanogaster*.

Results

Fertility

The data recorded for different parameters for control and treatment groups are given in Figures 1-4. The total number of flies counted for (10 replicates) fertility in estradiol treated group is 4341 in control, 4528, 4296, and 3815 for E1, E2 and E3, respectively. The percentages of female flies were more compared to males. Mean difference of fertility among treated and control groups are non-significant $F = 0.56$ ($P > 0.05$). DMRT revealed that there is significant difference between

E3 and control and also between E1 and E3. Figure 1 depicts the effect of estradiol on fertility. There is slight elevation in the fertility of E2 group, whereas it is reduced in E3 when compared to control group.

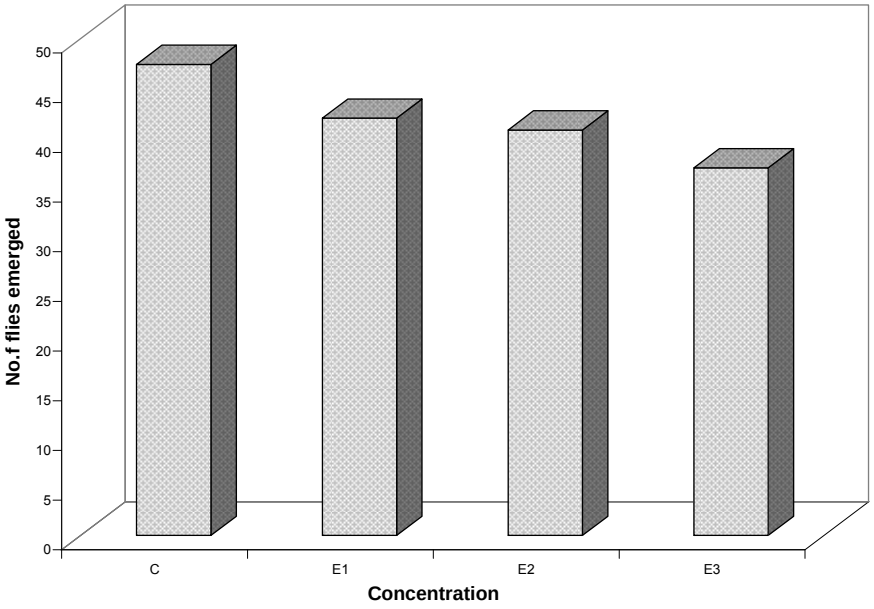

Figure 2. Effect of estradiol on viability of *D. melanogaster.*

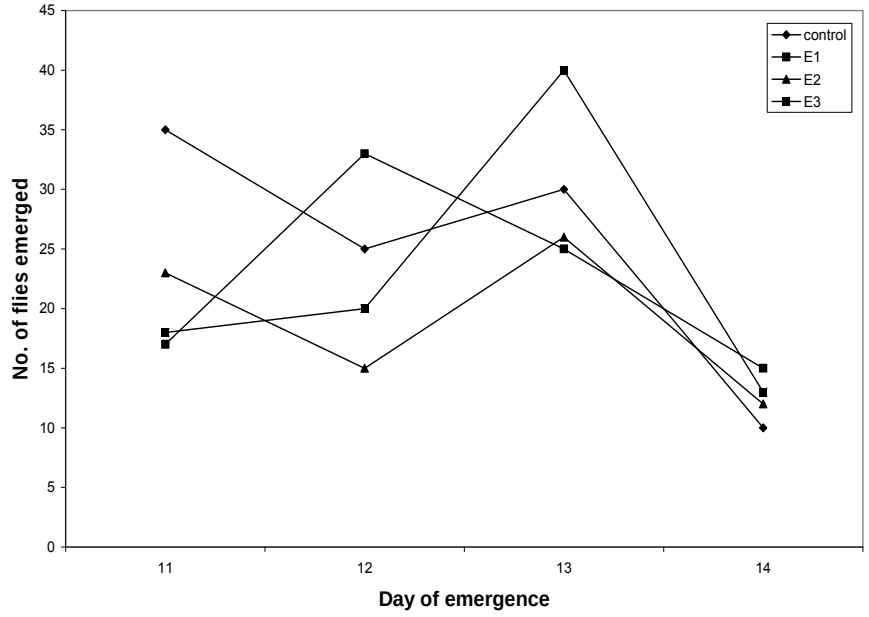

Figure 3. Effect of estradiol on pattern of emergence of *D. melanogaster* in control and treated groups.

Viability

Of the 500 eggs, the viability counted were 474 for control, 464 for E1, and 442 for E2 and E3. Percent of male and female flies emerged vary greatly in treated group compared to control. Female emergence is more compared to males. Pattern of emergence in Figure 3 shows that the emergence begins at 11th day and ends at 14th day in all the four groups, but there is difference in the peak of emergence. The peak of emergence on 13th day for E2 and E3, for E1 it is on 12th day and 11th day for control. Mean developmental time in days ranges from 16-18; there is significant difference between the numbers of days taken for development in different groups.

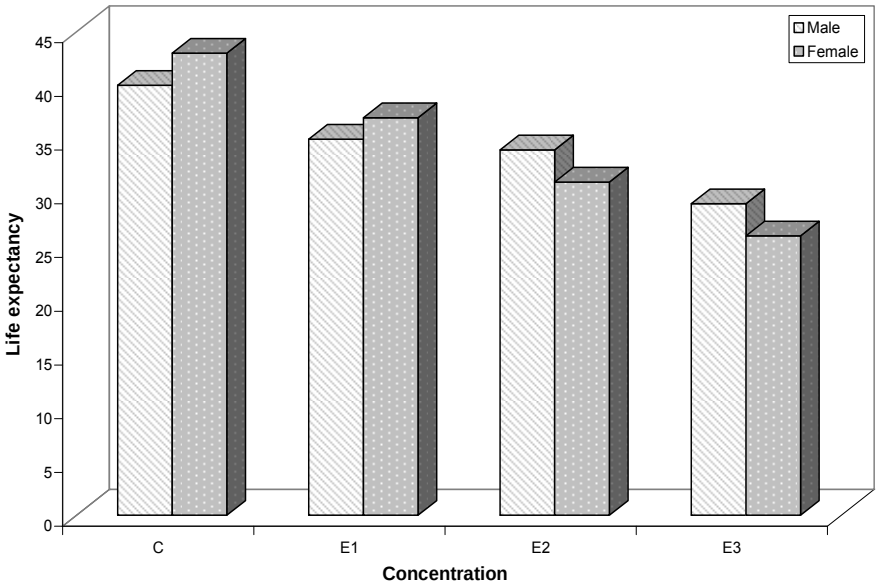

Figure 4. Effect of estradiol on male and female longevity of *D. melanogaster* in control and treated groups.

Longevity

Longevity, recorded for males, was not significant for different groups, but the life expectancy was enhanced in the E1 and E2 group, and it is decreased in E3 group. Female longevity (Figure 4) on the other hand, decreased with the increase in the concentration; there is a significant difference between the control and the treatment groups (Figure 4). Life expectancy is reduced greatly in E3 when compared to control. Pairwise comparison revealed the significant difference between control and all other groups.

Discussion

Estrogen and progesterone are two important female sex hormones, where rhythmic secretion determines the reproductive behavior, estrous cycle, ovulation, pregnancy, and parturition. The pattern of the secretion of these hormones has been well documented in cattle, buffaloes, sheep, goat, mare, pig, camel, and human (Deen *et al*, 2007). Recently, deficiency of the estrogen hormone or mutation of estrogen receptor gene or cytochrome P450 aromatase has an effect on skeletal maturation (Smith, *et al*., 1994; Carani *et al*., 2009)**.** Although the hormone is essential for vertebrate reproduction, the present studies have demonstrated that it can affect the reproduction of *Drosophila* flies. There are also reports that high levels of the hormone 17β-estradiol induces tumor in various organs of rats, mice, hamsters, and humans. Slight increase in the endogenous hormone production or by the therapeutic doses increase breast or uterine cancer risk (Liehr, 2000). The present study is the first report on estradiol effect on fitness parameters of *Drosophila*. The data recorded for different parameters have revealed that there is a dose dependent effect, both positive and negative effect of estradiol on *Drosophila*.

Organism's fertility is a quantitative trait determined by both environmental and genetic components (Clara and Luckinbill, 1985; Graves, 1993)**.** The estradiol treated flies have shown less fertility compared to that of control. Interestingly, at less than 50 µg concentration there is an increase in the fertility of the flies when compared to control, whereas, in the concentration of 150 µg fertility decreased. This is the first report of the effect of estradiol for *Drosophila*. Decreased fertility was also recorded at increased concentration of ether in *D. willistoni* (Fresia, *et al*., 2001). The viability in control and treatment groups varied greatly as also the percentage of male and female

flies in both control and treated groups. The percent of females emerged were more compared to that of the males. The estradiol probably has an effect on male embryos or there is a sex biased effect observed. Similarly, Triclosan treated *D. melanogaster* flies also affected more males compared to females (Anitha, *et al*., 2008). There is a decreased viability with increased concentration. The pattern of emergence also revealed different peaks of emergence in different treatment and control groups. The mean development time ranges between 16 to 18 days. E3 completed its development in 16 days, but E1 took 2 days more compared to E3. There was a synchrony in the emergence of flies in the control group. Toxicity of Dithane M-35 on *D. melanogaster* (Vasudev and Krishnamurthy, 1978) also revealed the effect of these chemicals on development and also suppress crossing over. Further prolongation of development time, lowering of viability and fecundity was observed. But the asynchrony is observed in the treated groups. There is also a negative report on fitness and survival rate on estradiol in fishes and other organisms (Johnstone *et al*., 1979; Hunter and Donaldson, 1983; Goryczko *et al*., 1991; Parks and Parks, 1991; Eh-Ghalid, 2009). The life expectancy of the male flies range between 28 to 35 days and for female 26 to 43 days. Flies in E1 group showed a maximum life expectancy when compared to E3 for both males and females. This is in accordance with the viability results where male flies emerged less compared to females. There is a linear relationship in the longevity and the concentration. The trade-off between longevity and reproduction prompted evaluation of *ova*, the disruption of which confers female sterility and lifespan extension (Sgro and Partridge, 1999). Further, *Drosophila* was fed with 4-phenylbutyrate (PBA) throughout adulthood, which significantly increased the life span without diminution of locomotor vigor, resistance to stress, or reproductive ability (Kang *et al*., 2001; Zivanov-Curlis *et al.*, 2004). But the effect of certain other chemicals on same fitness parameters is available. There is also a moderate increase in the life expectancy in low doses, when compared to the higher doses of estradiol and control. Similar results are also obtained by the cocoa supplementation on *Drosophila melanogaster*. The longevity increased in the treated groups when compared to control group, and there was no significant variation between the treated and control group. The changing pattern of longevity, fertility, viability, development time, and other life history variables exhibited by the flies may be perhaps due to short term exposure to the estradiol. A slight increase in fertility and longevity may be because lower doses of estradiol might have enhanced the energy levels in the flies, as it happens in humans.

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Genetic diversity of *Drosophila suzukii* in San Diego.

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The introduction of the fruit fly *Drosophila suzukii* has caused major problems in California's soft fruit industries (Cline 2009). The species is native to Asia and has been spotted on crops in Japan since 1916 (Kanzawa 1936). *Drosophila suzukii* was first introduced to Hawaii in the 1980's (Kaneshiro 1983), and it was spotted in 2008 in central California. Since 2008 it has spread throughout the western coast of the United States and to areas of Florida (Walsh *et. al*., 2010). Unlike other species of *Drosophila*, female *D. suzukii* have very large ovipositors that are capable of inserting eggs into unripe fruit. The puncture at the oviposition site is then susceptible to secondary microbial infections, ruining the commercial value of several thin-skinned summer fruits including cherries, pears, grapes, peaches, and blueberries (Walsh *et al*., 2010). The extent of the economic loss caused by these flies is not known.

Eight male *D. suzukii* were collected in a La Jolla, California residential yard during the summer of 2010. The DNA from these flies was extracted using DNeasy kits (Qiagen) following the manufacturer's protocol. A 631 bp fragment of the mitochondrial Cytochrome Oxidase I gene of each fly was sequenced to estimate the genetic diversity of the population. These sequences were then compared to sequences from a stock of flies from Hachijo Island, Tokyo, Japan caught in 1978 (UCSD Drosophila Stock Center) and to sequences on GenBank from flies collected in Washington, California, and Spain in 2009 (GenBank: HM803273.1 - HM803279.1 and HM636439.1, respectively). Sequences from this study can be found on GenBank under accession numbers: HQ646995-HQ646999.

The sequences from the La Jolla *D. suzukii* show an extremely high genetic diversity at the CO1 locus (Table 1), with five distinct haplotypes in only eight flies. As a comparison, well-established populations of *D. emarginata* and *D. sturtevanti* in Panama had haplotype diversity values of 0.205 and 0.406, respectively (Schumacher and Hooton, 2011). Such high levels of genetic diversity were unexpected in the La Jolla *D. suzukii* population, because it was reportedly introduced to the western United States only two years ago (Walsh *et al*., 2010). Moreover, a bottleneck or founder effect significantly decreases genetic diversity (England *et al*., 2003). Thus, *D. suzukii* either has been established in the western United States for a significant period of time, has been introduced multiple times, or was introduced only once but with enough individuals to maintain a genetically diverse pool.