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The Impact of *Piper longum* on Sex Ratio in *Drosophila melanogaster*: An Experimental Study

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Abstract

This study investigates the impact of *Piper longum* on the sex ratio of *Drosophila melanogaster*. Previous research has highlighted that food availability significantly influences maternal resource allocation, affecting offspring fitness and sex ratios. The sex ratio, defined as the male-to-female ratio in a population, is a crucial demographic factor with implications for reproductive capacity and genetic variability. While genetic and environmental factors are known to influence sex ratios, the specific impact of dietary components like *Piper longum* remains underexplored. Using *Drosophila melanogaster* as a model organism, this study examines how different concentrations of *Piper longum* in the diet affect the sex ratio of offspring. The results demonstrate that *Piper longum* significantly skews the sex ratio towards more female offspring compared to the control group, with both 10mg/l and 20mg/l concentrations showing a pronounced effect. These findings suggest that *Piper longum* can modulate reproductive outcomes, potentially offering applications in managing sex ratios in experimental populations.

Keywords: *Piper longum, Drosophila melanogaster*, sex ratio, male female

Introduction

Most previous studies on this topic focused on the food accessible to reproducing females and discovered significant maternal allocation responses to experimental manipulations of resource quantity, likely impacting the fitness of both the mother and her offspring (Meijer and Langer, 1995; Selman and Houston, 1996; Williams, 1996; Rutstein *et al*., 2005) [13, 23, 29, 21]. The availability of food can influence the relative investment patterns between male and female offspring (Rosenfeld and Roberts, 2004; Robertson *et al*., 2006) [20, 19] in addition to more apparent characteristics like the number and size of offspring. Theoretically, parents will allocate resources differently to the sexes when the fitness returns from producing males differ from those from producing females (Trivers and Willard, 1973) [26]. In such situations, parents often allocate greater resources to the sex that benefits the most from the current conditions (Komdeur, 1996; Nager *et al*., 1999; Kalmbach *et al*., 2001) [10, 14, 9]. These relative fitness returns are often dependent on the surrounding conditions. Parents may either.

i). Generate biased sex ratios of their offspring (Clutton-Brock *et al*., 1984; Dittus, 1998; Kalmbach *et al*., 2001) [5, 6, 9] or

ii). Maintain balanced sex ratios but give some offspring of one sex greater attention than the other (Velando, 2002) [27].

A lot of biological research focuses on the sex ratio, commonly referred to as the male-to-female ratio in a population (Skalski *et al*., 2005; Bailey, 2004) [1]. The ratio is typically expressed as males per 100 or 1000 females or as the percentage of the population that is male or female (Skalski *et al*., 2005; Qazi and Qazi, 2006; Prakash, 2008) [18, 17]. The female composition of the sex ratio indicates the reproductive capacity of the population (Schowalter, 2016) [22]. Along with other life history aspects, the sex ratio also provides information about the past, present, and future of a population, including the significance of the sexual mating system (Skalski *et al*., 2005; Schowalter, 2016) [22].

Several known genetic and environmental factors can affect the animal sex ratio. The male-to-female ratios may not match the theoretical 1:1 ratio due to factors influencing the primary sex ratio or post-fertilization mechanisms affecting the secondary sex ratio. Surprisingly, not much research has been conducted to examine the environmental impact on the sex ratio. In many organisms, including insects, the sex ratio is typically 1:1 (Prakash, 2008) [17]. This ratio maximizes genetic variability by increasing the availability of males relative to females (Schowalter, 2016) [22]. In the absence of changes, the sex ratio remains constant (Cherian, 2016) [2]. Numerous environmental factors eventually impact sex ratios (Schowalter, 2016; Cherian, 2016; Rosenfeld and Roberts, 2004) [22, 2, 20]. Physical, chemical, or biological environmental factors can influence sex ratios (Skalski *et al*., 2005;

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Schowalter, 2016; Hardy, 2002; Rosenfeld and Roberts, 2004; Wajnberg, 2008) [22, 30, 20].

In this work, *Drosophila melanogaste*r served as the model organism. Several factors led to the selection of this organism for the investigation. Firstly, *Drosophila* regularly produces a large number of progeny. Secondly, this organism has a short life cycle, reproduces quickly, and produces a large number of eggs. Thirdly, *Drosophila* has frequently been used as a model organism in numerous studies investigating various biological issues (Neethu *et al*., 2014) [15].

Piper longum Linn, often referred to as Indian long pepper, has been used to treat a number of respiratory disorders in Ayurvedic medicine (Kumawat *et al*., 2012) [11]. Many plant components, including the seed, root, leaf, and the entire plant, have been historically utilized to treat a variety of ailments (Sultana *et al*., 2019) [25]. Piperine, a bioactive alkaloid molecule isolated as a dietary phytochemical from the *Piper* species, has shown a wide range of beneficial effects, including anticancer, antioxidative, antibacterial, antiapoptotic, and antidiabetic properties (Buranrat and Junking, 2022; Choi *et al*., 2013; Derosa and Park, 2019; Jwa *et al*., 2016; Yang *et al*., 2015; Zarai *et al*., 2013) [4, 16, 8, 31, 32].

Research by Lakhmi *et al*. (2006) revealed that the hexane fraction of *Piper longum* causes animal death in addition to its strong anti-implantation effects. As noted by Garg in 1981, roots of *Piper longum* combined with *Embelia ribes* seeds demonstrated 100% anti-fertility activity in female albino rats. It is thought that *Piper longum* may enhance the contraceptive effects of other plant products; however, more research is necessary to determine whether this combination is feasible for developing a female contraceptive, as noted in Ayurveda Garbha Nivarana Aushadham, which is used for both genders and does not interfere with ovarian hormone activity in the uterus.

Materials and Methods

Preparation of *Piper* longum Media: The fruit extract powder of *Piper longum* was acquired from the Government *Ayurveda* Medical College and Hospital, Mysuru, Karnataka, India, and was used for preparing the experimental media.

Experimental Stock: The Oregon K strain of *Drosophila melanogaster* used in this study was obtained from the *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore, Mysuru. This stock was cultured in bottles containing wheat cream agar media. The media was prepared by boiling 100g of jaggery, 100g of wheat powder, and 10g of agar in 1000ml of distilled water, with 7.5ml of propionic acid added. The flies were maintained under laboratory conditions with 70% humidity, a 12-hour dark/12-hour light cycle, and a temperature of 22°C ± 1°C.

The flies obtained from this culture were used to establish the experimental stock with different diet media:

1. **Wheat Cream Agar Media:** Prepared by boiling 100g of jaggery, 100g of wheat rava powder, and 10g of agar in 1000ml of distilled water, with 7.5ml of propionic acid added.
2. ***Piper longum* Media**
   i). **For the 10mg/l Concentration:** Prepared by boiling 100g of jaggery, 100g of wheat powder, and 10g of agar in 1000ml of distilled water, with 7.5ml of propionic acid and 10mg of *Piper longum* powder added.
   ii). **For the 20mg/l Concentration:** Prepared by boiling 100g of jaggery, 100g of wheat powder, and 10g of agar in 1000ml of distilled water, with 7.5ml of propionic acid and 20mg of *Piper longum* powder added.

Flies emerging from the wheat cream agar media and other experimental media were maintained under the same laboratory conditions and were used to study sex ratio experiments in *Drosophila melanogaster*.

Sex Ratio Experiments: Virgin male and female flies were collected from the wheat cream agar, 10mg *Piper longum*, and 20mg *Piper longum* media. After mating, these pairs were transferred to vials containing their respective media. The pairs were transferred to new vials once every seven days until their death. The number of male and female offspring emerging from each diet was recorded, and the experiments continued until the death of the flies. A total of five pairs were made separately for each of the wheat cream agar, 10mg *Piper longum*, and 20mg *Piper longum* media.

Statistical Analysis

The data obtained were analyzed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, and Tukey's Post-Hoc test were carried out for the data obtained for sex ratio. A graph of concentration v/s mean sex of male and female (in number) was plotted for *Piper longum*. The graph of the two was compared. And another method used i.e. the data subjected to Chi square.

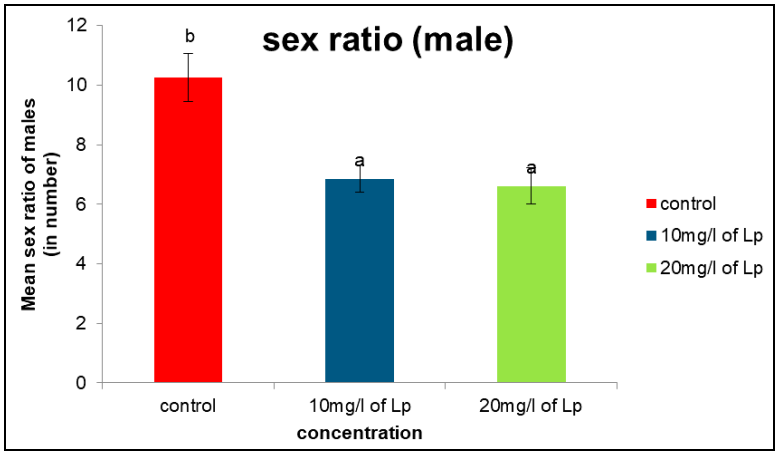

Fig 1: The graph plotted using concentration v/s mean sex ratio of males (in number) showing more numbers of males in the control than the other treated media in *Drosophila melanogaster* showing d=2, f= 9.997 P<0.05.

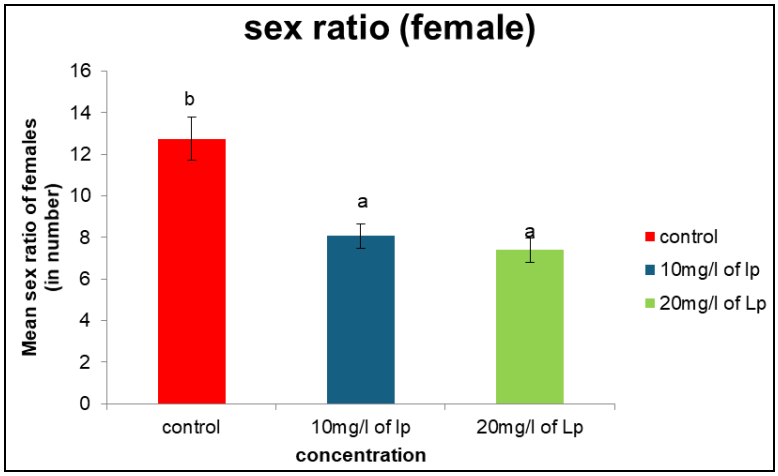

Fig 2: The graph plotted using concentration v/s mean sex ratio of females (in number) showing more numbers of females in the control than the other treated media in *Drosophila melanogaster* showing d=2 f=14.381 P<0.05

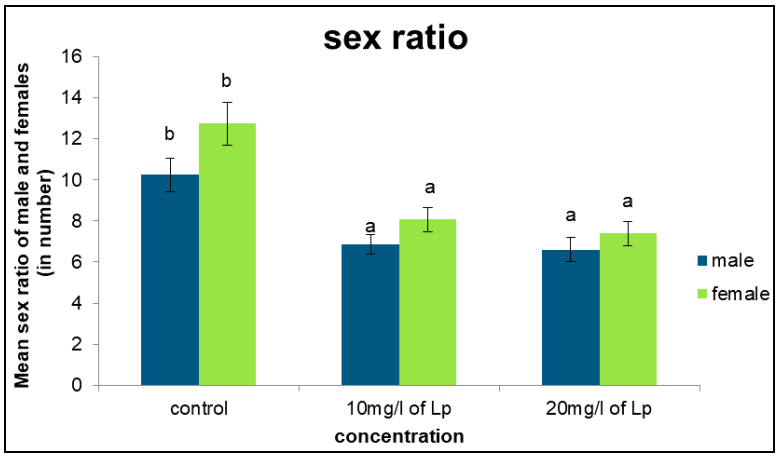

Fig 3: Comparison of effect of *Piper longum* on sex ratio of males and females in *Drosophila melanogaster.*

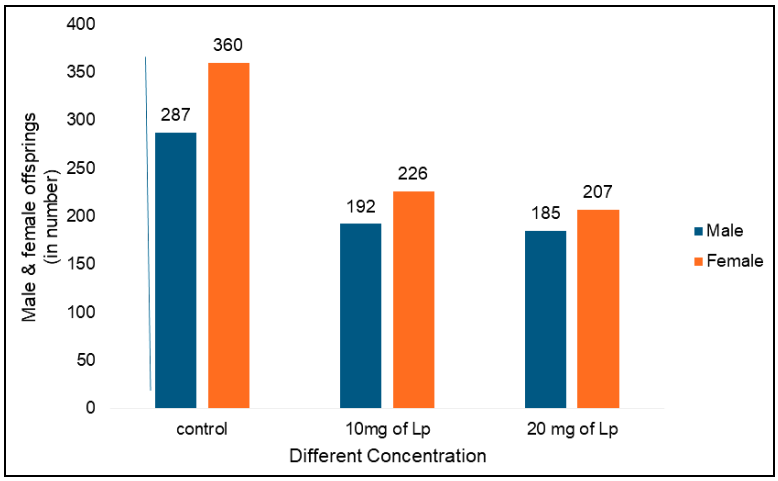

Fig 4: The graphs showed the comparison between the male and female adult flies numbers in *Drosophila melanogaster* raised on the different diets (Wheat cream agar, 10mg/l *Piper longum*, *20mgl Piper longum media)*

The Figure 4 suggests that the female offspring are produced more than the male offspring in all the three diets. When the Data subjected to the Chi square analysis showed the insignificant between the male and female sex ratio in control media, and significant between the male and female sex ratio in both different concentrations of *Piper longum.*

Table 1: The effect of wheat cream agar, 10mg/l *Piper longum* and 20mg/l *Piper longum* media on the male offsprings sex ratio of *Drosophila melanogaster.*

Treatment (Media)	No. Adults	No. Females	No. Males	F:M Ratio
Control	647	360	287	1:0.797
1omg/l	418	226	192	1:0.849
20mg/l	392	207	185	1:0.893

Table 2: The effect of wheat cream agar, 10mg/l *Piper longum* and 20mg/l *Piper longum media.* On the female offsprings sex ratio of *Drosophila melanogaster*.

Treatment (Media)	No. Adults	No. Females	No. Males	M:F Ratio
Control	647	360	287	1:1.254
10mg/l	418	226	192	1:1.153
20mg/l	392	207	185	1:1.118

Table 3: The effect of wheat cream agar, 10mg/l *Piper longum* and 20mg/l *Piper longum* media on the female and male offsprings sex ratio of *Drosophila melanogaster* and Chi square analysis value.

Treatment (Media)	Sex Ratio F:M	Chi Square Value	Significant
Control	1.254:0.797	4.118	P>0.05
10mg/l	1.153:0.849	1.382	P<0.05
20mg/l	1.118:0.893	0.617	P<0.05

Figure 1 shows the mean sex ratio of males (in number) in Drosophila melanogaster, indicating that the control group produced more males compared to the groups treated with *Piper longum* media showing significant results (P<0.05, df=2, F=9.997).

Figure 2 displays the mean sex ratio of females (in number) in *Drosophila melanogaster*, suggesting that the control group also produced more females than the treated groups showing significant results (P<0.05, df=2, F=14.381).

Figure 3 compares the effect of *Piper longum* on the sex ratio of both males and females in *Drosophila melanogaster*.

Figure 4 presents the comparison between the number of male and female adult flies raised on different diets (wheat cream agar, 10mg/l *Piper longum*, 20mg/l *Piper longum* media). This figure indicates that female offspring are more numerous than male offspring across all three diets. Chi-square analysis showed an insignificant difference in the male and female sex ratios in the control media, but a significant difference in both *Piper longum* media concentrations.

Discussion

The results indicate that the control group (wheat cream agar media) produced a higher number of both male and female offspring compared to the groups treated with *Piper longum* media. Specifically, the F ratio in the control group was 1:0.797 for males and 1:1.254 for females, suggesting a relatively balanced sex ratio. In contrast, the sex ratios in the 10mg/l and 20mg/l *Piper longum* media groups were lower, with fewer males being produced relative to females.

According to the Chi-square analysis, the control group's male-to-female sex ratio difference was not statistically significant (P>0.05). But in the 10 mg/l and 20 mg/l *Piper longum* media groups, it was significant (P<0.05), suggesting that the *Piper longum* media had a noteworthy impact on the offspring's sex ratio.

The effect of several physical conditions on *D. melanogaster's* sex ratio has been the subject of numerous studies. These variables include the temperature of the surrounding air, the amount of light present, and electromagnetic field (EMF) radiation. Dietary nutrition is one of the primary external environmental factors limiting population growth. Alterations in sex ratios in response to food availability and other environmental conditions have been well-documented in insects, reptiles, and birds. For instance, the dramatic skewing of the sex ratio in the Samoan butterfly *Hypolimnas bolina* due to male-selective mortality caused by *Wolbachia* infection (Hardy, 2002) [30]. In the current study altered sex ratio by *Piper longum* supports the antifertility activity demonstrated by many studies. As noted by Garg in 1981, roots of *Piper longum* combined with *Embelia ribes* seeds demonstrated 100% anti-fertility activity in female albino rats. It is thought that *Piper longum* may enhance the contraceptive effects of other plant products; however, more research is necessary to determine whether this combination is feasible for developing a female contraceptive. Future studies may focus light on these aspects.

Conclusion

Sex Ratio Impact: The study demonstrates that the *Piper longum* media significantly affects the sex ratio of *Drosophila melanogaster*, with a tendency to produce more female offspring compared to the control group.

Concentration Effects: Both concentrations of *Piper longum* (10mg/l and 20mg/l) significantly altered the sex ratio, with the higher concentration showing a slightly more pronounced effect.

Control Group Comparison: The control group produced a more balanced sex ratio compared to the *Piper longum*-treated groups, indicating that the dietary inclusion of *Piper longum* influences the reproductive outcomes.

Implications for Future Research: These findings suggest that *Piper longum* has potential applications in modulating sex ratios in experimental populations of Drosophila melanogaster. Further research could explore the underlying mechanisms and potential applications in other species or contexts.

Overall, the study highlights the significant impact of *Piper longum* on the sex ratio of *Drosophila melanogaster* and underscores the importance of considering dietary components in reproductive biology studies.

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References

1. Bailey RO. 'Sex ratio and sexual conflict in the spotted hyena (*Crocuta crocuta*)', Behavioral Ecology and Sociobiology. 2004; 56(4):366-373. Doi: 10.1007/s00265-004-0784-4.
2. Cherian A, Rose A, Gupta D, Minz S, Prasad J, George K. Whither Sex Ratios in a Low Mortality Setting. *IJWHR*, 2016, 64-67.
3. Chivers ML, Rieger G, Latty E, Bailey JM. A sex difference in the specificity of sexual arousal. Psychological science. 2004; 15(11):736-744.
4. Choi S, Kim Y, Han CK, Lee S, Lee S. 'Piperine enhances the bioavailability of curcumin in rats', Planta Medica. 2013; 79(11):971-976. Doi: 10.1055/s-0032-1328181.
5. Clutton-Brock TH, Albon SD and Guinness FE. Maternal dominance, breeding success and birth sex ratios in red deer. Nature. 1984; 308(5957):358-360.
6. Dittus WP. Birth sex ratios in toque macaques and other mammals: integrating the effects of maternal condition and competition. Behavioral Ecology and Sociobiology. 1998; 44:149-160.
7. Garg SK. Antifertility effect of *Embelia ribes* and *Piper longum* in female rats. Fitoterapia. 1981; 52(4):167-169.

8. Jwa H, Shin NH and Choi JW. 'Piperine inhibits inflammatory responses in lipopolysaccharide-stimulated RAW 264.7 macrophages', *International Journal of Molecular Medicine*. 2016; 38(5):1531-1538. Doi: 10.3892/ijmm.2016.2716.
9. Kalmbach E, Nager RG, Griffiths R and Furness RW. Increased reproductive effort results in male-biased offspring sex ratio: an experimental study in a species with reversed sexual size dimorphism. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2001; 268(1481):2175-2179.
10. Komdeur J. Facultative sex ratio bias in the offspring of Seychelles warblers. Proceedings of the Royal Society of London. Series B: Biological Sciences. 1996; 263(1370):661-666.
11. Kumawat NS, Chaudhari MB and Wanjari MM. 'Piper longum Linn: A review on its pharmacological and therapeutic potential', *Journal of Pharmacy and Bioallied Sciences*. 2012; 4(3):237-245. Doi: 10.4103/0975-7406.99435.
12. Lakshmi V, Kumar R, Agarwal SK, Dhar JD. Antifertility activity of *Piper longum Linn*. In female rats. Natural Product Research. 2006; 20(3):235-239.
13. Meijer T, Langer U. Food availability and egg-laying of captive European starlings. The Condor. 1995; 97(3):718-728.
14. Nager RG, Monaghan P, Griffiths R, Houston DC, Dawson R. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Sciences. 1999; 96(2):570-573.
15. Neethu BK, Babu YR, Harini BP. Flavors supplemented in diet regulate the hatchability and viability in Drosophila. Dros. Inf. Serv [Internet], 2014, 24-28.
16. Park SH, Khan N, Lee S, Zimmermann K, DeRosa M, Hamilton L, Hudson W, Hyder S, Serratos M, Sheffield E and Veludhandi A. Biodiesel production from locally sourced restaurant waste cooking oil and grease: synthesis, characterization, and performance evaluation. ACS Omega. 2019; 4(4):7775-7784.
17. Prakash R. 'Sex ratio and sexual size dimorphism in the Indian bullfrog, *Hoplobatrachus tigerinus* (Daudin)', Journal of Biosciences. 2008; 33(2):257-264. Doi: 10.1007/s12038-008-0026-6.
18. Qazi JI and Qazi R. 'Sex ratio and sex allocation in the invasive species, *Phyla canescens* (Scrophulariaceae)', Journal of Ecology. 2006; 94(5):931-938. Doi: 10.1111/j.1365-2745.2006.01143.x
19. Robertson BC, Elliott GP, Eason DK, Clout MN and Gemmell NJ. Sex allocation theory aids species conservation. Biology letters. 2006; 2(2):229-231.
20. Rosenfeld CS and Roberts RM. Maternal diet and other factors affecting offspring sex ratio: a review. Biology of reproduction. 2004; 71(4):1063-1070.
21. Rutstein AN, Gilbert L, Slater PJ and Graves JA. Sex-specific patterns of yolk androgen allocation depend on maternal diet in the zebra finch. Behavioral Ecology. 2005; 16(1):62-69.
22. Schowalter TD. 'Insect Ecology: An Ecosystem Approach', Academic Press, 2016, 147-150.
23. Selman RG and Houston DC. The effect of pre breeding diet on reproductive output in zebra finches. Proceedings of the Royal Society of London. Series B: Biological Sciences. 1996; 263(1376):1585-1588.
24. Skalski JR, Ryding KE and Millspaugh J. Wildlife demography: analysis of sex, age, and count data. Elsevier, 2010.
25. Sultana N, Ashraf M, and Swaleha Z. 'Pharmacological and therapeutic potential of medicinal plants: A review', *Journal of Pharmacy and Bioallied Sciences*. 2019; 11(3):238-254. Doi: 10.4103/jpbs.JPBS_33_19.
26. Trivers RL and Willard DE. Natural selection of parental ability to vary the sex ratio of offspring. Science. 1973; 179(4068):90-92.
27. Velando A. Experimental manipulation of maternal effort produces differential effects in sons and daughters: implications for adaptive sex ratios in the blue-footed booby. Behavioral Ecology. 2002; 13(4):443-449.
28. Wajnberg E, Bernstein C, Van Alphen J. eds. Behavioral ecology of insect parasitoids: from theoretical approaches to field applications. John Wiley & Sons, 2008.
29. Williams TD. Variation in reproductive effort in female zebra finches (*Taeniopygia guttata*) in relation to nutrient-specific dietary supplements during egg laying. Physiological Zoology. 1996; 69(5):1255-1275.
30. Wilson K and Hardy IC. Statistical analysis of sex ratios: an introduction. Sex ratios: concepts and research methods. 2002; (1):48-92.
31. Yang W, Wang Y, Li X, Chen X. 'Piperine attenuates oxidative stress and inflammation in streptozotocin-induced diabetic rats', *European Journal of Pharmacology*. 2015; 761:241-248. Doi: 10.1016/j.ejphar.2015.10.045.
32. Zarai Z, Boujelbene M, Ben Salem M and Ben Salah G. 'Piperine inhibits cell proliferation and induces apoptosis in breast cancer cells', *Journal of Cancer Research and Therapeutics*. 2013; 9(3):456-464. Doi: 10.4103/0973-1482.119301.