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ORIGINAL ARTICLE

Evaluation of Iron, Ferritin levels and the Toxic effect of Datura metel in Drosophila melanogaster model.

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ABSTRACT

Introduction: The toxic effect of *Datura metel* have been well documented in humans however litter is known on its effect on iron profile status. The objective of this study was to evaluate the effect *Datura metel* on some iron profile parameters using *Drosophila melanogaster* model.

Materials and Methods: *Drosophila melanogaster* flies were allocated into four classes (ethanolic leaf extract, ethanolic seed extract, aqueous seed extract and aqueous leaf extract) with six (6) different concentrations. Each concentration were performed in triplicates with twenty-five (25) flies each. Aqueous and ethanolic extract of *Datura metel* leaf and seed were extracted and different concentrations was administered to *Drosophila melanogaster*. A survival assay was done for 14 days while Serum iron and Serum ferritin assay was done after 7 days on the various treatment group. Iron and ferritin were estimated using enzyme linked immunosorbent assay (ELISA), ANOVA was used to compare between the different concentrations of the extract and the control group and result reported in mean±SEM.

Results: The results obtained showed a significant decrease ($p<0.001$) in iron concentration in the experimental group treated with different concentrations of aqueous leaf extract of *Datura metel* when compared with the control. Similarly, there was a significant decrease in iron ($P = 0.001$) and ferritin ($P < 0.001$) in the experimental group treated with different concentration of ethanolic extract of *Datura metel* seed when compared to the controls. The median survival rate

shows that after 14 exposure to different concentrations of *datura metel,* aqueous and ethanolic leaves extracts had a median survival rate of 8 flies while aqueous and ethanolic seed extract had a median survival rate of 11 and 12 flies respectively.

Conclusion: The results suggest that ethanolic and aqueous extract of *Datura metel* seed and leaf decreases iron and ferritin levels in *Drosophila melanogaster*. And the leaves of *Datura metel* maybe more toxic than the seed of the plant.

Keywords: *Datura metel, Drosophila melanogaster,* iron, ferritin

INTRODUCTION

Plants have always played a major role in the treatment of human traumas and diseases worldwide. The demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines. For the past few years, there has been a great increase in the use of medicinal plant for curative purposes. The therapeutic activities of most plants are due to the presence of one or more of such components like alkaloids, tannins, saponins, cardiac glycosides, steroids, alkaloids, flavonoids, phenols and glycosides (1).

Datura metel is an erect shrub with spreading branches. A perennial herbaceous plant, belonging to the Solanaceae family can reach a height of 1.5m. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. Its common names are: Thorn apple, Devil's apple, Jimson weed and Angel's trumpet. Its indigenous names in Nigeria include: Igbo –Myaramuo; Hausa – Zakami; Yoruba – Apikan (2). Flowers are large, solitary, and trumpet-shaped with a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colours, ranging from white to yellow and light to dark purple. The flowers are hermaphrodite and are pollinated by insects. The fruit is in the form of a capsule covered with short spines. Datura can tolerate average soil but prefers soil which is rich and moist or even very alkaline soil but hardly survives under shade. It prefers a warm temperature and is distributed in warmer regions of the world (3).

In 2016, Imo and his colleagues observed the haematological effects of ethanolic leaf, seed and fruit extracts of *Datura metel* in male albino rats and postulated that the decrease in RBC, PCV and Hb levels in Groups 3 administered high dose (600 mg/kg bw) of leaf extract compared with the normal control and especially 5 administered high dose (600 mg/kg bw) of seed extract is believed to be as a result of high contents of the secondary metabolites (mostly tropane alkaloids) contained in the leaf and seed of *Datura metel*. Other plants such as soybean have also been reported to interfere with iron homeostasis (4). Furthermore, the

way in which *Drosophila melanogaster* acquires iron from the diet remains poorly understood despite iron absorption being of vital significance for larval growth (5)

Hence, this study is aimed at investigating the effects of *Datura metel* leaf and seed extract on serum iron and ferritin of healthy *Drosophila melanogaster.*

MATERIALS AND METHODS

IDENTIFICATION OF PLANT

The plant materials (leave and seed) were harvested in Ekosod in village, Benin City which is situated in south of Nigeria. It was further transported to the Plant Biotechnology Department, Faculty of Physical Sciences, University of Benin for authentication and given a Voucher Number UBH-321 (leaf) and 322 (seed).

METHOD OF EXTRACTION

Plant (*Datura metel*) extraction was carried out at the Faculty of Pharmacy, University of Benin. The leaf and seed were air dried for a period of 3 days in a cool temperate environment. The leave and seed sample was then dried after the initial time at 40^0c for 2 days then it was grinded in British milling machine into powdered sample. The weight of the plant sample was recorded as 395.98g for leaf and 37.45g for seed. For Aqueous extract, the plant sample was soaked in chromatographic tank with 900ml of distilled water for 24hours, while 900ml of ethanol was used for the ethanolic extract. The liquid-soaked sample was then filtered with filter paper and funnel to differentiate the filtrate from the residue. The filtrate was then concentrated using a rotary evaporator and stored in a sample bottle for preservation in a refrigerator at 4^0C.

Drosophila melanogaster and Treatments

Both genders (1-3 days old) of *Drosophila melanogaster* (Harwich stain) were cultured in the *Drosophila melanogaster* Research Laboratory, Department of Physiology, Faculty of Basic Medical Sciences, University of Benin, Edo state, Nigeria. The flies were obtained from the Federal University of Santa Maria, Brazil. The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosed into young adult fruit flies under a natural photoperiod of about 12 hours light and 12 hours dark daily for the period of administration of the chemical compound under investigation. Flies were collected and separated into four experimental groups with five vials of 30 flies in each group and the flies were then treated as stated below in the experimental design.

PREPARATION OF FEED FOR *Drosophila melanogaster*

PROCEDURE

Six hundred fifty (650) ml of water was measured and turned into a pot and heated till boiling. Agar was then added to the boiling water. Yeast was dissolved using boiling water in another container. The mixture was stirred for 10 minutes. The cornmeal was dissolved with 150 ml of water with glucose added and poured into the pot and stirred continuously for 10 minutes. Yeast was then added to the mixture and stirred for another 15 minutes. The remaining 50 ml of water was used to rinse the remnant in the plate used. The meal was brought down and allowed to cool to 60^0C. The preservative methylparabine was then added after it was dissolved in absolute alcohol and

stirred. The meal was then transferred into appropriate vials.

EXPERIMENTAL DESIGN

This experiment consisted of four (4) class which were given different concentrations of ethanolic and aqueous extracts of *Datura metel* leaf and seed.

Class 1 was (*Datura metel* ethanolic seed extract)
Class 2 (*Datura metel* ethanolic leaf extract)
Class 3 (*Datura metel* aqueous seed extract) and
Class 4 (*Datura metel* aqueous leaf extract)

In this experiment, 1800 flies were allocated into the four classes (ethanolic leaf extract, ethanolic seed extract, aqueous seed extract and aqueous leaf extract). Each of the classes were further divided into six (6) groups with different concentrations (controls, 5% *Datura metel*, 10% *Datura metel*, 20% *Datura metel*, 40% *Datura metel*, 100% *Datura metel*) each. Each concentration was made in triplicates vials with twenty-five (25) flies each. The concentrations of *Datura metel* introduced at different concentrations into the diet in each group are presented below;

Group I: Control flies fed on diet mixed with water
Group II: Flies administered 5% *Datura metel* (ethanolic and aqueous extract)
Group III: Flies administered 10% *Datura metel* (ethanolic and aqueous extract)
Group IV: Flies administered 20% *Datura metel* (ethanolic and aqueous extract)
Group V: Flies administered 40% *Datura metel* (ethanolic and aqueous extract)
Group VI: Flies administered 100% *Datura metel* (ethanolic and aqueous extract)

The flies were monitored for 14 days and daily mortality records were taken.

ANAESTHETIZING FLIES BY USING CO_2 GAS

Carbon dioxide is a non-flammable odorless gas which is an excellent alternative to volatile and toxic anesthetics. It is non-toxic in low quantity. When using CO_2 gas, the flies are transferred to an empty vial and the CO_2 gas piston is shot into the vial to release the gas before they are transferred to the CO_2 gas panel to keep them anesthetized long enough to do each experiment.

PREPARATION OF SAMPLES FOR BIOCHEMICAL ASSAY HOMOGENIZATION

The flies were collected followed by anesthetizing, and homogenising in a homogenizing buffer (0.1M phosphate buffer, pH 7.4), and centrifuged at 4000 rpm for 10 minutes. Subsequently, the supernatant was then separated from the pellet into a labelled eppendorf tubes and used for the determination of serum iron and ferritin.

BIOCHEMICAL ASSAYS

Iron and ferritin were analyzed using colorimetric and Enzyme Linked Immunosorbent Assay (ELISA) methods respectively.

Determination of Iron

Method: Colorimetric method

Procedure

Test tubes/cuvettes: "Blank", "Standard", "Control and "Sample" were labelled. To all tubes, 2.5 ml Iron Buffer reagent was added. To respective tubes, 0.5 ml (500 µl) of sample was added and mixed. To blank, 500 µl iron-free water was added. The spectrophotometer was

blanked at 560 nm with the reagent blank. The absorbances of all tubes were read and recorded (A1 reading). To all the tubes, 0.05 ml (50 µl) Iron color reagent was added and mixed. All tubes were placed in the heating bath at 37°C for 10 minutes. The spectrophotometer was blanked at 560 nm with the reagent blank. The absorbances of all tubes were read and recorded (A2 reading)

Determination of Ferritin

Method: Enzyme Linked Immunosorbent Assay (ELISA)

Procedure:

The microplates' well was formatted for each serum reference, control, and test specimen to be assayed in duplicate. To each assigned microtitre well, 0.025 mml (25 µl) of appropriate serum reference, control and test specimen was added. To each well, 0.100 ml (100 µl) of the ferritin biotin reagent was added. The microplate was swirled for 20-30 secs, covered and incubated for 30 minutes at room temperature. The content of the microplate was decanted and a paper was used to blot the microplate dry. To the microplate well, 350 µl of wash buffer was added and aspirated. This step was repeated thrice. To each well, 0.100 ml (100 µl) of ferritin enzyme conjugate was added. The content of the microplate was decanted and a paper was used to blot the microplate dry. To well microplate well, 350 µl of wash buffer was added and aspirated. This step was repeated thrice To each well, 0.100 ml (100 µl) of working substrate solution was added. The microplate well was incubated for 15 minutes at room temperature. To each well, 0.050 ml (50 µl) of stop solution was added, the microplate was mixed gently for 15-20 secs. The absorbance was read at a wavelength of 450 nm.

RESULTS

Toxic effect of *Datura metel* on *Drosophila melanogaster*

To determine the effect of *Datura metel on Drosophila melanogaster*, a survival analysis was done and to also determine which was more potent leaves or seeds of *Datura metel*. Figure 1 panel A shows the effect of aqueous seed extract of *Datura metel* on the survival of for the period of 14 days. For the controls there was 41.66% survival rate, 5% concentration showed 36.34% survival rate, 10% showed 27.69% survival rate, 20% showed 13.26% survival rate, 40% showed 25% survival rate and 100% showed 7.03%. Panel B shows the effect of the ethanol seed extract of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days. For the controls there was 41.66% survival rate, 5% concentration showed 15.75% survival rate, 10% showed 15.48% survival rate, 20% showed 17.01% survival rate, 40% showed 6.22% survival rate and 100% showed 6.25% survival rate. Figure 2 panel shows the effect of the aqueous leaf extract of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days. For the controls, there was 41.66% survival rate, 5% concentration showed 42.42% survival rate, 10% showed 36.45% survival rate, 20% shower 38.5% survival rate, 40% showed 33.45% survival rate and 100% showed a 23.02% survival rate. Figure 2 panel B shows the effect of the ethanol extract leaf of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days. For the controls there was 41.66% survival rate, 5% concentration showed

35.71% survival rate, 10% showed 30.38% survival rate, 20% showed 37.43% survival rate, 40% showed 7.36% survival rate and 100% showed 7.98% survival rate. Table 1 shows the median survival rate of *Drosophila melanogaster* in response to the different concentrations of *Datura metel.* The results shows that at 100%¸ the aqueous seed extract showed a median survival rate of 11 flies, the ethanolic extract at 100% showed a median survival rate of 12 flies. Both the ethanolic and aqueous leaf extracts showed a median survival rate of 8 flies.

Some Iron profile parameters in *Drosophila melanogaster* treated with *Datura metel*

To evaluate the effect of the different concentrations of *Datura metel* on some iron profile parameters, iron and ferritin levels were estimated in both seed and leaf extracts. Figure 3 shows the effect of different concentrations (5%, 10%, 20%, 40% and 100%) of leaf extract of *Datura metel* on some iron profile parameters in *Drosophila melanogaster* for the period of 7 days. Figure 3 Panel A shows the effect of aqueous extract of *Datura metel* on iron. The results shows that iron was significantly lower ($p=0.04$) in the 100% group when compared to control. The control group also had a significantly higher iron when compared to 40% group ($p=0.003$). Ferritin was also significant higher in the control group when compared to 40% ($p=0.0024$) administered group. 10% ($p=0.016$) and 20% ($p=0.019$) administered group also had a significantly higher ferritin levels when compared to the 40% group (figure 3 panel B). The ethanolic leaf extract of *Datura metel* shows that iron ($p=0.0027$) and ferritin ($p=0.033$) levels were significantly higher in the control group when compared 100% administered group as shown in figure 3 panel C and D respectively. Figure 4 shows the effect of the seed extract of *Datura metel* on iron and ferritin levels in *Drosophila melanogaster.* Panel A shows that there was no significant difference in iron levels when comparing control to all experimental groups. However, figure 4 panel B shows that there ferritin was significantly higher ($p<0.001$) in the control group when compared to all experimental groups. 5% administered experimental group was significantly than 40% ($p=0.001$) and 100% ($p=0.001$) administered groups. Ferritin levels appear to be in a dose-dependent manner with the higher concentration having lower ferritin levels. Similarly, the ethanolic seed extract showed that iron levels was significantly higher ($p<0.001$) in the control group when compared to other experimental groups and this appear to be in a dose dependent manner (Figure 4 panel C). In the ethanolic seed extract, ferritin levels was significantly higher the control group when compared the 40% ($p<0.001$) and 100% ($p<0.001$) experimental groups. 5% experimental group was also significantly higher than 40% ($p=0.003$) and 100% ($p<0.001$) experimental groups (Figure 4 panel D).

Figure 4: Panel A and B shows the effect of different concentrations of aqueous seed extract of *Datura metel* on iron and ferritin levels in *Drosophila melanogaster.* Panel C and D shows the effect of different concentrations of ethanolic seed extract of *Datura metel* leaf on iron and ferritin in *Drosophila melanogaster*

DISCUSSION

The objective of this study was to investigate the effect of aqueous and ethanolic extract of *Datura metel* leaf and seed on iron and ferritin

levels in *Drosophila melanogaster*. Plants have a very great potential for the treatment and management of certain disease. A large number of plants have been used by tribal and folklore: in many countries for the treatment of different diseased conditions. The medicinal value of plants lies in their bioactive phytochemical constitution that produce definite physiological actions on human body and other animals. These chemical constituents include flavonoids, alkaloids, essential oils, saponins, terpenoids, tannins and phenolic compounds. *Datura metel* possess potent, toxic, anticholinergic properties. The leaves are most commonly used as a narcotic, either smoked or boiled and eaten; seeds are similarly used. Roots, seeds or leaves are added to alcoholic drinks to increase the intoxicating effect. Side effects include dry mouth and throat, eye pain, blurred vision, restlessness, dizziness, arrythmia, flushing and faintness Datura species are well known because of their high concentration of tropane alkaloids, which has led to poisoning episodes when Datura is accidentally mixed with edible crops (6). In this study, results shows that the consumption of aqueous seed extract of *Datura metel* on the survival rate of *Drosophila melanogaster* for a period of 14 days, there was a moderate reduction in the survival rate of the flies (*Drosophila melanogaster)* on a dose dependent rate. However, there was massive reduction in the survival rate of the flies on a dose dependent rate when administered with ethanolic leaf and aqueous leaf extracts of *Datura metel respectively.* The median survival rate data from this study shows that the leaves of *Datura metel* seem to be more potent than the seed of the plant. This suggest that *Datura metel* may contain certain chemical that are toxic to the flies and this toxicity is increased as the dose is increased. Although, it has been postulated that all parts of the plant are toxic because of their high tropane alkaloid level (7), the survival curve shows a massive reduction in survival rates on the ethanolic leaf extract therefore pointing to the fact that the leaves (both ethanolic and aqueous) maybe more toxic than the seeds. From the survival assay, there is an indication that the toxic constituent (mostly the tropane alkaloids) of *Datura metel* is more soluble in ethanol than water hence a higher toxicity levels in ethanol when compared to aqueous extract. Similarly works reporting the toxic nature of datura have been reported. Fatal poisoning with *Datura metel* have been reported and some people have also used it as a "suicide drug" (8,9,10).

To evaluate the effect of *Datura metel* on some iron profile parameters, iron and ferritin were estimated. The results shows that aqueous leaf extract of *Datura metel* had a significant difference in the iron and ferritin concentrations among the experimental groups against the control group. There was also a significant decrease in serum iron concentration when the control group was compared to various experimental groups of different concentration. Similarly, aqueous seed extract of *Datura metel* had no significant effect on iron however, ferritin levels was significantly lower in the experimental groups when compared to the control group and this appear to be dose dependent. Ethanolic leaf extract of *Datura metel* had a significant effect on iron and ferritin concentration while ethanolic seed extract of *Datura metel* also significantly reduced iron and ferritin levels in the experimental groups as compared to the control group. The reduction in iron and ferritin levels is probably due to early reported observations that *Datura* extract

may cause a significant disruption in hepatic and renal lipid peroxidation and this may also suggest mechanism of toxicity of *D. metel* in the heart which involves lipid peroxidation (11). Lipid peroxidation is a free radical-mediated oxidation of polyunsaturated fatty acids involving chain reactions that ultimately cause deleterious effect to critical biological macromolecules (12). These oxidative reactions often lead to the formation of lipid hydroperoxide which are further degraded into several aldehydic compounds including hydroxy-alkenals (13). In comparison to free radicals, these aldehydes are more stable and have the tendency to diffuse and escape from the cell thereby attacking targets cells far away. Interesting, there is a correlation between iron metabolism and lipid peroxidation which may explain our results. Similarly Okpara *et al.* (14) have recently reported that a significant decrease in RBC, PCV, HB as well as in the erythrocytic indices (MCV, MCH and MCHC), in albino rats administered *Datura metel* extract, which was attributed to RBC susceptibility to lipoperoxidative changes which is also directly association with molecular oxygen, high content of metal ions catalysing oxidative reactions and availability of high amount of polyunsaturated fatty acids (PUFA's). They further reported that the decrease in the erythrocyte parameters and indices in the *D. metel* group could also be attributed to possible disruption of haemopoisis due to intracellular oxidative stress. Haemoglobin biosynthesis takes place in the mitochondria and toxic inhibition of the activities of enzymes such as alpha-aminolevulinic acid synthase (ALA-synthase) and prophobilinogen synthase will affect the haemoglobin synthetic pathway (15). The disruption of the activities of any of these enzymes due to oxidative damage to macromolecules may interfere with haemoglobin production, thus defective and decreased erythrocyte production. Therefore, the decrease in (MCV, MCH and MCHC) values in the *D. metel* treated groups could also be attributed to the disruption in haeme biosynthetic pathway, hence in haemopoiesis leading to the production of microcytes. The results of iron and ferritin reported in this study could also be as result in a disruption in the heme biosynthesis pathway due to the fact that iron is a key player in the formation of heme molecule. Furthermore, the decrease in iron and ferritin levels can result in microcytic anaemia, ineffective erythropoiesis due to nutritional depletion of iron (Fe) and finally decreased haematocrit levels and haemoglobin concentration.

Most free alkaloids are lipophilic and soluble in polar solvents like methanol and ethanol (16). This may be the reason of the active effect of the ethanolic extract than the aqueous extract.

CONCLUSION

Different parts of *Datura metel* have been reported to have therapeutic activities because of its phytochemical. Although all parts have also been reported to be toxic. The result obtained shows that ethanolic and aqueous extracts of *Datura metel* leaf and seed decreases iron and ferritin in experimental groups observed. Also the ethanolic extract maybe more toxic than the aqueous extract and the leaves were potent than the seeds as shown in the median survival rate.

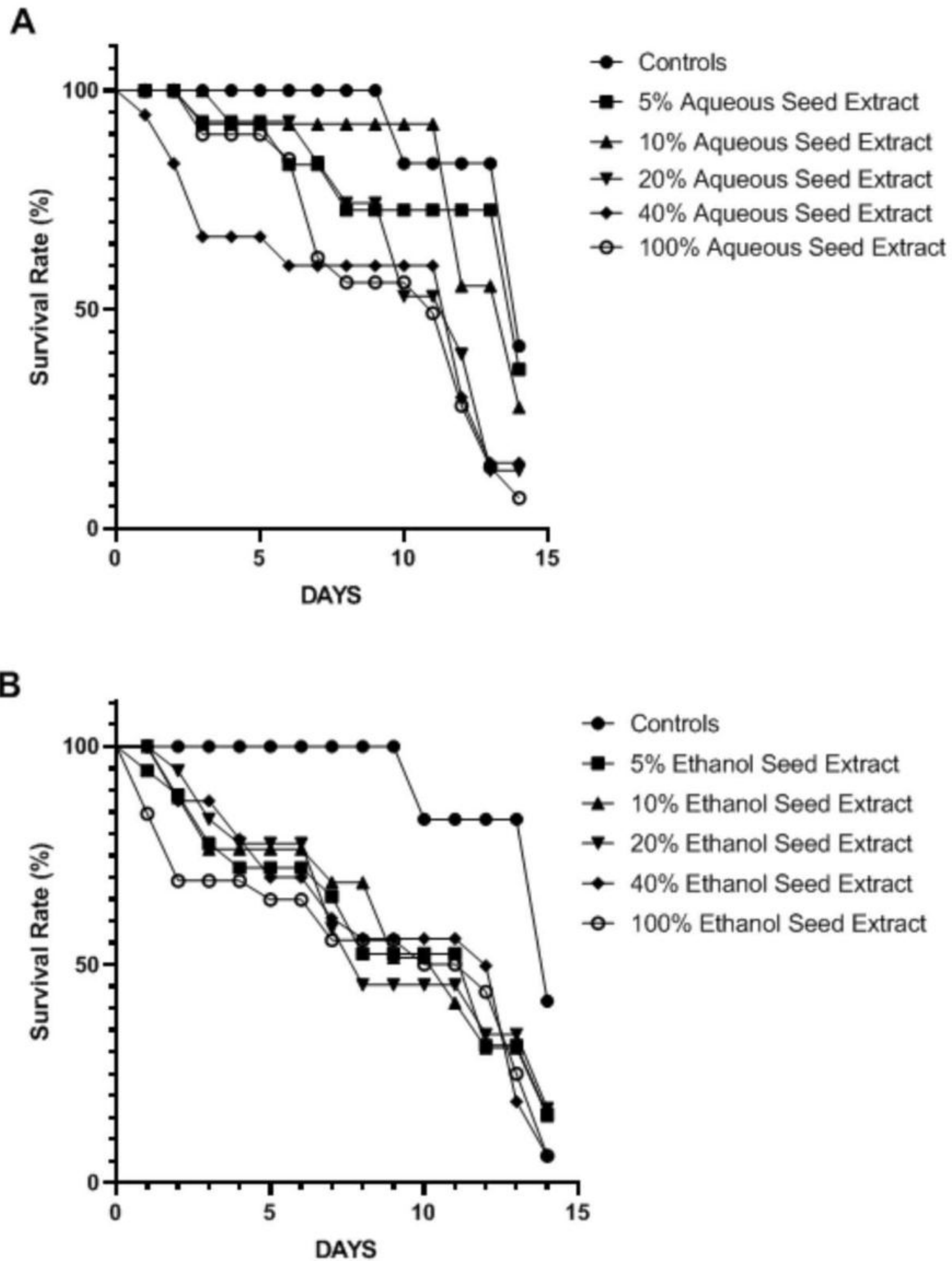

Figure 1: Panel A shows the effect of aqueous seed extract of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days. Panel B shows the effect of the ethanol seed extract of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days.

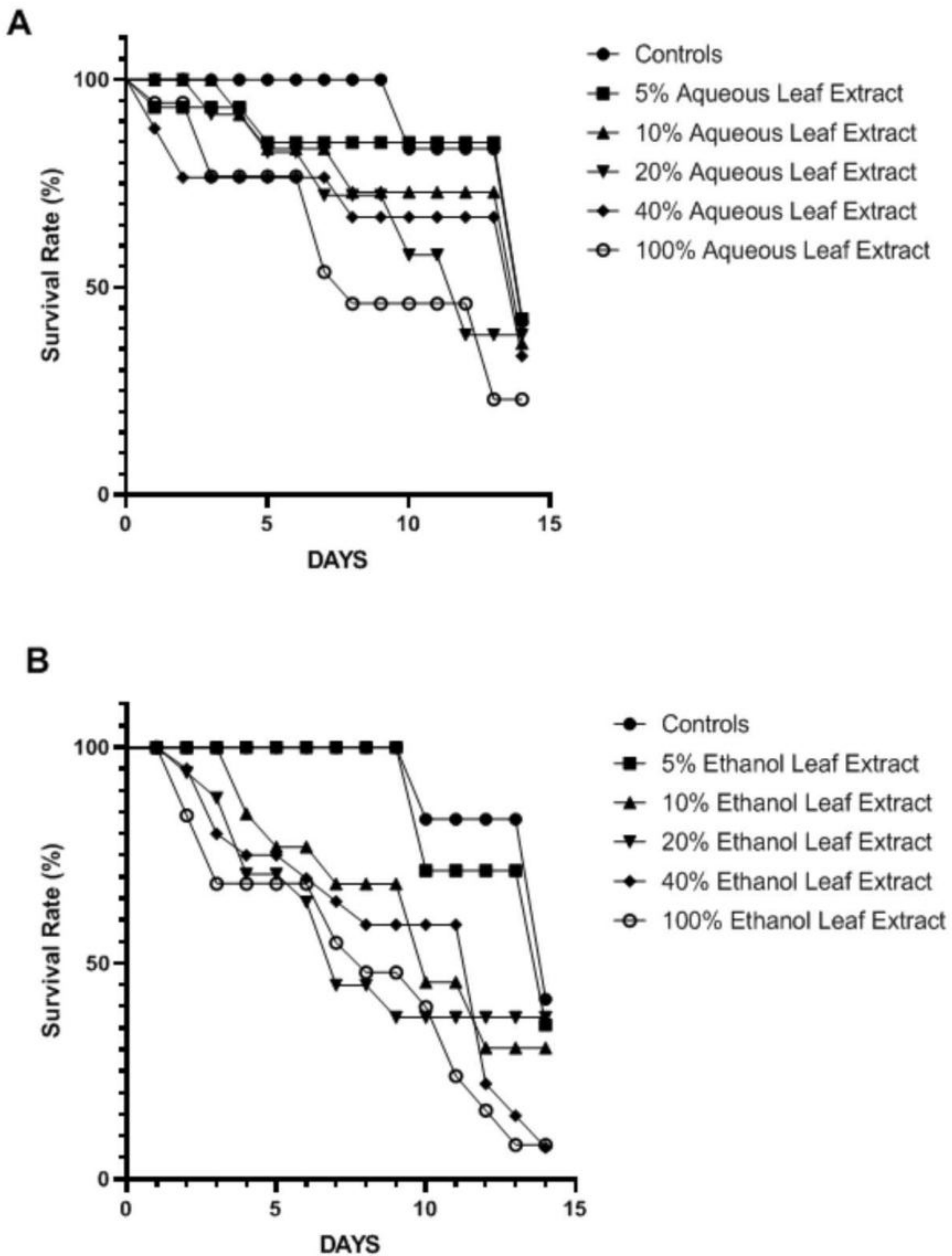

Figure 2: Panel A shows the effect of the aqueous leaf extract of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days. Panel B shows the effect of the ethanol extract leaf of *Datura metel* on the survival of *Drosophila melanogaster* for the period of 14 days.

Table 1: Median survival rate of *Drosophila melanogaster* treated with different concentration of *Datura metel*

Concentrations	Aqueous Seed Extract	Aqueous Leaf Extract	Ethanol Leaf Extract	Ethanol Seed Extract
Control	14	14	14	14
5%	14	14	14	12
10%	14	14	10	11
20%	12	12	7	8
40%	12	14	12	12
100%	11	8	8	12

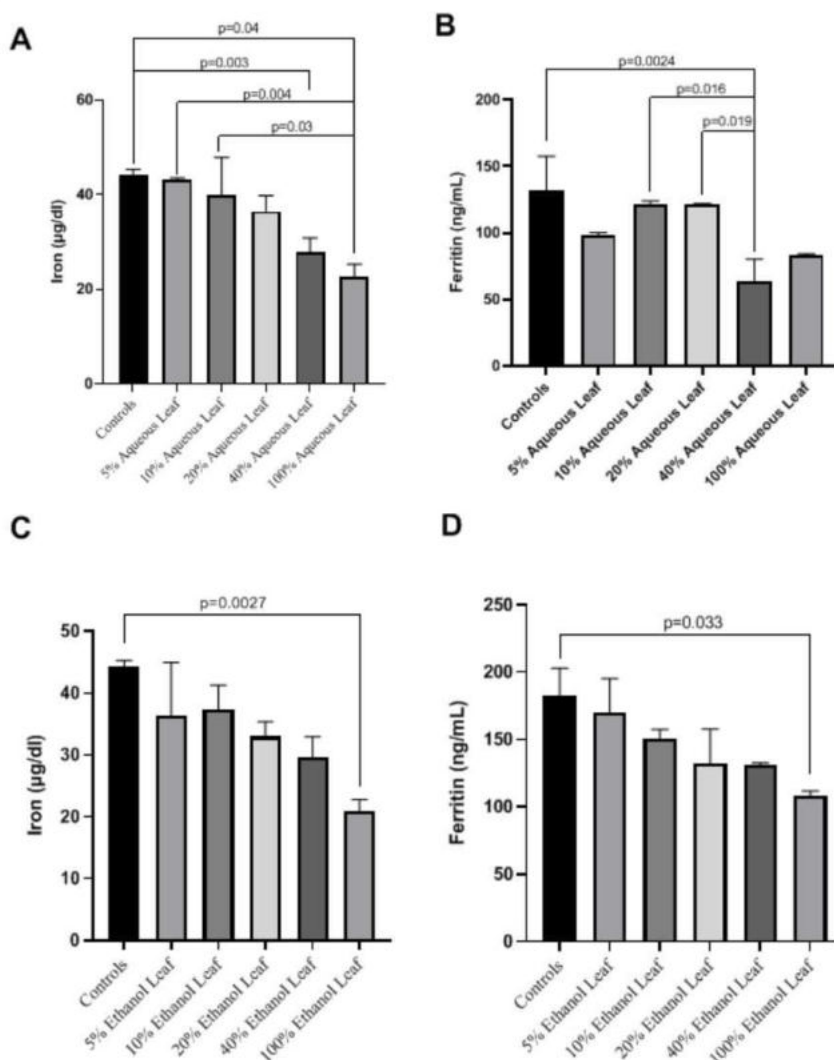

Figure 3: Panel A and B shows the effect of different concentrations of aqueous leaf extract of *Datura metel* on iron and ferritin levels in *Drosophila melanogaster.* Panel C and D shows the effect of different concentrations of ethanolic leaf extract of *Datura metel* leaf on iron and ferritin in *Drosophila melanogaster.*

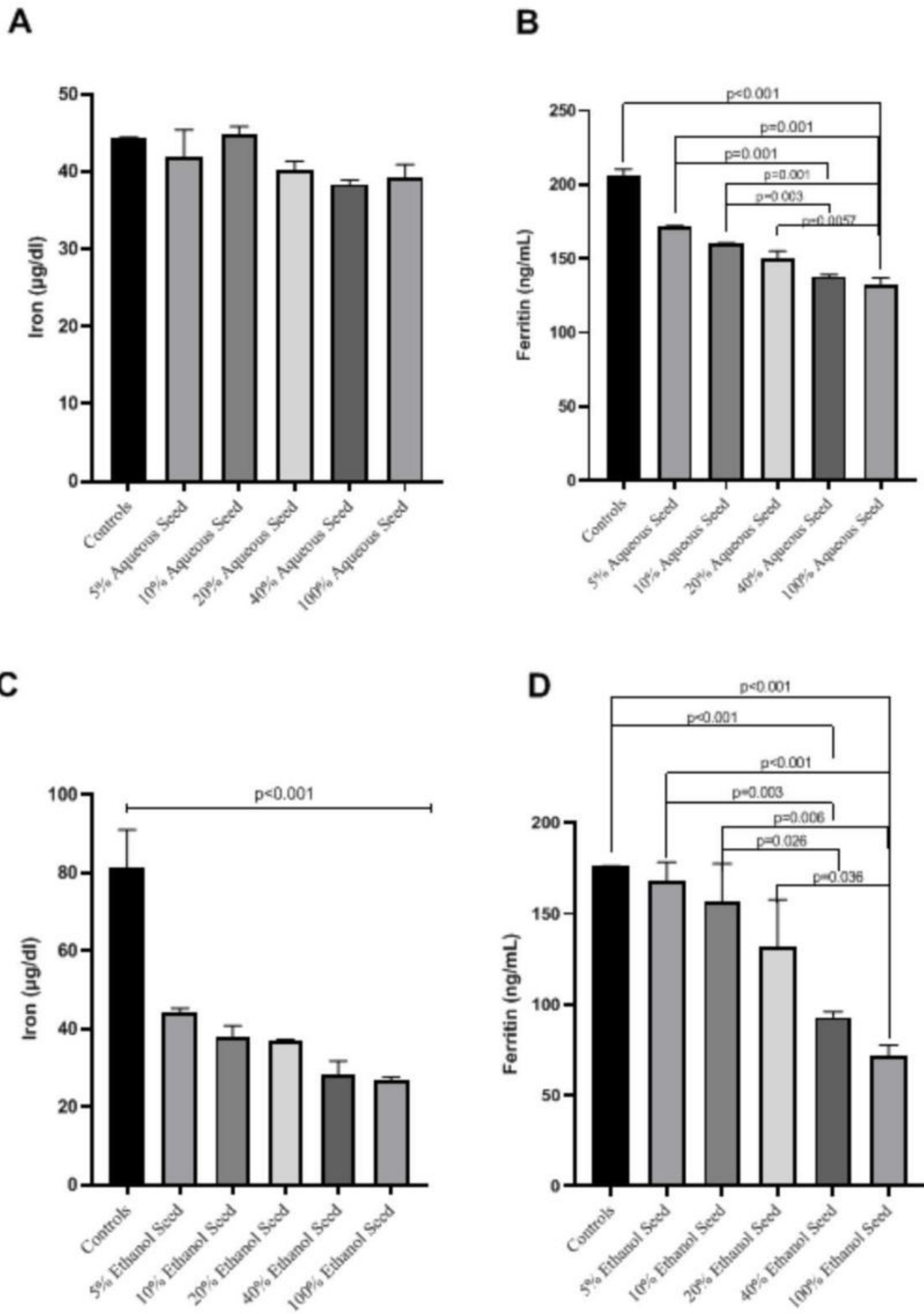

Figure 4: Panel A and B shows the effect of different concentrations of aqueous seed extract of *Datura metel* on iron and ferritin levels in *Drosophila melanogaster.* Panel C and D shows the effect of different concentrations of ethanolic seed extract of *Datura metel* leaf on iron and ferritin in *Drosophila melanogaster*

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