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PROTECTIVE EFFECT OF *NIGELLA SATIVA* L. SEEDS EXTRACT ON REPRODUCTIVE TOXICITY INDUCED BY FLUORIDE, ALUMINIUM AND THEIR COMBINATION IN SWISS ALBINO MALE MICE

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Keywords:

Fluoride, Aluminium, Testis, Epididymis, *Nigella sativa* seeds, Male mice

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ABSTRACT: The aim of the present study was to investigate the possible role of *Nigella sativa* L. seeds extract on fluoride, aluminum and their combination induced reproductive toxicity in Swiss albino male mice. *Nigella sativa* L. known as black seeds is used as a form of traditional medicinal plant. Fluoride and aluminum are omnipresent metals and are tremendously used in industries, pharmaceuticals, and food additives. The combination study was planned as the two metals co-exist in the environment. The exposure of the combination of metals may cause a hazardous effect on different systems of the body, especially the male reproductive system. The experimental design of the study consisted of different groups. Group 1: control, Group 2: NaF treated (10 mg/kg), Group 3: NS with NaF treated (300 mg/kg + 10 mg/kg), Group 4: $AlCl_3$ treated (100 mg/kg), Group 5: *Nigella sativa* with $AlCl_3$ treated (300 mg/kg + 100 mg/kg), Group 6: NaF + $AlCl_3$ treated (5 mg/kg + 50 mg/kg), Group 7: *Nigella sativa* with NaF + $AlCl_3$ treated (300 mg/kg + 5 mg/kg + 50 mg/kg). Various antioxidant and biochemical parameters on the testis and epididymis were studied after 45 and 60 days of exposure. The results revealed that fluoride, aluminum, and their combination treatment induced a significant decline in the body weight and organ weight, GSH, SOD, CAT levels, and a significant increase in the activity of TBARS level in the testis and epididymis of mice. Cholesterol and glycogen levels significantly increased, and a significant decline in the levels of protein, phosphorylase, and sialic acid as compared to the values of control mice was observed. Treatment with ethanolic extract of *Nigella sativa* seeds resulted in amelioration of reproductive toxicity induced by fluoride and aluminum, indicating its therapeutic potential.

INTRODUCTION: Metals have a crucial effect on the reproductive system of males directly, by targeting the reproductive organs, or indirectly by acting on the neuroendocrine system. The level of toxicity produced by a metal depends on several factors like the health status of an individual, gender, age, and genetics and also on the chemical composition, dose and route of exposure of metal [1].

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Fluoride and aluminum are widely distributed metals found on earth. Fluoride is physiologically active when it penetrates into organs, tissues, and cells due to its high biological activity and is present in plants, micro-organisms, animals, and human beings [2].

Fluoride is the monovalent anion of fluorine and is present in the form of sodium fluoride and aluminum fluoride. Sodium fluoride, which is the common fluoride salt, is used for the prevention of dental caries. The previous research done [3, 4] suggest that less than 1 ppm of fluoride is useful in the prevention of dental caries, but if the concentration of fluoride increases more than 1.5 ppm, it results in fluorosis.

The aluminum does not occur in its pure form, but mostly it is present in its combined form with oxygen, fluorine, silicon, sulphate, phosphate, hydroxide *etc*. It exists in trace amounts in the biological material; it does not seem to be a useful element and is usually considered to have harmful effects on human health [5]. Various forms of aluminum compounds are used for different purposes such as in consumer appliances, food packaging, and food additives, in treatment of water, paper making, fire retardant, fillers, and also in pharmaceuticals [6].

In this study, the toxic effect of fluoride, aluminum, and its combination on oxidative stress parameters in male reproductive organs (Testis and epididymis) of male Swiss albino mice is reported. Oxidative stress is the imbalance between the reactive oxygen species and the antioxidants which defend our body. When the concentration of reactive oxygen species is not controlled by the internal defense system, it causes damage to protein, lipids, and DNA, which leads to toxicity in the body. Medicinal plants are the major source of a therapeutic agent since ancient times to cure diseases. *Nigella sativa* (Black seeds) is one of the most popular herbs in many parts of the world. It is an annual flowering plant, and it belongs to the family of Ranunculaceae. It is grown in different parts of the world. It is considered one of the main sources of nutrition for all living beings. It is also known as Kalonji or black seeds. The seeds and oil of this plant have been used in making of food and medicines. The studies performed on *Nigella sativa* L. have proved that most of its pharmacological actions are due to its ability to scavenge free radicals and/or inhibit lipid peroxidation [7].

Animal studies have shown that the extract of *Nigella sativa* seeds has many therapeutic effects such as gastroprotective, anti-tumor antianxiety, anti-inflammatory, and anti-oxidant [8]. Black seeds are used as an anticonvulsant, laxative, diuretic to cure infections and wounds, hair treatment, headache, ear pain, parturition diseases, toothache, digestive system disturbances, glands diseases, fraction healing, liver, spleen, eye diseases, and muscle relaxant [9, 10]. Thymoquinone, is the major constituent of oil and seeds of *Nigella sativa*, which has medicinal properties [7, 11]. Based on these facts, our study was designed to investigate the protective effects of *Nigella sativa* L. seed extract (NSSE) on fluoride, aluminum and their combination induced reproductive toxicity in mice.

MATERIALS AND METHODS:

Chemical: Fluoride as sodium fluoride and aluminum as aluminium chloride was procured from HIMEDIA Laboratories Private Limited. It was dissolved in distilled water.

Test Animal: Swiss albino male mice with an average weight of 25-35 g were used in this experiment. The animals were kept in IIS (Deemed to be University) animal house approved by CPCSEA (Registration no: 1689/PO/a/13/ CPCSEA). The animals were housed in cages in a ventilated animal room of the University.

Water and food in the form of a standard pellet was given *ad libitum* to the mice. Wooden shavings were used as bedding to absorb urine. The bedding was changed on an average of every three days.

Metals and Their Doses:

- Fluoride as sodium fluoride (10 mg/kg b. w.).
- Aluminium as aluminium chloride (100 mg/kg) chloride.
- Sodium fluoride + aluminium chloride (5 mg/kg + 50 mg/kg).
- *Nigella sativa* seed extract (NSSE) (300 mg/ kg b. w.).

The Experiments were Planned on the Following Groups:

1. Control with distilled water.
2. Sodium fluoride (10 mg/kg b.w.) treated.
3. *Nigella sativa* with sodium fluoride treated (300 mg/kg + 10 mg/kg).
4. Aluminium chloride treated (100 mg/kg) treated.
5. *Nigella sativa* with aluminium chloride treated (300 mg/kg + 100 mg/kg).
6. Sodium fluoride + aluminium chloride treated (5 mg/kg + 50 mg/kg).
7. *Nigella sativa* with sodium fluoride + aluminium chloride treated (300 mg/kg +5 mg/kg + 50 mg/kg).

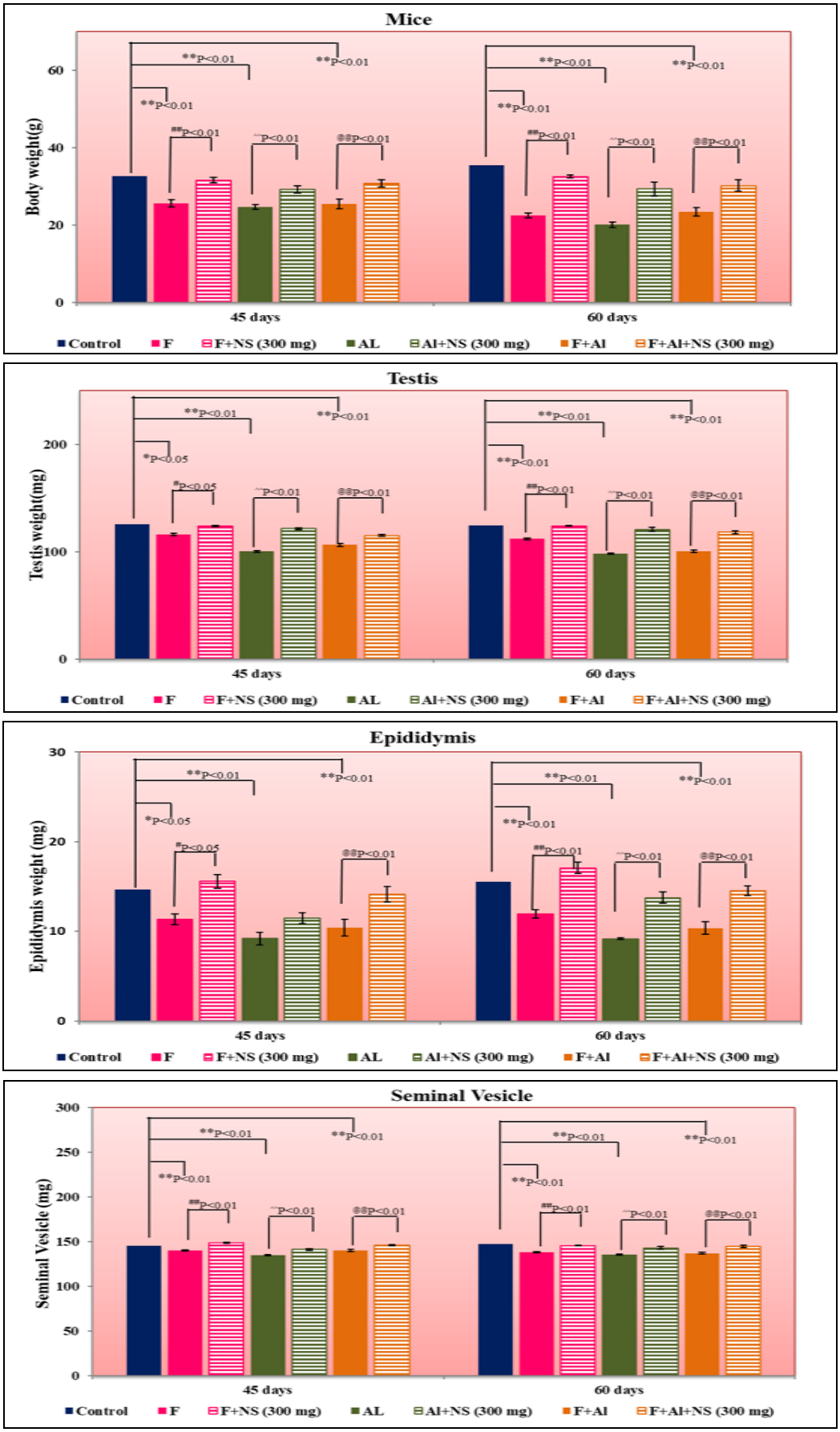

FIG. 1: BAR CHART DEPICTING BODY WEIGHT, TESTIS WEIGHT, EPIDIDYMIS WEIGHT AND SEMINAL VESICLE WEIGHT OF MICE OF DIFFERENT GROUPS EXPOSED TO FLUORIDE, ALUMINIUM, F-AL COMBINATION, *NIGELLA SATIVA* FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

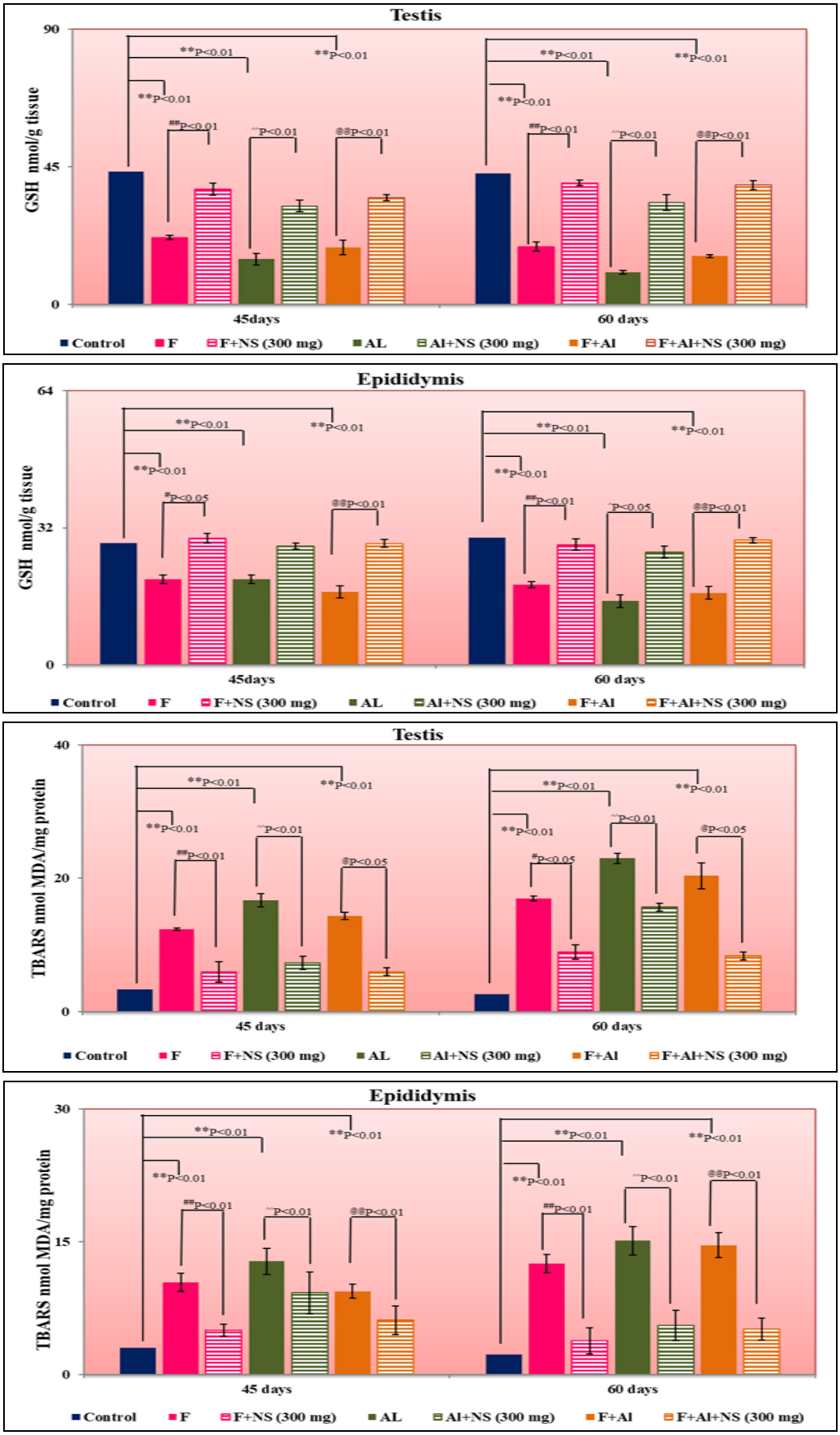

FIG. 2: BAR CHART DEPICTING GSH AND TBARS LEVELS ON DIFFERENT GROUPS OF MICE AFTER TREATMENT FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

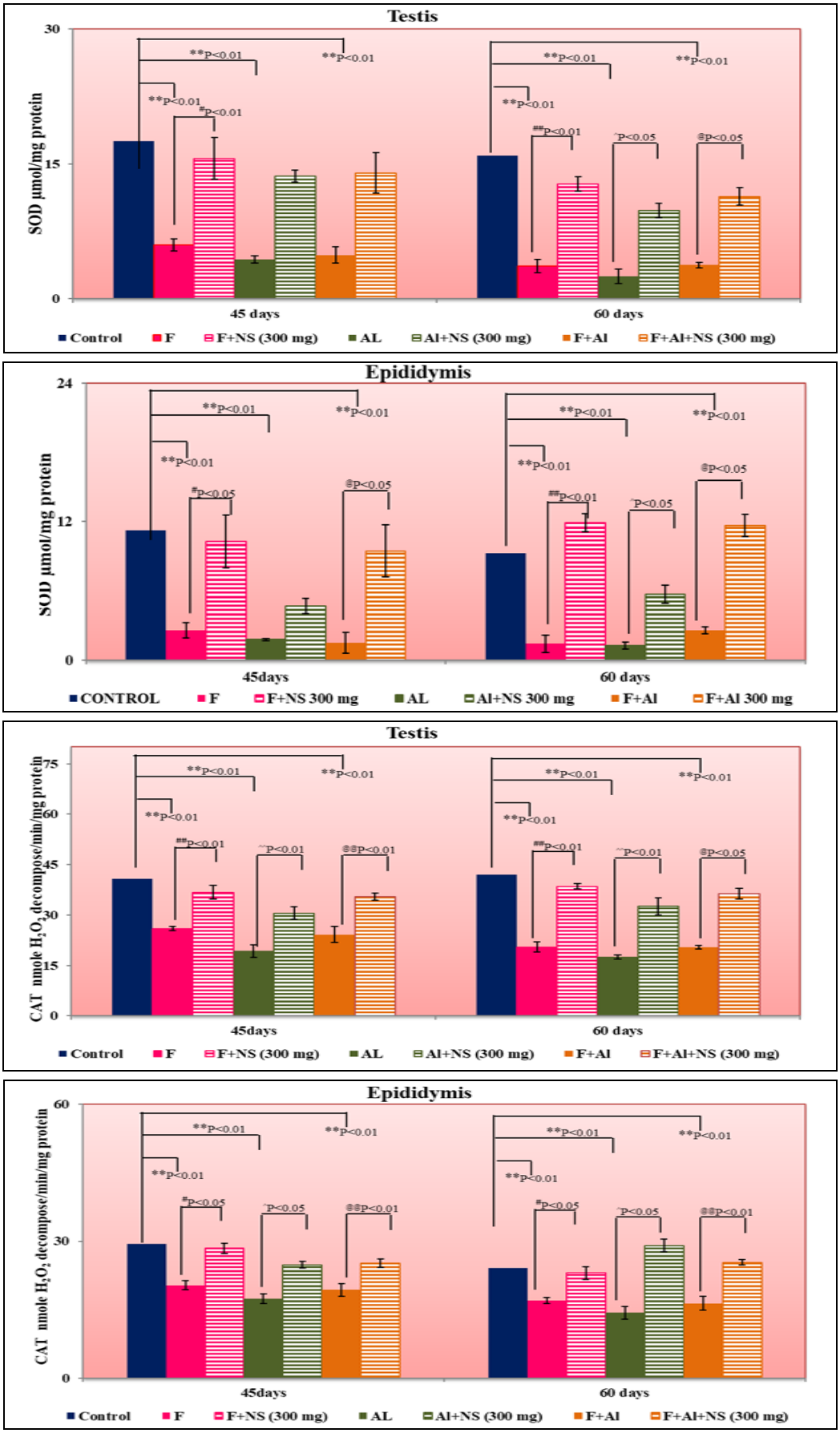

FIG. 3: BAR CHART DEPICTING SOD AND CAT LEVELS ON DIFFERENT GROUPS OF MICE AFTER TREATMENT FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

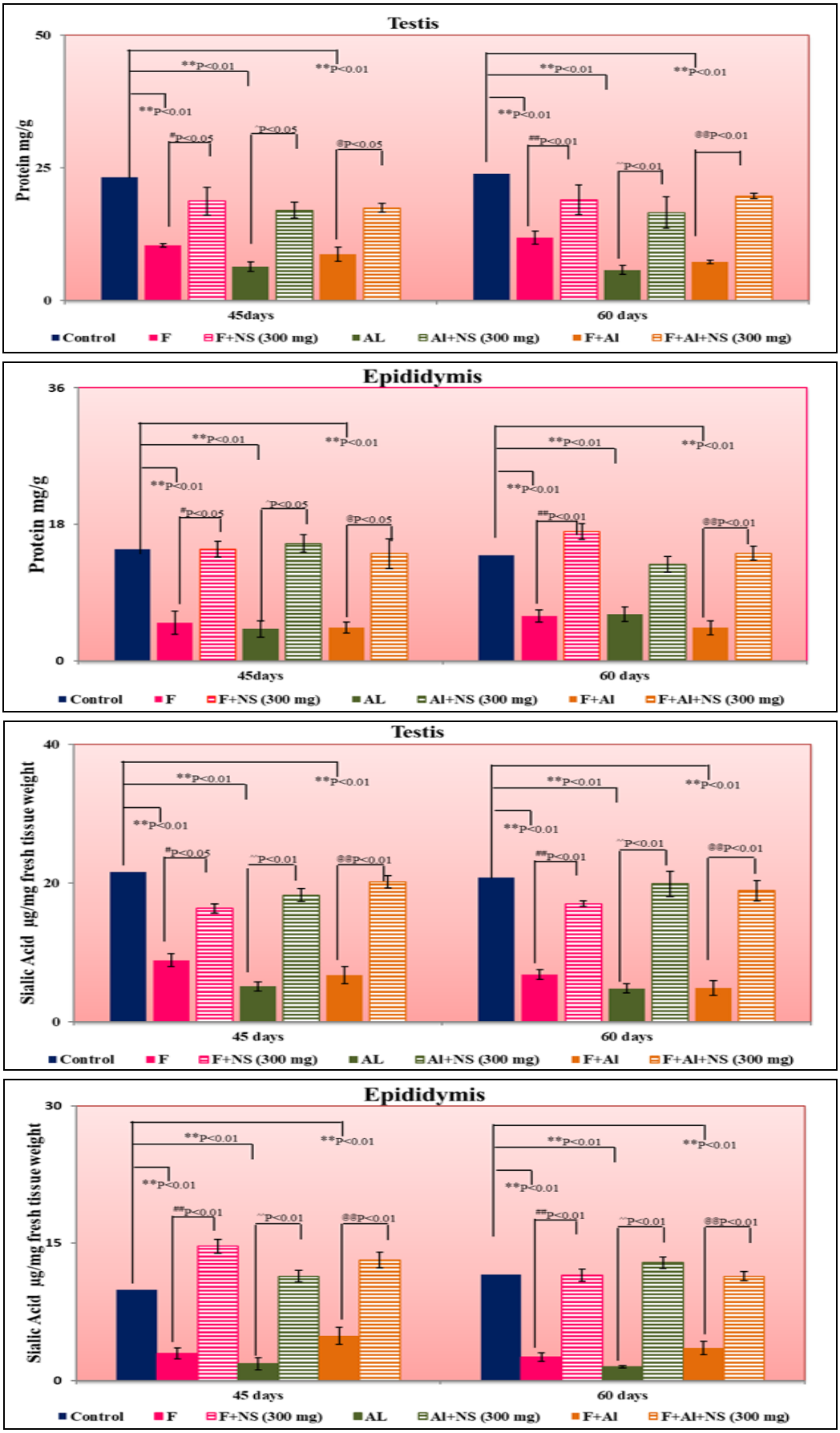

FIG. 4: BAR CHART DEPICTING PROTEIN AND SIALIC ACID LEVELS ON DIFFERENT GROUPS OF MICE AFTER TREATMENT FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

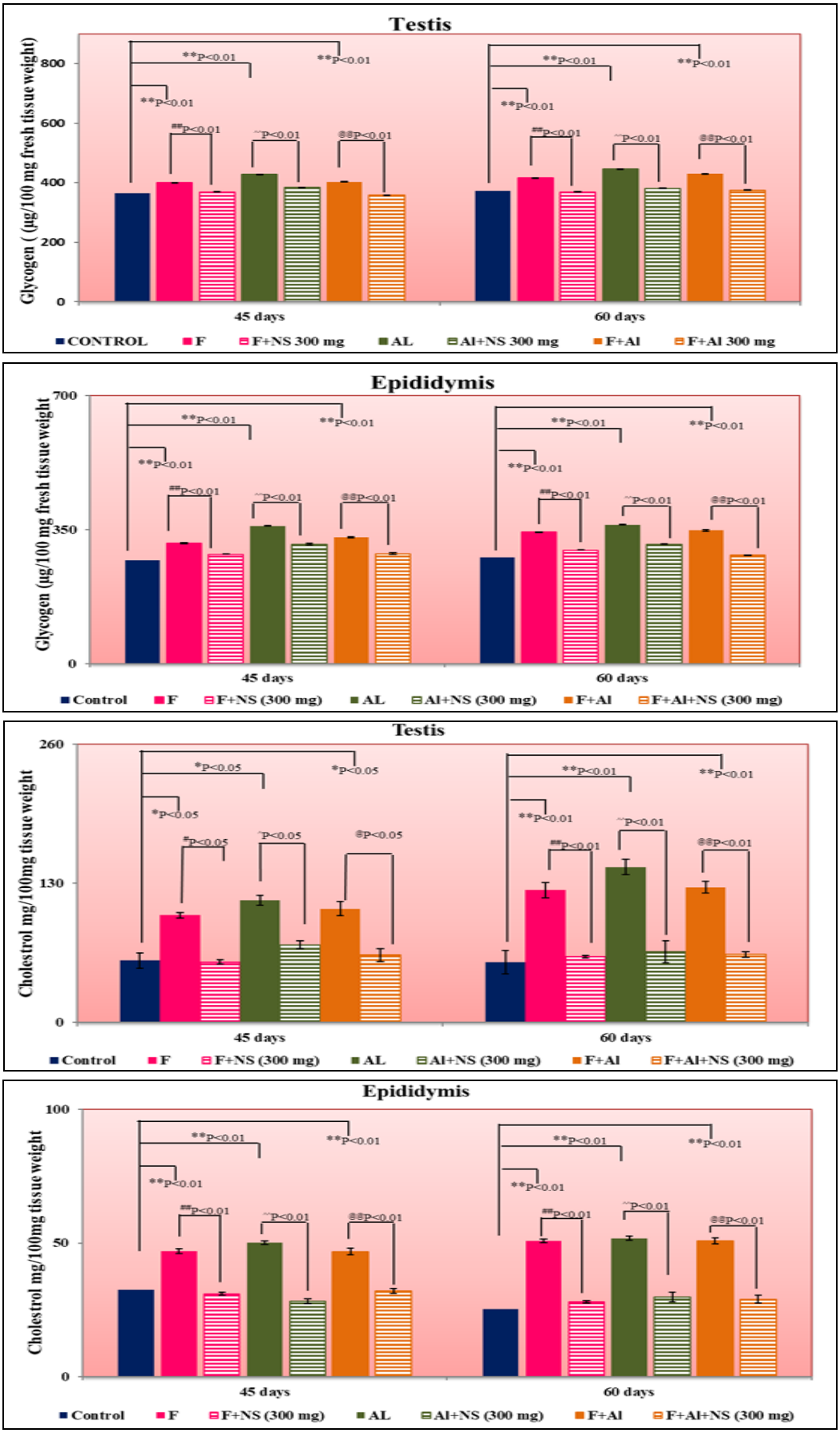

FIG. 5: BAR CHART DEPICTING GLYCOGEN AND CHOLESTEROL LEVELS ON DIFFERENT GROUPS OF MICE AFTER TREATMENT FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

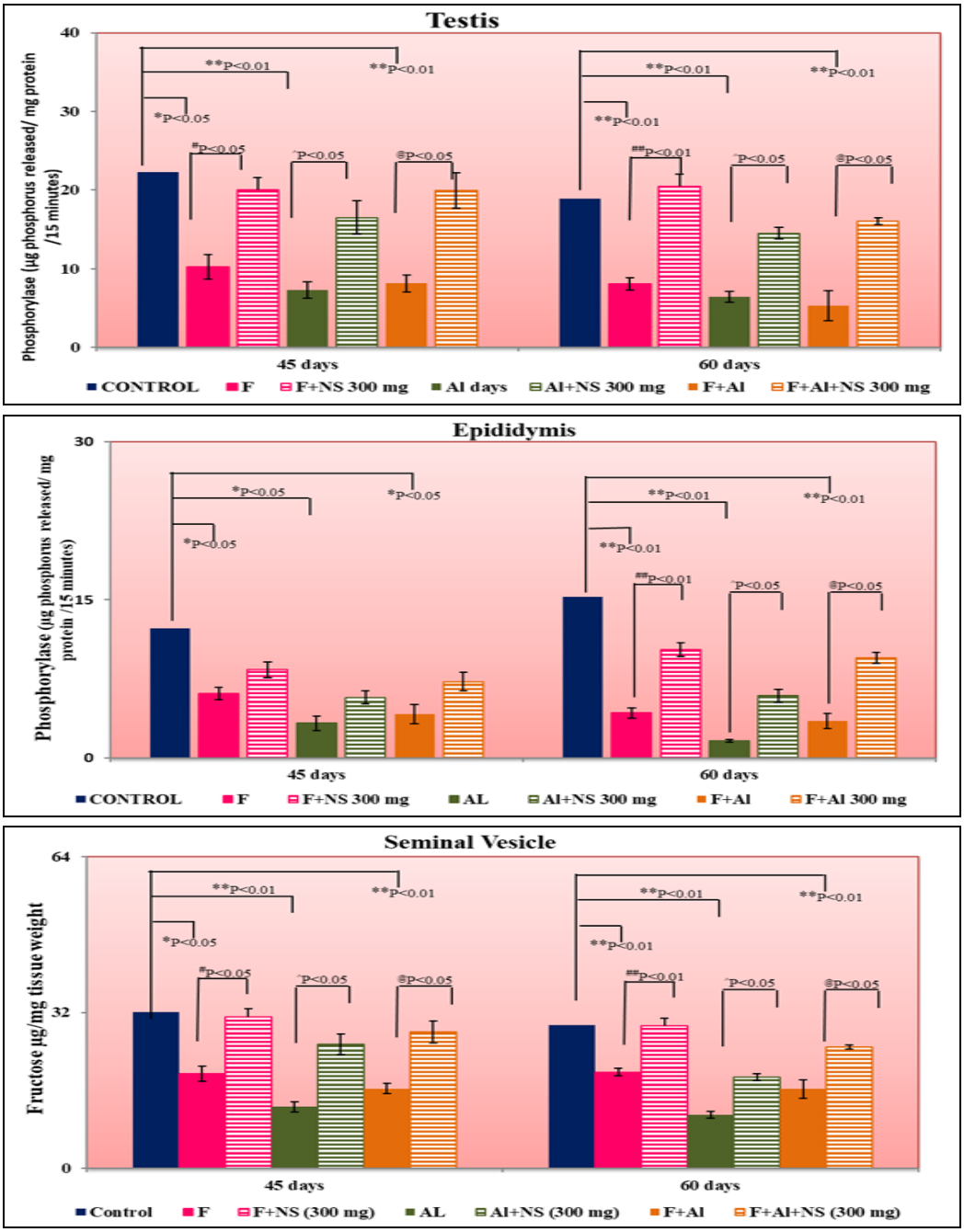

FIG. 6: BAR CHART DEPICTING PHOSPHORYLASE AND FRUCTOSE LEVELS ON DIFFERENT GROUPS OF MICE AFTER TREATMENT FOR 45 AND 60 DAYS. Data are shown as mean ± SEM

In each group minimum, six animals were taken. The various doses were administered orally to the male mice, and they were sacrificed after 45 and 60 days of treatment, then testis and epididymis were collected for evaluation of oxidative indices and biochemical parameters.

Plant Material and Extraction Procedure: *Nigella sativa* L. seeds were used and purchased from a local grocery market in Jaipur, India. The ethanolic extract was prepared according to the WHO protocol CG-04. For the preparation of an ethanolic extract, the seeds were washed, air-dried, powdered, and then subjected to Soxhlet apparatus for extraction with 50% ethanol. The extract obtained was filtered and then evaporated to dryness under reduced pressure, which yielded about 8.5% of solid residue.

Statistical Analysis: The data were expressed as mean ± SE (Standard error) and was also analyzed for statistical comparison using SPSS software using two-way ANOVA (Analysis of variance) followed by posthoc Tukey's test.

TABLE 1: STATISTICAL COMPARISONS

Normal control treated with distilled water *v/s.* sodium fluoride (10 mg/kg b.w.), aluminum chloride (100 mg/kg b.w.), sodium fluoride + aluminum chloride treated (5 mg/kg + 50 mg/kg) (*, **)
Sodium fluoride (10 mg/kg b.w.) *v/s. Nigella sativa* with sodium fluoride treated (300 mg/kg + 10 mg/kg) ($^{\#}$, $^{\#\#}$)
Aluminum chloride (100 mg/kg b.w.) *v/s. Nigella sativa* with aluminum chloride treated (300 mg/kg + 100 mg/kg) (^, ^ ^)
Sodium fluoride + aluminum chloride treated (5 mg/kg + 50 mg/kg) *v/s. Nigella sativa* with sodium fluoride + aluminum chloride treated (300 mg/kg + 5 mg/kg + 50 mg/kg) ($^{@}$, $^{@\,@}$)

RESULTS AND OBSERVATION: The result of physical parameters after the treatment of fluoride, aluminum, and their combination in mice is shown in **Fig. 1.** The result of LPO and antioxidant enzymes after the treatment of fluoride, aluminum and their combination in mice is shown in **Fig. 2** and **3.** The result of biochemical parameters after the treatment of fluoride, aluminum and their combination in Mice is shown in **Fig. 4, 6**.

Fig. 1 shows the effects of fluoride, aluminum, and their combination on mice's body and organ weight. In the current work, mice that were continuously treated with fluoride (10 mg/kg b.w.), aluminum (100 mg/kg b.w.), and F-Al combination for 45 and 60 days showed a significant decline ($P<0.01$) in the body weight and in the weight of testis, epididymis and seminal vesicle as compared to their control group. Simultaneous administration of NSSE 300 mg/kg b.w. with fluoride, aluminum, and their combination resulted in significant elevation ($P<0.01$) in the body weight and organ weight as compared to their individual treatment.

The treatment of fluoride, aluminum and its combination to the mice for 45 and 60 days resulted in a significant decrease ($P<0.01$) in levels of CAT, SOD, GSH and a significant increase ($P<0.01$) in TBARS levels in the testis and epididymis as compared to the control group. When the fluoride, aluminum, and its combination were treated with *Nigella sativa* for 45 and 60 days, a significant increase in the levels of GSH, CAT, SOD and a significant decrease in the level of TBARS level was observed as compared to their individual treated group **Fig. 2, 3**.

A significant decrease (**$P<0.01$) in protein, sialic acid, phosphorylase level whereas significant increase (**$P<0.01$) in the cholesterol and glycogen in the testis and epididymis after 45 and 60 days of treatment as compared to their respective controls was observed. A significant decline in the fructose level was also observed in the seminal vesicle after the treatment of fluoride, aluminum, and their combination for 45 and 60 days. *Nigella sativa* seed extract treatment resulted in a significant increase in protein, sialic acid, and phosphorylase level, while cholesterol and glycogen values showed a decline. An increase in the fructose values in the seminal vesicle was also recorded **Fig. 4-6**.

DISCUSSION: The aim of the current work was to evaluate the effect of sodium fluoride (NaF), aluminum chloride ($AlCl_3$) and its combination ($NaF+AlCl_3$) on the physical parameters, anti-oxidant status, and biochemical parameters of testis and epididymis of adult male Swiss albino mice (Mus musculus). Ameliorative efficiency of ethanolic extract of *Nigella sativa* seed extract against effects of fluoride, aluminum and their combination in testis and epididymis of male Swiss albino mice was also studied.

Mice that were continuously treated with fluoride (10 mg/kg b. w.), aluminum (100 mg/kg b. w.), and F-Al combination for 45 and 60 days showed a significant decline in the body weight and testis, epididymis, and seminal vesicle weight. Comparable results are observed by Rao and Bhatt *et al.*, 2012 [12], Chinoy and Sharma *et al.*, 1998 [13], Vani and Reddy, *et al.*, 2000 [14] and Basha 2011 [15] in rats and mice treated with different doses of fluoride.

Our results are in consensus with those obtained [16,] who observed a decrease in body weight by sodium fluoride treatments in male and female rats and mice at different doses and different intervals. It is also demonstrated that sodium fluoride treatment reduces body weight gain and organ weight gain due to less food intake [17]. Numerous scientists, [18, 19, 20, 21, 7] also reported that when mice treated with aluminum showed a decline in body weight. It was concluded that some metals could be accountable for the decline in body weight due to disturbance occur in metabolism, which results in a decrease of

feed intake and inhibition of growth [22, 23]. According to Yamamoto 2002, [24] aluminum has potential to cause decline in the production of ATP and protein active transport, therefore resulting in decreased body weight and less feed intake.

The treatment of fluoride, aluminum, and its combination to the mice for 45 and 60 days produces oxidative stress by inhibiting the activity of SOD, GSH, and CAT and by increasing the level of TBARS in the testis and epididymis of Swiss albino mice. Similar results were indicated by Rao and Bhatt *et al.*, 2012 [25] in which fluoride (10 mg/kg b.w.) treatment for 60 days caused severe oxidative stress as evidenced by a reduction in the level of SOD, CAT, and GSH whereas elevation in the level of TBARS in the testis of mice [26]. concluded that fluoride and aluminum resulted in a decrease in free radical scavenging enzymes *viz*., SOD, GSH-Px, and catalase, whereas increased LPO values in the testis, cauda epididymis, liver, muscle, and brain of male mice [27, 28, 29]. Fluoride exposure leads to an increase in the level of LPO in testis, epididymis indicating oxidative stress [30, 31].

Comparable results were observed by [32] in which they reported depletion of antioxidant defense system in epididymis after exposure of aluminum chloride leading to disruption of structural and functional integrity of epididymis of adult rats. Declination in the activity of SOD and level of GSH may be due to increased utilization in scavenging free radicals formed due to toxic effect of aluminum in epididymis [33]. It has been reported that aluminum leads to abnormal metabolism of zinc and copper, resulting in the decrease of SOD values as observed in our present study. *In-vitro* study on reproductive toxicity done by Yousef *et al.*, 2007 [34] reported that aluminum exposure caused enhancement of free radicals and alterations in the enzyme activities. Elevation in the TBARS level and declination in the SOD and catalase level was observed in the incubation medium. Simultaneous administration of *Nigella sativa* seed extract when co-administered with fluoride, aluminum and its combination for 45 and 60 days provided evidence for a positive role of *Nigella sativa* by decreasing the toxicity in testis and epididymis by a significant reduction in TBARS level while significantly increasing the level of SOD, CAT and GSH in testis and epididymis [35, 36]. Ingestion of aluminum produced oxidative stress, and *Nigella sativa* seed oil prevent the formation of reactive oxygen species, causing reduction of lipid peroxidation levels and enhances the levels of antioxidants. The black seed may be successful in the protection of rat liver necrosis [37]. Studies done by Al-Mahasneh and Ragheb *et al.*, 2008 [38, 39] indicated that *Nigella sativa* decreases the LPO levels and increases antioxidant enzymes.

Rabbits treated with sodium fluoride (50 mg/kg b.w.) resulted in a significant decrease in protein levels of many tissues such as liver, kidney, testis and stomach, skeletal and cardiac muscles [40]. Poly-acrylamide gel electrophoresis of testis and cauda epidiymis protein level in sodium fluoride-treated rats showed a withdrawal of some proteins and production of new proteins [41].

Aluminum and fluoride cause alteration in the level of protein in various tissues of treated animals alone or in combination, and this alteration may be due to the changes in the metabolism of protein or maybe by the formation of complexes with proteins [42, 43, 44]. Our results are in accordance with the finding of Pandey and Jain 2013 [45] who observed that continuous administration of aluminum in rats induced damage to testis and epididymis of rats indicated by the decline of tissue sialic acid level leading to damage in the sperms of testis and epididymis. This decline might be associated with disturbed steroid genesis resulting in the alteration of the integrity of the sperm acrosome membrane, and this in turn influences metabolism, motility, viability, and fertilizing ability [46]. Fluoride treatment resulted in a decrease in the activity of glucose-6-phosphate dehydrogenase in rats and also changes in the metabolism of glycogen [47]. According to Aumuller and Riva 1992 [48] the deficiency of androgens caused a decline in the seminal vesicle fructose, and the exposure with male hormones restored the capacity of the accessory glands to form fructose.

Nigella sativa seeds and oil of this plant have low toxicity and have various properties such as anti-inflammatory, analgesic, anticarcinogenic, anti-diabetic, antiulcer, antimicrobial, and antiparasitic activities [49, 50]. *Nigella sativa* shows a protective effect by regulating various activities of oxidative enzymes, enzyme levels of liver, markers of renal

function, and lipid profile of blood [37, 51-54]. The positive role of *Nigella sativa* seed extract was observed when co-administered with fluoride, aluminum, and their combination.

The testis' toxicity was decreased, indicated by a significant increase in the protein, sialic acid, fructose, phosphorylase levels, and a significant reduction in the cholesterol and glycogen level, which was altered by treatment of fluoride, aluminum, and their combination. Rats treated with *Nigella sativa* oil (1 mL/kg/day) for 12 weeks resulted in a decrease in the serum cholesterol, triglyceride, glucose levels, and leucocyte and platelet count [55].

When alloxan-induced diabetic rats were given *Nigella sativa* seed and its oil fraction diet, resulted in a significant reduction of total cholesterol levels when compared to diabetic control mice, where a non-significant decrease was observed for LDL-cholesterol [56]. A similar result when *Nigella sativa* seed powder or oil was given to mice for Arnold and Elvove 1942 [2, 4, 6,] and 8 weeks resulted in a significant reduction in the total cholesterol and LDL-cholesterol levels [57]. Hypocholestrolemic effect of *Nigella sativa* may be due to synergistic effect of various constituents present in it, presence of flavonoids, polyunsaturated fatty acids may lead to a reduction in cholesterol absorption and elevation of synthesis of bile acids [58].

Animals and human studies also showed that *Nigella sativa* seeds and thymoquinone, one of the main constituents of seeds, have the ability to treat male infertility, and their antioxidant activities have recently gained greater attention due to their role as dietary supplements with minimal side effects [59]. *Nigella sativa* also has the capability to protect different organs and tissues such as kidney, liver, gastrointestinal, lung, heart, blood, brain, and reproductive system against different toxins [60].

CONCLUSION: Sodium fluoride and aluminum chloride alone and in combination induced profound changes in the reproductive parameters evidenced by changes in various biochemical parameters and antioxidant parameters. *Nigella sativa* seed extract brought about an amelioration of toxicity induced due to its antioxidant and detoxifying properties.

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CONFLICTS OF INTEREST: The author declares no conflicts of interest.

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