Effect of *Echinacea purpurea* extract on immunological, biochemical and reproductive performance of male Egyptian buffaloes

O.M. El-Malky[1], H.S. Abou-Seri[1], M.M.E. Ibrahim[1], Doaa S. Abdel-Hady[1], Nashwa M. Kamel[1], R. Nagy[1], P.E. Mehanny[2], Shimaa M. Ali[1*]

[1]Animal Production Research Institute (APRI); [2]Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.
*Correspondence author Email: dr.shimaa8582@gmail.com

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Abstract

The present study aimed to evaluate the effect of *Echinacea purpurea* extract (EPE) supplementation on immunological and biochemical aspects, as well as the reproductive performance of male Egyptian buffaloes. Ten adult male buffalo weighed 300-340±5.25 kg and aged 2-3 years were divided into two comparable groups (5 each). The 1st group received a basal diet and was given two pellet/ head/d by dosing gun orally and served as a control. While the 2nd group received the basal diet and were given 1 g of EPE in the form of capsules (2 capsules / head /d) by dosing gun orally. Each capsule of the immulant contains 500 mg of dry EPE. Blood and semen samples were collected biweekly from all animals before morning feeding during the experimental period. The post-thaw quality of buffalo semen was done for the assessment of motility, viability and intact acrosome of spermatozoa. The current results declared that treated buffaloes with EPE showed a performance improvement, whereas live body weight showed a significantly higher value than that of the control group. Serum immunoglobulins (IgG & IgM) concentrations were highly significantly ($P<0.01$) affected by EPE supplementation in the treated group than in the untreated one. Pro-inflammatory cytokines and all acute-phase proteins studied were significant ($P< 0.05$) reduced in the supplemented group compared to the non-supplemented group in all experimental months. In association with that, there was a significant increase in hematological parameters, including RBCs, Hb, Ht, and neutrophils, by EPE treatment. While leucocyte count and most of its fractions showed significantly ($P< 0.05$) higher values in the untreated group compared to the treated one. Analysis of blood serum constituents revealed that the treated group had greater concentrations of total protein, glucose, triiodothyronine, thyroxin, and testosterone levels. No significant variations ($P> 0.05$) were noted in AST and creatinine between groups, but a significantly lower ALT was observed in the treated group than that was found in the control group. Malondialdehyde level decreased and antioxidant biomarkers improved significantly ($P< 0.01$) with EPE treatment. Testicular parameters, libido, and most physical semen properties were enhanced significantly ($P< 0.01$) by the beneficial effect of EPE supplementation. Higher conception rate of buffaloes artificially inseminated with thawed-semen produced from animals treated with EPE.

Key words: *Echinacea purpurea*; immunology; semen characteristics; buffalo.

Introduction

Male Egyptian buffaloes play a prominent role in animal production systems. They contribute about 50% to animal production through their genetic resources and represent part of the Nile Valley and Upper Egypt ecosystems (Abdel-Aziz et al., 2009). They are characterized by thick skin and a lack of development of sweat glands, hence, they are more susceptible to heat tolerance, which makes buffaloes have a low tolerance to heat stress and thus need greater alertness to compete in such hostile conditions (Vo and Wang 2007). Summer season in Egypt is characterized by high ambient temperature and relative humidity, which greatly affects the reproductive performance of male buffalo, which means that the animal suffers from heat stress and becomes outside the animal's "comfort zone" (Hady et al., 2018). This is associated with an imbalance in the animal's physiological metabolism, affecting its temperament, altering endocrine status, and physiological responses (Bernabucci and Mele, 2014; Akhtar et al., 2016), a decrease in fertility, and a physiological state, which are among the main factors determining the animal's performance throughout its life and which are affected by surrounding environmental influences (El-Masry et al., 2010). Improving the reproductive performance of animals is one of the main goals of the success of the livestock industry and ensuring its sustainability due to its economic importance (Abdelnour et al., 2020). Buffalo farming is important source of income of marginal farmers in developing and under developed countries (Gautam et al 2021), but most of the research was focused on improving the reproductive performance of female buffaloes (Gaur & Purohit, 2020) and not on male buffaloes. Farmers' economics depend on optimal fertility, and any deficiency in the reproductive performance of buffaloes is ultimately reflected in the farms' production and profitability (Khan et al., 2009). On the other hand, male infertility is a multifactorial disease, caused by genetic and acquired factors such as nutritional imbalances and exposure to chemicals, which increase the levels of oxidative stress in spermatozoa and could produce pituitary-hypothalamic or sex hormonal effects resulting in functional or structural impairments of the male sperm cells; also, there is an increased probability of testicular damage (Mazzilli et al., 2023).

Echinacea purpurea (EP) is a medicinal herb that contains many components such as alkamides, caffeic acid derivatives, non-saturated acids, glycoproteins, flavonoids, and polysaccharides (Bauer and Wagner, 1991), utilized to treat viral, urinary tract, upper respiratory infections, cutaneous irritations, and chronic illnesses brought on by a lack of immune responses (Stanisavljevic et al., 2009). It has been proven to be an immunostimulant (improves immunity through its effects on the body's immune system), anti-inflammatory (resistant to most diseases), and improves animal performance and health (Chang, 2000). So, EP and its active components are considered safe and cheap products in nurturing and protecting both human and animals through different mechanisms like supporting immune system response and improving antioxidant state (Mwamatope et al., 2023). Several reports cleared the effects of Echinacea extracts on animal's immune systems. They showed that *Echinacea* may modulate innate and adaptive immune responses and generate significant impacts on immune cells, including stimulating lymphocytes (Robbers and Tyler, 1999), production of cytokines (Cundell et al., 2003), immune cell proliferation, and phagocytosis (Goel et al., 2002).

Use of medical herbs extract for improved animal performance has a positive effect on body organisms, rumen microflora, acts as metabolic regulators, enhances the absorption of essential nutrients, serves protective functions, increases the resistance of animals exposed to stress, and increases the degree of antioxidant protection, which plays an important role in cell protection against oxidative damage (Sakowski et al., 2015; Panchasara et al., 2019; Kolling et al., 2022; Rochfort et al., 2022). In the last years, medical herbs extract has been used as a cheap, economical, anti-inflammatory properties, lower toxicity, and natural antioxidant source to enhance the reproductive performance of farm animals. It has become a new approach with promising results and positive impacts on preserved semen, post-thawed semen, and fresh semen (Ahmed et al., 2020; Azimi et al., 2020). The limitations of natural insemination procedures, which wear out bulls, and improper semen freezing for artificial insemination are some of the factors that affect the potency of semen production from Egyptian buffalo bulls. So, the usage of natural herbal extracts to enhance the reproductive performance of bulls is recommended. Pamungkas et al. (2019) indicated that using medicinal herbs boosted the synthesis of steroidogenesis and testosterone production, which improved sexual activity and increased the fertility of bulls and exhibited improved volume, concentration, viability, and motility of sperm and effects on male reproductive functions. Scarce studies are available for the effect of Echinacea purpurea extract, an herb, on male buffalo's performance under Egyptian summer environmental conditions.

Thus, the present research aimed to ascertain the impact of using *Echinacea purpurea* extract as a natural compound and immuno-stimulating additive on blood metabolites, an immunity marker profile, antioxidant status, sperm characteristics and the fertility of male buffaloes raised under Egyptian summer environmental conditions.

Materials and Methods

Experimental animals, feeding, management and treatments

The present experimental work was carried out at Sids Animal Production Research Experimental Station, Beni-Suef Governorate, which latitudes 28°90′81″N 30°94′62″E. belonging to the Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt throughout August to November, 2023.

Data in the present study were obtained from ten proven, healthy, sexually mature, typically normal, Saidi Egyptian buffalo males used as semen donors, free of external and internal parasites (averaged an initial live body weight ranged from 300-340±5.25 kg) and aged 2-3 years old enrolled and randomly assigned to two groups (5 each). The 1st group received a basal diet and was given two pellets/ head/d by dosing gun orally and served as a control. While the 2nd group received the basal diet and gave 1 g of *Echinacea purpurea* extract (EPE) in the form of capsules (2 capsules / head /d) by dosing gun orally according to Reklewska et al. (2004). Each capsule of the immulant contains 500 mg of dry EPE.

All of them were fed individually according to their body weight as recommended by the requirements of APRI (1997) for adult buffalo at 8.00 a.m. and 3.00 p.m., on ration composed of a concentrate feed mixture consisting of 33% un-decorticated cotton seed meal, 21% wheat bran, 22% corn, 14% rice bran, 3% limestone, 3% molasses, 2.8% calcium and 1.2% common salt plus clover hay and rice straw. Every month, the amount of diets provided was modified based on the progression of the animals' weight. Animals had access to multi-mineral licking blocks and fresh water in troughs freely for all day times, and they were housed individually under semi-open shed cages during the day and kept outdoors on slotted concrete floors, under semi-shed stables during the night and darkness periods.

Two months before the commencement of the experiment, every bull was taught how to serve an artificial vagina to collect semen and that period was considered a preparatory period. Fasting live body weight measurement was recorded biweekly for each animal individually before morning meal by the same technician using a large animal electronic scale.

Blood sampling and biochemical analysis

During the trial period, 10 milliliters of blood were drawn from each animal's jugular vein every two weeks and placed into vacutainer tubes before feeding. The blood sample was divided into two parts. The smaller part was placed in a tube containing EDTA as an anticoagulation for haematological assay by an electronic cell counter according to Feldman et al. (2000). The other part of the blood was transferred to a sterilized vacutainer tube without any anticoagulant substance and centrifuged at 1.400 × g for 20 min. for serum preparation and kept at -20ºC until analysis. Using ready-made kits, serum was analyzed to determine serum total protein, glucose, and aminotransferases (ALT & AST) according to Young (2000). Creatinine was analyzed with commercially available kits using a UV spectrophotometer (model Slim SEAC, Firenze, Italy). The radioimmunoassay method was used to measure the levels of serum thyroxine (T4), triiodothyronine (T3), and testosterone using kits from "Diagnostic Products Corporation, Los Angeles, USA". Serum malondialdehyde (MDA) content, total antioxidant capacity (TAC) level, serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities were determined by using diagnostic kits supplied by Biodiagnostic® Company, Egypt, according to Draper and Hadley (1990), Koracevic et al. (2001), Lin et al. (1988), Goth (1991), and Kakkar et al. (1984), respectively.

Serum haptoglobin (HP) was measured throughout the haemoglobin-binding assay according to Makimura and Suzuki (1982). Serum amyloid-A (AA) was determined by a solid-phase sandwich ELISA with a commercially available ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. Interleukin (IL-6 and IL-1α) levels were determined from undiluted serum samples using commercially available ELISA kits (Biosource, Diagnostic Corporation, USA). The plates were read at 450 nm on a computerized automated microplate ELISA reader (BioTEC, ELX800G, USA).

For estimation of the immunoglobulin A, G, and M concentration in blood serum, the following reagent kits were used: MIKROANALIZ 46 IgG, quality standard TY 9398-337-00155866-99; MIKROANALIZ IgA, quality standard TY 9398-335-00155866-99; and MIKROANALIZ IgM, quality standard TY 9398-336-00155866-99. The kits were made in the joint stock company "НПО СИНЕКОЗ", and they are applicable for estimation of immunoglobulin's A, G, and M by turbidimetry method.

Testicular parameters

Every two weeks, the testicular assessments were carried out and they included the following characteristics: Testicular length and the scrotal circumference was determined by a flexible, described by Mickkelson et al. (1982). The water displacement method was used to calculate the testicular

volume (ml). According to Wildeus and Hammond (1993), the testis tone score (TTS, obtained by manual palpation; ranked from 1, very soft to 9, very hard) was ascertained.

Libido

The sexual activity (libido) was determined by measuring the sexual reaction time (RT) for each ejaculate, which was measured in seconds as the time elapsed from the first approach of the bull to the teaser animal till complete ejaculation. Latency period (LP) estimated in seconds after ejaculation to return activity again.

Semen collection

During this study, semen was collected when animals reached to maturity and all animals were healthy, and fertile. Two false mounts were made by the bulls atop a trained teaser steer, before actual semen collection. Semen was collected from each animal biweekly by using artificial vagina and semen evaluation was recorded during the collecting experimental period.

Semen characteristics

Following collection, each bull's ejaculates were examined to ascertain the following: volume of ejaculate semen (ml) was measured to the nearest 0.1 ml using a graduated collection tube. Hydrogen ion concentration (pH) was measured using the pH meter. Vigor (0 to 5), was evaluated according to Santos et al. (2014). Sperm mass activity was estimated using a five-grade (0-4) score. The sperm characteristics were determined according to Ewuola and Egbunike (2010). The sperm concentration was evaluated by means of a hemocytometer as $n\times10^6$/ml. Total and motile sperm outputs ($\times10^9$) were calculated. Sperm abnormality (%), and acrosome integrity (%) were determined according to Salisbury et al. (1978), and Jankovicova et al. (2006), respectively. Finally, seminal fructose concentration (mg/ml) was measured according to Toragall (2019).

Pregnancy and fertility rate

A field study was conducted to compare control and treated semen efficiency and estimate fertility rates. We used 20 normally cyclic Saidi buffaloes (2-4 parities) from a herd raised at a private farm located in Beni-Suef Governorate, divided into two groups (10 each), to compare and examine the control and treated post-thawed semen efficiency. Every buffalo was synchronized through i.m. injection once in the morning to induce estrus using 3 milliliters of Estrumate (PGF2α-Essex Animal Helth Friesoythe, Germany) for each animal. All buffaloes showed signs of estrus within 2-3 days, then buffaloes in heat were artificially inseminated with 0.25 ml of frozen-thawed semen containing 20×10^6 motile spermatozoa/straw as well as, receiving two services within a 10-12-hour interval.

The diluted semen was inserted into the uterus utilizing the recto-vaginal insemination method (Salisbury et al., 1978). Pregnancy rate (PR) was determined based on pregnancy diagnosis by the rectal palpation after 60 days from the date of insemination.

Statistical analysis

Data were analyzed using a factorial experimental design (2 treatments, 4 months) using the general linear model approach defined by SAS software (SAS, 2010). Before the analysis, all percentages were logarithmically transformed to equalize data distribution. Duncan's new multiple range test (Duncan, 1955) was used to determine the significant differences among treatments. Chi-square analyses were used to calculate the conception rate as a percentage. The following statistical model was used:

$$Y_{ijk}= \mu + T_i + M_j + (TM)_{ij} + E_{ijk}$$

Where, Y_{ijk} is the dependent valuable; μ is the overall mean, T_i is the effect of treatment (i= control, and treatment), M_j is the effect month post-treatment (j= 1st, 2nd, 3rd, and 4th month post-treatment); TM_{ij} is the effect of interaction between treatment and month post-treatment, E_{ijk} is the residual experimental error.

Result and discussion

Effect of EPE on Live body weight changes

Live body weight (LBW) changes of the experimental animals are presented in Figure 1. EPE treatment had a significant ($P < 0.01$) higher effect on LBW and their values gradually increased across all treated months. The final values of LBW increased significantly ($P < 0.01$) compared to the initial one by 68.8kg and 42.4kg in treated and control groups, respectively. Treated animals attained 7.95% higher final body weight than untreated one. This increase in body weight changes can be due to an improvement in digestibility both of crude protein and fiber and also feeding values, and /or polyphenolic compounds present in chelated minerals with oxidative properties in the intestinal tract which play an important role in generating less lipid peroxides through limited intestinal absorption, which improves growth traits (Aboulthana et al., 2018). Also, Recoquillay (2006) mentioned that *Echinacea* can improve its performance by stimulating the secretion of digestive enzymes leading to enhancement of nutrient digestion and absorption. In beef cattle, addition of herbal mixture containing

Echinacea increased daily weight gains, with concurrently improved feed consumption for every kilogram increase in body weight and improved cattle's performance (Klebaniuk et al., 2012; Klebaniuk et al., 2016). Łozicki et al. (2006) and Klebaniuk, (2011) reported that there are many beneficial qualities to using herbal mixtures in cattle

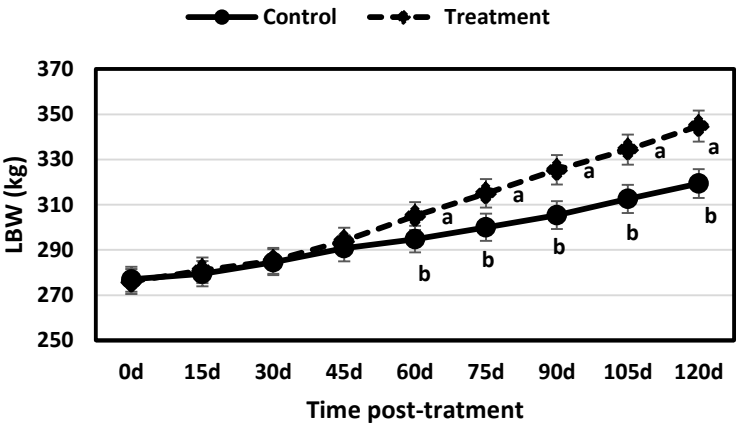

Figure 1. Live body weight (LBW) changes of male buffalo as affected by *Echinacea purpurea* extract treatment.

Table 1. Haematological parameters of male buffalo as affected by *Echinacea purpurea* extract treatment.

Factors		RBCs traits			WBCs ($\times10^3$/mm^3)	Leukocyte fractions (%)				
		Hgb (g/dl)	RBCs ($\times10^6$/ mm^3)	HT (%)		NUT	LYM	MON	EOS	BAS
Treatment effect										
Control		12.97^{b}	8.27^{b}	38.74^{b}	8.50^{b}	44.24^{b}	44.90^{b}	4.10	3.29^{a}	0.130^{a}
Treatment		13.39^{a}	8.62^{a}	39.64^{a}	9.79^{a}	47.82^{a}	48.24^{a}	4.13	3.04^{b}	0.112^{b}
±SEM		0.08	0.10	0.23	0.14	0.29	0.28	0.66	0.06	0.003
Month effect										
1st		12.39^{d}	7.59^{b}	36.47^{d}	9.66^{a}	45.87	46.33	4.09	3.58^{a}	0.127
2nd		12.91^{c}	7.96^{b}	37.63^{c}	9.08^{ab}	45.79	46.47	4.21	3.41^{ab}	0.116
3rd		13.52^{b}	8.97^{a}	40.53^{b}	9.18^{ab}	45.87	46.78	4.07	3.16^{b}	0.123
4th		13.90^{a}	9.26^{a}	42.13^{a}	8.66^{b}	46.57	46.72	4.09	2.50^{c}	0.117
±SEM		0.11	0.14	0.32	0.20	0.40	0.40	0.09	0.09	0.005
Interaction effect										
Control	1st	12.31^{f}	7.61^{c}	37.25^{d}	9.57^{a}	45.35^{cd}	45.68^{c}	4.02	3.51^{ab}	0.140^{a}
	2nd	12.77^{cd}	7.77^{c}	37.82^{cd}	8.46^{b}	44.61^{d}	45.48^{c}	4.23	3.59^{ab}	0.123^{abc}
	3rd	13.32^{bc}	8.82^{b}	39.03^{c}	8.48^{b}	44.19^{de}	45.47^{c}	4.15	3.44^{ab}	0.126^{abc}
	4th	13.47^{bc}	8.87^{b}	40.88^{b}	7.49^{c}	42.81^{e}	42.98^{d}	3.99	2.62^{cd}	0.129^{ab}
Treatment	1st	12.47^{ef}	7.57^{c}	35.69^{e}	9.75^{a}	46.40^{bc}	46.98^{bc}	4.15	3.66^{a}	0.114^{bc}
	2nd	13.04^{cd}	8.15^{c}	37.45^{d}	9.70^{a}	46.99^{bc}	47.45^{b}	4.18	3.24^{b}	0.109^{bc}
	3rd	13.73^{b}	9.12^{ab}	42.03^{b}	9.87^{a}	47.55^{b}	48.09^{b}	3.99	2.87^{c}	0.119^{abc}
	4th	14.33^{a}	9.64^{a}	43.38^{a}	9.83^{a}	50.35^{a}	50.45^{a}	4.19	2.38^{d}	0.104^{c}
±SEM		0.15	0.20	0.45	0.28	0.57	0.56	0.13	0.13	0.007
P value:										
Treatment		0.0002	0.0149	0.0066	<0.0001	<0.0001	<0.0001	0.7238	0.0065	0.0015
Month		<0.0001	<0.0001	<0.0001	0.0087	0.4873	0.8382	0.7077	<0.0001	0.4310
Interaction		<0.0001	<0.0001	<0.0001	0.0040	<0.0001	<0.0001	0.5368	0.0203	0.0385

Values with different superscripts within the same column are significantly different (P < 0.05). Hgb = Haemoglobin; RBCs = Red blood cell count; HCT = Haematocrit; WBCs = White blood cells; NUT = Neutrophil; LYM = Lymphocyte; MON= Monocyte; EOS= Eosinophil; BAS= Basophil.

rations; the biologically active compounds found in medicinal herbs (such as glycosides, alkaloids, saponins, phenols, coumarins, tannins, bitterness, locks, and essential oils) or preparations made from herbs influence certain physiological factors that help enhance the rumen microflora and its function or boost the metabolic process in the organism. Moreover, some studies reported that cows treated with 10 to 200 g/day/head herbs showed a significant increase in digestion coefficients of dry matter and organic matter, feed efficiency, average daily gain, maintenance of good health status, and improved efficiency of dairy production and performance (Zmora et al., 2012; Qiao et al., 2013; Ghafaria et al., 2015). McNeil et al. (2023) reported that EP treatment improved the health and growth performance of calves associated with greater post-weaning weekly body weight.

Kraszewski et al. (2008) found that feed intake and conversion, daily weight gains and final body weight rates increased significantly by adding herbs (1.0 and 2.0%) to concentrated feed ration. In Ossimi lambs, Tantawi et al. (2023) reported that oral supplementation of EP from 4 to 12g per head/day had a significant increased the final body weight and decreased feed intake and conversion compared to untreated one. In lactating Zaraibi goats, Gabr et al. (2023) found that EP supplementation showed a significant increase in body weights and feed conversion ratios, attributed that to improvement in nutrient digestibility of crude protein and crude fiber, as well as the feeding values. Contrary, Nowak et al. (2005) found that EPE improved weight gain, but there was no significant effect on the intake of the concentrate. Ayrle et al. (2021) found no significant effect of EP oral administration on body weight and general health, regardless of the dose.

Effect of EPE on haematological parameters

Table 1 demonstrated that the least square means for all periods of red blood cells (RBCs) count, haemoglobin (Hgb). and haematocrit (Ht), were significantly ($P< 0.001$) increased by treated with EPE compared to the control group. Also, leukocyte count and percentages of neutrophils, lymphocytes, basophils, and eosinophils showed significantly ($P< 0.01$) higher values in the treated group compared to the untreated one. These results were in accordance with those reported by many authors (Ezz, 2011; Skaudickas et al., 2003; Chaves et al., 2007; Dehkordi and Fallah, 2011; Khattab et al., 2019). This improvement is due to the EPE contents of cichoric acid and echinacea, both of which stimulate bone marrow and hematopoietic stem cells (Goel et al., 2022). Other investigations also found that there was a rise in the leukocyte count (Agnew et al., 2005; Ali, 2008). The potential benefits of echinacocide and polysaccharides to stimulate bone marrow and white blood cell counts, as well as the potential of echinacin and cichoric acid to boost macrophages, might be responsible for this result. Furthermore, it was discovered that the percentage of lymphocyte subpopulations changed as a result of *Echinacea*, suggesting that it may influence both innate and adaptive immune activities (Zhai, 2008).

Gabr et al. (2023) found that the goat group treated with EP showed significant differences ($P<0.05$) in hematological parameters, especially Hgb, and lymphocytes, compared to the control group. McNeil et al. (2023) reported that The addition of EP to the calves' diet was related to decreased segmented neutrophil counts, a greater number of lymphocytes, and a reduced segmented neutrophil per lymphocyte ratio. No effect was observed on the number of leukocytes, band neutrophils, basophils, or monocytes. They mentioned that EP has been reported to significantly improve the immunity, health and performance of animals due to its immunostimulating and anti-inflammatory effects. It was found that treating dairy calves with EP was associated with immunomodulation and reduced inflammation. Vieira et al. (2023) reported that EP played a significant role in macrophage regulation due to it containing alkylamides in its components. Also, in ewes, Barbour et al. (2015) found that an elevation of the antibody production when EP administrated orally. Kozyr et al. (2019) found an elevation of T-lymphocytes by 46%. Contrary, Ayrle et al. (2021) reported that EPE treatment did not show any significant effect or changes on blood cell counts, whereas eosinophil granulocytes, Hgb, and Ht were reduced treated groups.

Effect of EPE on Humoral immune responses and acute-phase proteins

Immunoglobulins (IgG, IgM & IgA)

Least square means of serum immunoglobulins (IgG, IgM & IgA) of male buffaloes are presented in Table 2. Data cleared that the concentration of serum immunoglobulins, especially IgG and IgA, in the supplemented group were greater ($P < 0.01$) than untreated one (2.26 vs. 2.14 and 0.064 vs. 0.056 g/dl), respectively. Serum IgG of treated and untreated buffaloes increased gradually to a higher value (2.81 and 2.65 g/dl, respectively) at the end of the fourth month. Also, serum IgA followed the same manner. In both groups, IgM showed an insignificant ($P > 0.05$) increase trend towards the end of the experimental time, whereas the treated group recorded relatively higher values than that of the control group (0.54 vs. 0.51 g/dl, respectively). Najafzadeh et al. (2011) explained that *Echinacea* extracts included strong antioxidants, polyphenols, minerals, and vitamins that have immune-modulating activity and thus caused an improvement in the level of immunoglobulins. Kozyr et al. (2019) mentioned that medicinal plants had a vital role in calves' health status and found that blood IgG, IgM, and IgA concentrations increased by 15% on average. Duvvu et al. (2018) cleared that Extra diet mixes herbs raise IgA, IgM, and IgG levels, which in turn enhance macrophage phagocytic activity and boost the quantity of B and T cells that are stimulated. Recent investigations reported that EP has proven immune modulation efficacy and showed a significant effect on immune regulation due to it containing alkylamides and polysaccharides (Liu et al., 2022; Vieira et al., 2023; Wang et al., 2024). In calves, Nowak et al. (2005) found that EPE increased significantly IgA concentration. EP supplementation seems more appropriate to modulate immune functions, due to significant differences recorded in IgG between treated and non-treated groups with different dosages of EP after 9 days post-

treatment (Seckina et al., 2018). Teleb et al. (2009) reported that goats treated with EPE showed higher values of serum γ-globulin, which reflected in improving the immune status and stimulating the defense system of animals. Ayrle et al. (2016) mentioned that *Echinacea purpurea* is one of the most promising herbs for boosting the immune system in ruminants. Whereas, Ayrle et al. (2021) found that IgG counts did not show any significant effect or change values by EPE treatment.

Cytokines (interleukins; IL-1 & IL-6)

The current results (Table 2) indicated that there were no significant ($P > 0.05$) variations in overall mean values for all periods of examined pro-inflammatory cytokine (IL-6), while there was a significant ($P < 0.001$) decreased in IL-1 for the treated group of male buffalo compared to control one (69.35 vs. 69.60 and 5.19 vs. 5.73 pg/ml, respectively). Serum concentrations of IL-1 and IL-6 for the treated group maintained the highest values from the first month of the experiment until the end of the second month. After that, these concentrations decreased from the third month until the end of the experiment, where the untreated group showed higher concentrations.

Wang and He (2018) mentioned that The adipose tissue's macrophages secrete IL-6, which controls adipocyte proliferation and apoptosis, encourages lipolysis, prevents lipid synthesis, and lowers blood lipid levels. Ayrle et al. (2021) reported that no differences were obtained for IL-1β and IL-8 by EPE treatment in calves. On the other hand, many studies reported that Alkylamides have been associated with the anti-inflammatory properties of *Echinacea* species, and their modulation of IL-1β and IL-8 in different species and models of disease has been established (Zhai et al., 2007; Sharma et al., 2009; Fusco et al., 2010; Ritchie et al., 2011). McNeil et al. (2023) found that EP supplementation in the diet of calves did not significantly affect IL-10, IL-6, and TNF-α levels. Vieira et al. (2023) reported that EP plays a significant role in anti-inflammatory properties due to it containing alkylamides in its components. Also, caffeic acid derivatives and polysaccharides present in EP showed antioxidant and anti-inflammatory impacts (Ravazzolo et al., 2022; Liu et al., 2022; Wang et al., 2024). Seckina et al. (2018) found significant differences in IL-1β and IL-4 concentrations of calves treated and non-treated with EP. Yu et al. (2013) found that essential oil in EP decreased significantly cytokine productions. Several studies have shown the positive significant effect of EP treatment on immune cells, like a rise in the immune cell count, lymphocyte activation, macrophages, phagocytosis, and cytokine production (Robbers and Tyler, 1999; Goel et al., 2002; Cundell et al., 2003). Kong et al. (2021) found that EP supplementation did not significantly affect the IL-6 concentrations, and the values were not statistically different between the control and EP group, and add to that, EP can reduce the level of cytokines.

Acute-phase proteins (serum amyloid-A, haptoglobin & fibrinogen)

The data referring to the concentrations of evaluated serum amyloid-A (SAA), haptoglobin (HP), and fibrinogen (FBG) during the experimental period are presented in Table 2. The treated group showed relatively lower values ($P < 0.05$) of acute-phase proteins (SAA, HP & FBG) than those recorded in the untreated one in all experimental times. The concentrations of SAA, HP, and FBG showed significant differences between the two groups, whereas higher values were noticed in the untreated group than in the treated groups during the experimental period (Table 2). The highest and lowest values of SAA were observed in the fourth month in control and treated groups, respectively, whereas the lowest FBG concentrations were recorded in the same month for the two experimental groups. These results may be due to EPE treatment being correlated with decreased inflammation (reduced SAA, HP & FBG) and stimulated immunity (raised interleukins and leukocyte counts) through active components of EPE. Among these are polysaccharides that stimulate the immune system (Steinmüller et al., 1993), alkamides, which have anti-inflammatory properties (Chen et al., 2005), and phenolics, which have anti-oxidative properties (Dalby-Brown et al., 2005). Ayrle et al. (2021) proposed that the local intestinal immune system could be activated by EPE. McNeil et al. (2023) found that EP supplementation to dairy calves' diet was associated with lower haptoglobin. To confirm this, SAA, HP, & FBG were slightly significantly fewer in the EPE-treated bulls, suggestive of reduced inflammation (Murray et al., 2014). The findings of Fararh et al. (2017) provide support for the current results, as they observed that haptoglobin levels were lower in diseased calves supplemented with Tulathromycin and an essential oil mixture, including EP, than in diseased calves treated with Tulathromycin alone.

Effect of EPE on serum biochemical parameters

As shown in Table 3, serum total protein and glucose concentrations of the treated group were significantly ($P < 0.05$) greater than the control group. Total protein (TP) values were within the normal range as reported by Boyd (2011). Some studies reported that the rise in serum TP values may be attributed to the increase in CP digestibility (Youssef and Zaki, 2001; Shahen et al., 2004). Gabr et al. (2023) reported that EP supplementations to goat diet significantly increased ($P < 0.05$) blood metabolites concentrations (TP, ALT, AST, and creatinine). In calves, medicinal plants especially EPE

increased serum total protein concentration significantly (Nowak et al., 2005; Kozyr et al., 2019). Also, Nazar (1994) and El-Ashry et al. (2006) suggested that animals treated with medicinal herbs revealed a notable rise in serum glucose values than the control one. In Ossimi lambs, Tantawi et al. (2023) found that treatment with different levels of EP affected significantly (P<0.05) total protein and glucose concentrations, whereas AST and ALP values decreased.

Table 2. Humoral immune responses and acute-phase proteins of male buffalo as affected by *Echinacea purpurea* extract treatment

Factors		Immunoglobulin's (g/dl)			Cytokines IL (pg/ml)		Acute-phase proteins		
		Ig G	Ig A	Ig M	IL-1	IL-6	AA (µg/ml)	HP (mg/ml)	FBG (g/l)
Treatment effect									
Control		2.14^{b}	0.056^{a}	0.51	5.73^{a}	69.60	80.13^{a}	0.16^{a}	2.39^{a}
Treatment		2.26^{a}	0.064^{b}	0.54	5.19^{b}	69.35	76.02^{b}	0.14^{b}	2.10^{b}
±SEM		0.03	0.002	0.02	0.09	0.68	1.04	0.01	0.05
Month effect									
1st		1.84^{c}	0.029^{d}	037^{c}	6.25^{a}	69.91^{ab}	79.07	0.19^{a}	2.41^{a}
2nd		1.93^{c}	0.046^{c}	0.43^{c}	5.65^{b}	69.07^{ab}	78.42	0.16^{b}	2.33^{a}
3rd		2.29^{b}	0.074^{b}	0.59^{b}	5.06^{c}	71.07^{a}	78.12	0.14^{c}	2.22^{a}
4th		2.73^{a}	0.090^{a}	0.71^{a}	4.89^{c}	67.84^{b}	76.69	0.12^{c}	2.02^{b}
±SEM		0.04	0.003	0.02	0.13	0.97	1.47	0.01	0.07
Interaction effect									
Control	1st	1.84^{d}	0.029^{e}	0.37^{c}	6.42^{a}	69.61^{ab}	79.91^{a}	0.18^{ab}	2.59^{a}
	2nd	1.90^{d}	0.042^{de}	0.41^{c}	5.95^{a}	66.59^{bc}	79.80^{a}	0.17^{abc}	2.52^{ab}
	3rd	2.18^{c}	0.068^{c}	0.57^{b}	5.26^{b}	71.61^{a}	80.56^{a}	0.16^{bc}	2.39^{abc}
	4th	2.65^{a}	0.085^{ab}	0.70^{a}	5.30^{b}	70.57^{ab}	80.26^{a}	0.14^{cd}	2.07^{d}
Treatment	1st	1.85^{d}	0.030^{e}	0.38^{c}	6.09^{a}	70.21^{ab}	78.23^{ab}	0.20^{a}	2.23^{bcd}
	2nd	1.97^{d}	0.050^{d}	0.45^{c}	5.35^{b}	71.54^{a}	77.05^{ab}	0.15^{bc}	2.14^{cd}
	3rd	2.40^{b}	0.080^{bc}	0.60^{b}	4.86^{bc}	70.54^{ab}	75.69^{ab}	0.12^{d}	2.05^{d}
	4th	2.81^{a}	0.095^{a}	0.72^{a}	4.49^{c}	65.11^{c}	73.12^{b}	0.11^{d}	1.97^{d}
±SEM		0.01	0.003	0.06	0.18	1.37	2.08	0.01	0.09
P value:									
Treatment		0.0114	0.0267	0.2947	<0.0001	0.8003	0.0067	0.0491	<0.0001
Month		<0.0001	<0.0001	<0.0001	<0.0001	0.0234	0.7079	<0.0001	0.0011
Interaction		<0.0001	<0.0001	<0.0001	<0.0001	0.0083	0.0491	0.0018	0.0081

Values with different superscripts within the same column are significantly different (P < 0.05). IL= Interleukins**;** AA= Amyloid-A; HP= Haptoglobin; FBG= Fibrinogen**.**

Table 3. Serum biochemical parameters of male buffalo as affected by *Echinacea purpurea* extract treatment.

actors		Biochemical parameters				
		TP (g/dl)	GLU (mg/dl)	ALT (IU/dl)	AST (IU/dl)	Creatinine (mg/dl)
Treatment effect						
Control		7.73^{b}	69.20^{b}	39.44^{a}	77.03	0.88
Treatment		8.32^{a}	70.71^{a}	37.28^{b}	76.07	0.84
±SEM		0.12	0.51	0.47	0.38	0.01
Month effect						
1st		7.36^{c}	64.34^{d}	36.63^{b}	74.31^{c}	0.90^{a}
2nd		7.60^{bc}	69.12^{c}	36.99^{b}	76.14^{b}	0.89^{a}
3rd		8.03^{b}	71.95^{b}	38.32^{b}	77.58^{ab}	0.87^{a}
4th		9.12^{a}	74.42^{a}	41.51^{a}	78.18^{b}	0.79^{b}
±SEM		0.17	0.73	0.67	0.53	0.02
Interaction effect						
Control	1st	7.28^{d}	64.24^{d}	36.81^{c}	74.72^{cd}	0.89^{a}
	2nd	7.25^{d}	68.50^{c}	37.17^{c}	76.14^{bcd}	0.92^{a}
	3rd	7.55^{d}	71.20^{bc}	40.02^{b}	77.56^{ab}	0.88^{a}
	4th	8.84^{ab}	72.87^{b}	43.77^{a}	79.69^{a}	0.83^{ab}
Treatment	1st	7.45^{d}	64.43^{d}	36.46^{c}	73.89^{d}	0.89a
	2nd	7.95^{cd}	69.73^{bc}	36.80^{c}	76.14^{bcd}	0.87^{a}
	3rd	8.51^{bc}	72.70^{b}	36.62^{c}	77.60^{ab}	0.86^{a}
	4th	9.40^{a}	75.99^{a}	39.25^{bc}	76.67^{bc}	0.75^{b}
±SEM		0.23	1.03	0.94	0.76	0.03
P value:						
Treatment		0.0006	0.0414	0.0017	0.0774	0.0545
Month		<0.0001	<0.0001	<0.0001	<0.0001	0.0009
Interaction		0.0005	0.0105	0.0217	0.0029	0.0028

Values with different superscripts within the same column are significantly different (P < 0.05).
TP= Total protein; GLU= Glucose; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase.

However, there were no notable variations (P>0.05) found in AST and creatinine between experimental groups, and the fact that the readings fell within the normal range suggested that the animals' livers and kidneys and overall nutritional status were both in good condition. These findings could help to explain why the EPE supplementation had no negative effects on liver and kidney tissues and was safe for their function. However, a significantly lower blood serum ALT was observed in the treated group than that was found in the control group (Table 3). In lactating Damascus goats, El-Basiony et al. (2015) found that EPE treatment by 4 g/head/day exhibited slightly elevated total protein values and had no significant effect ($P \geq 0.05$) on blood glucose concentrations. Ganjavi et al. (2022) mentioned that blood metabolite concentrations in Holstein's calves were not significantly affected by 350, 700, and 1050 mg of Echinacea/day. Kong et al. (2021) found that ALT and AST concentrations showed similar values in both EP and control groups. However, blood glucose and creatinine levels were reduced by EP extract.

Effect of EPE on serum hormones

As shown in Table 4, average concentrations of blood serum of thyroid hormones (T3, and T4) and testosterone hormone were significantly (P< 0.01) affected by EPE treatment, which recorded higher values in the treated group than that in the untreated one. This observation may be attributed to the fact that the treatment with EPE resulted in additional secretion of thyroid hormones, as the thyroid gland can accumulate it into thyroxine. The variations in each group's metabolic activity as a result of receiving EPE treatment can be partially blamed for the variations in thyroid hormone concentrations seen in this study. In ruminant species, blood thyroid hormone levels have been associated with feed consumption and are thought to be reliable markers of an animal's nutritional health (Riis and Madsen, 1985). T3 and T4 concentrations showed a corresponding relationship to live body weight, with greater values in heavier lambs and lower values in lighter lambs (Abd El-Hafeez et al., 2014; Mohamed et al., 2016). This might be due to the polyphenol compound of EPE as a particular class of antioxidants called caffeoyl derivatives can act as an effective defense against the negative effects of harmful substances (Tsai et al. 2012). This result is compatible with the enhancement attained in body weight during experimental period since these hormones influence the body's entire metabolic system (Husveth, 2011). Hart et al. (1981) found the correlation between the metabolism of certain nutrients and the release of thyroid hormones.

Serum testosterone (TST) concentrations were found to be 0.87 vs. 1.09 ng/ml in the control and treated group, respectively. A significant (P< 0.001) difference was observed between values in both groups. Differences between treatments and months of the experimental period were highly significant (P< 0.001). TST helps to create protein, which is necessary for regular sexual desire and erections, which may be the reason for its advantages on libido (Hafez, 1987). Jana and Bhattacharya (1994) reported that thyroid hormones play an important role in the synthesis, release, and circulation of testosterone. Concerning TST hormone levels, it is the main hormone that regulates the testicular sperm production process (Sharpe et al., 1990). The enhancement in TST due to EPE treatment as an antioxidant source may have increased the proliferation of Leydig cells (Glade et al., 2015) since testosterone synthesis primarily comes from these cells. This finding is in accordance with reported by Mao et al. (2021), who found that TST level was raised with EPE administration compared to the control. The reason is that an enzyme known as 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the synthesis of testosterone. This promotes androstenedione's conversion to TST (Mindnich et al., 2005). The enhancement in TST concentration by antioxidant treatment is in agreement with the reports by El-Kholy et al. (2008) and Swelum et al. (2021). They found that TST concentrations were considerably raised by the administration of phytogenic natural substances as antioxidants. Age-related changes in testicular circumference and body weight may be related to rising testosterone secretion. Larger testes on rams result in higher TST production. The increase in testosterone concentration at all times were significantly ($P < 0.001$) better with treated group than the control group. The increase of testosterone levels might relate to improve in digestible energy. This finding is in agreement with what was revealed by Scott et al. (2011), who discovered that sexually distinct differentiation of the brain areas controlling energy expenditure may be the reason behind this sex-specific action of TST. Contrary to this result, Novika et al. (2024) found that male androgen expression can be reduced by EP administration. Other investigations indicate similar results, in which EP treatment for up to four weeks showed decreasing the TST levels gradually produced by the male sexual hormone organ, which could be related to the vegetative sterols found in the components of the EPE (Skaudickas et al., 2003; 2004).

Effect of EPE on serum MDA and antioxidant enzymes

The least square means of serum MDA, TAC, GPX, SOD and CAT levels between experimental groups has been depicted in Table 5. MDA concentrations was significantly (P< 0.001) lower in treated group than that untreated group during all experimental times. In the control group, the

MDA concentration increased significantly (P< 0.001) over time until it reached its highest level in the fourth month of the experiment, while it took the exact opposite trend in the treatment group. Serum TAC level followed the contrary pattern of MDA with the treatment group in all experimental months since its level increased significantly (P< 0.001) over time with EPE treatment. All studied antioxidant enzymes (GPX, SOD, & CAT) activities were found to be significantly higher (P< 0.05) in the treated

Table 4. Thyroid hormones and testosterone levels of male buffalo as affected by *Echinacea purpurea* extract treatment.

actors		Thyroid hormones		Testosterone (ng/ml)
		T3 (ng/ml)	T4 (µg/dl)	
Treatment effect				
Control		59.17^{b}	7.33^{b}	0.87^{b}
Treatment		62.13^{a}	8.72^{a}	1.09^{a}
±SEM		0.85	0.09	0.02
Month effect				
1st		44.10^{d}	6.69^{d}	0.69^{d}
2nd		51.80^{c}	7.54^{c}	0.84^{c}
3rd		69.41^{b}	8.08^{b}	1.04^{b}
4th		77.65^{a}	9.80^{a}	1.34^{a}
±SEM		1.21	0.12	0.03
Interaction effect				
Control	1st	44.05^{e}	6.86^{e}	0.67
	2nd	51.02^{d}	6.90^{e}	0.79
	3rd	66.70^{c}	7.42^{d}	0.91
	4th	74.90^{b}	8.15^{c}	1.09
Treatment	1st	44.16^{e}	8.51^{e}	0.72
	2nd	52.59^{d}	8.19^{c}	0.90
	3rd	72.12b	8.74^{b}	1.17
	4th	80.39^{a}	11.45^{a}	1.59
±SEM		1.71	0.17	0.04
P value:				
Treatment		0.0111	<0.0001	<0.0001
Month		<0.0001	<0.0001	<0.0001
Interaction		0.2892	<0.0001	<0.0001

Values with different superscripts within the same column are significantly different (P < 0.05).
TP= Total protein; GLU= Glucose; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase

Table 5. Oxidative stress and antioxidant status of male buffalo as affected by *Echinacea purpurea* extract treatment.

Factors		MDA (nmol/ml)	TAC (mM/l)	Antioxidant enzymes		
				GPX (mU/ml)	SOD (U/ml)	CAT (U/ml)
Treatment effect						
Control		204.03^{a}	2.49^{b}	29.42^{b}	3.41^{b}	14.35^{b}
Treatment		183.44^{b}	2.71^{a}	30.40^{a}	3.51^{a}	15.09^{a}
±SEM		0.90	0.03	0.30	0.04	0.19
Month effect						
1st		199.00^{a}	2.05^{d}	28.17^{c}	2.99^{d}	13.78^{c}
2nd		199.48^{a}	2.52^{c}	29.76^{b}	3.34^{c}	14.38^{b}
3rd		190.76^{b}	2.79^{b}	30.67^{ab}	3.64^{b}	15.17^{a}
4th		185.72^{c}	3.04^{a}	31.05^{a}	3.87^{a}	15.57^{a}
±SEM		1.27	0.05	0.42	0.06	0.27
Interaction effect						
Control	1st	200.58^{bc}	2.01^{d}	28.52^{cd}	3.09^{de}	13.62^{d}
	2nd	203.63^{ab}	2.47^{c}	29.19^{cd}	3.33^{cd}	14.36^{c}
	3rd	203.80^{ab}	2.62^{c}	29.97^{bc}	3.56^{bc}	14.71^{bc}
	4th	208.11^{a}	2.85^{b}	29.99^{bc}	3.66^{b}	14.71^{bc}
Treatment	1st	197.41^{c}	2.08^{d}	27.82^{d}	2.90^{e}	13.94^{cd}
	2nd	195.32^{c}	2.56^{c}	30.33^{bc}	3.34^{cd}	14.39^{c}
	3rd	177.71^{d}	2.96^{b}	31.36^{ab}	3.72^{b}	15.62^{ab}
	4th	163.33^{e}	3.23^{a}	32.11^{a}	4.07^{a}	16.42^{a}
±SEM		1.80	0.07	0.60	0.08	0.39
P value:						
Treatment		<0.0001	<0.0001	0.0221	0.0064	0.0107
Month		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction		0.0002	0.0062	0.0079	0.0060	0.0086

Values with different superscripts within the same column are significantly different (P < 0.05).
MDA = Malondialdehyde; TAC= Total antioxidant capacity; GPX= Glutathione peroxidase; SOD= Superoxide dismutase; CAT= Catalase.

group than the untreated one. Results pointed to significant (P< 0.05) higher antioxidant enzymes activities observed in both experimental groups with advanced months of the experiment, values recorded in the fourth month were higher than the values in the first month. Generally, it can be seen that EPE treatment reduces serum MDA and increases serum TAC levels, and GPX, SOD, and CAT superoxide dismutase activities, which play substantial roles in combating oxidative stress. Related investigations also record comparable findings for dairy cows (Sharma et al., 2011). These findings are in disagreement with those reported by Kong et al. (2021). Lipid peroxidation produces MDA, a byproduct that is utilized as a gauge for the tissue reaction chain's speed. MDA is also employed as a marker of oxidative stress in cells. Ahmed et al. (2010) suggested that due to their increased susceptibility to stress, buffaloes may benefit from supplementary intakes of antioxidant medicinal herbs in addition to their natural diet to enhance performance in stressful situations. They also reported that MDA promotes cross-linking bonds in the membranes of the body, leading to changes in the permeability of ions and the activity of enzymes. On the other hand, Sotek et al. (2018) revealed that EP has strong antioxidant properties, so it plays an important role in neutralizing free radicals in the animal body generated under cellular stress if it is present at low concentrations in animal feed mixtures. Kong et al. (2021) reported that EP can reduce the creation of reactive oxygen species (ROS) by increasing the synthesis of antioxidants. Kumar et al. (2012) mentioned that GPX, SOD, and are classified as enzymatic antioxidants that function by eliminating internal and extracellular superoxide radicals and stopping the plasma membrane's lipid peroxidation. SOD uses spontaneous dismutation to change ROS produced by the cell into hydrogen peroxide (Brookes, 2005). Kumar et al. (2018 a & b) stated that herbs supplementation significantly (P <0.05) decrease the oxidative stress in buffalo bulls, whereas, lipid peroxidation was decreased, while SOD and GPX activities increased during supplementation and post-supplementation phases.

Effect of EPE on testicular measurements and sexual activity

The effect of EPE treatment on testicular length, scrotal circumference, testicular volume, testicular tone firmer score, reaction time, and latency period are shown in Table 6. The present results revealed that the least square means of testicular measurement values for all the experimental animals didn't really change (P> 0.05) at the beginning of the experiment. Testicular Length, scrotal circumference, and testicular volume increased gradually over time and were significantly (P< 0.01) higher in the treatment group than in the control group. These parameters increased with the advancement of age and increased progressively with the duration of the experimental period in treated and non-treated groups. However, the testis tone score increased insignificantly (P> 0.05) with EPE treatment, while it was affected significantly (P< 0.05) over time until it reached the highest score in the treated group (6.70) and control group (6.40). The significant variations between the experimental groups for testicular parameters in the current study might be attributed to the variation in energy supply by EPE treatment. The growth rate and nutritional status may have an impact on scrotal circumference (Cameron et al., 1988). In both Buffalo and Friesian bulls, Abd El-Moty et al. (2001) observed positive relationship significantly between BW, testicular measurements and TST hormone. Ammar et al. (2021) reported that there are a positively correlation among age, scrotal circumference, and testosterone level in Gayo buffalo. Also, testosterone hormone associated with scrotal circumference and semen volume (Sajjad et al. 2007). Variations between experimental groups were significant for most reproductive traits of supplemented bulls than the un-supplemented bulls. Supplemented bulls reached reaction time earlier than the control bulls. Heavier body weight (Fig. 1) and larger testicular size than the control was, also, recorded. This positive variation might explain the increase in serum testosterone (Table 4). These traits have an association with each other (Obande et al., 2015). Many studies cleared that the testicular size, volume and scrotal circumference were important parameters in assessing the reproductive efficiency and spermatogenic capabilities and play a major role in the sperm production rate of buffalo males and positively correlated in young buffalo bulls (Osman, 1972; Ahmad et al., 1989; Pant et al., 2003; Elmaz et al., 2008; Perumal, 2014). Ruiz-Olvera et al. (2017) suggested that Spermatogenesis and blood TST levels have relationships with testicular volume and body and testes mass index. The reproductive potential of domestic male animals was predicted using testicular weight and their circumference (Sarder, 2005; Maurya et al., 2010).

Sexual parameters were better in treated bulls compared with the untreated ones. Reaction time (RT) and latency period (LP) were significantly (P< 0.01) improved in treated bulls by EPE compared to control ones. Pre-copulatory behavior was correlated with ejaculation rate, which supports the hypothesis of Price et al. (1992) proposing that rams' recurrent pre-copulatory behavior is indicative of their underlying sexual desire. Sexual desire and sex drive are influenced by a variety of factors, such as season, genetics, breed variations, hormones, temperature, post-weaning care, and diet (Mickelsen et al., 1982). Estimates of RT and LP indicated a quick response to EP supplemented group compared to

Table 6. Reproductive parameters of male buffalo as affected by *Echinacea purpurea* extract treatment.

Factors		Testicular traits				Libido	
		TL (cm)	SC (cm)	TV (ml)	TTS (1-9)	RT (sec)	LP (sec)
Treatment effect							
Control		16.21^{b}	22.65^{b}	472.55^{b}	6.09	92.03^{a}	121.61^{a}
Treatment		16.41^{a}	23.22^{a}	506.10^{a}	6.26	81.80^{b}	114.59^{b}
±SEM		0.06	0.13	3.84	0.08	0.25	2.01
Month effect							
1st		15.83^{d}	19.53^{d}	406.20^{d}	5.79^{c}	51.67^{d}	90.22^{d}
2nd		16.17^{c}	21.66^{c}	462.20^{c}	6.06^{bc}	71.69^{c}	107.26^{c}
3rd		16.46^{b}	24.03^{b}	513.40^{b}	6.30^{ab}	99.17^{b}	128.97^{b}
4th		18.80^{a}	26.51^{a}	575.70^{a}	6.55^{a}	125.16^{a}	145.91^{a}
±SEM		0.08	0.19	5.44	0.12	0.36	2.84
Interaction effect							
Control	1st	15.81^{e}	19.38^{e}	403.80^{e}	5.75^{c}	60.03^{g}	98.11^{d}
	2nd	16.09^{de}	21.39^{d}	449.80^{d}	6.00^{c}	80.05^{e}	110.12^{c}
	3rd	16.34^{bcd}	23.71^{c}	490.60^{c}	6.19^{abc}	102.11^{c}	132.12^{b}
	4th	16.62^{b}	26.12^{b}	545.50^{b}	6.40^{ab}	126.04^{a}	146.11^{a}
Treatment	1st	15.85^{e}	19.69^{e}	408.60^{e}	5.83^{c}	43.30^{h}	82.34^{e}
	2nd	16.25^{cd}	21.94^{d}	474.40^{c}	6.11^{bc}	63.32^{f}	104.33^{cd}
	3rd	16.58^{bc}	24.36^{c}	535.80^{b}	6.40^{ab}	96.36^{d}	125.84^{b}
	4th	16.99^{a}	26.90^{a}	605.60^{a}	6.70^{a}	124.29^{b}	145.82^{a}
±SEM		0.12	0.26	7.69	0.17	0.51	4.03
P value:							
Treatment		0.0171	0.0029	<0.0001	0.1492	<0.0001	0.0160
Month		<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
Interaction		0.0056	0.0015	0.0040	0.0245	<0.0001	0.0046

Values with different superscripts within the same column are significantly different (P < 0.05).
TL= Testicular Length; SC= Scrotal circumference; TV= Testicular volume; TTS= Testis tone score; RT= Reaction time; LP= Latency period.

Table 7. Physical semen characteristics and seminal fructose concentration of male buffalo as affected by *Echinacea purpurea* extract treatment.

Factors		Physical semen characteristics											SF (mg/ml)
		VOL (ml)	pH	VIG (0-5)	MM (0-4)	PM (%)	SCO (×10^{6})	TSO (×10^{9})	MSO (×10^{9})	SV (%)	AB (%)	IA (%)	
Treatment effect													
Control		2.54^{b}	6.99^{a}	3.31^{b}	3.06^{b}	59.51^{b}	625.43^{b}	1.59^{b}	0.95^{b}	66.09^{b}	19.12^{b}	67.50^{b}	4.51^{b}
Treatment		3.26^{a}	6.92^{b}	3.79^{a}	3.46^{a}	71.63^{a}	733.08^{a}	2.33^{a}	1.67^{a}	69.62^{a}	14.53a	79.50^{a}	5.00^{a}
±SEM		0.03	0.02	0.03	0.02	0.49	4.14	0.03	0.02	0.39	0.29	0.39	0.05
Month effect													
1st		2.49^{d}	6.82^{c}	3.21^{d}	3.05^{d}	59.56^{d}	621.61^{d}	1.55^{d}	0.92^{d}	63.62^{c}	17.57^{a}	69.00^{c}	3.71^{d}
2nd		2.78^{c}	6.93^{b}	3.49^{c}	3.15^{c}	64.64^{c}	669.23^{c}	1.86^{c}	1.20^{c}	66.46^{b}	17.07^{a}	73.50^{b}	4.59^{c}
3rd		3.06^{b}	7.02^{a}	3.67^{b}	3.35^{b}	67.92^{b}	702.93^{b}	2.15^{b}	1.46^{b}	69.13^{b}	16.93^{a}	75.25^{a}	5.08^{b}
4th		3.28^{a}	7.05^{a}	3.85^{a}	3.49^{a}	70.17^{a}	723.25^{a}	2.37^{a}	1.66^{a}	72.23^{a}	15.73^{b}	76.25^{a}	5.65^{a}
±SEM		0.03	0.37	0.04	0.03	0.69	05.85	0.04	0.03	0.99	0.41	0.56	0.08
Interaction effect													
Control	1st	2.25^{g}	6.83^{de}	3.04^{f}	2.90	54.29^{g}	565.44^{f}	1.27^{f}	0.69^{g}	63.34^{d}	19.52^{a}	66.00^{e}	3.69^{e}
	2nd	2.50^{f}	6.94^{cd}	3.25^{e}	3.00	58.26^{f}	619.37^{e}	1.55^{e}	0.90^{f}	65.40^{d}	19.46^{a}	67.00^{de}	4.36^{d}
	3rd	2.59^{ef}	7.07^{ab}	3.43^{d}	3.14	61.88^{e}	649.74^{d}	1.68^{e}	1.04^{e}	66.93^{cd}	19.41^{a}	68.00^{de}	4.82^{c}
	4th	2.81^{d}	7.10^{a}	3.52^{d}	3.18	63.60^{de}	667.18^{cd}	1.87^{d}	1.19^{d}	68.71^{bc}	18.09^{a}	69.00^{d}	5.18^{b}
Treatment	1st	2.74^{de}	6.81^{e}	3.37^{de}	3.19	64.84^{d}	677.78^{c}	1.86^{d}	1.21^{d}	63.89^{d}	15.61^{b}	72.00^{c}	3.72^{e}
	2nd	3.05^{c}	6.91^{cde}	3.72^{c}	3.30	71.02^{c}	719.10^{b}	2.19^{c}	1.56^{c}	67.52^{bcd}	14.67^{bc}	80.00^{b}	4.81^{c}
	3rd	3.52^{b}	6.96^{bc}	3.90^{b}	3.56	73.95^{b}	756.11^{a}	2.66^{b}	1.97^{b}	71.32^{b}	14.45^{bc}	82.50^{a}	5.33^{b}
	4th	3.74^{a}	6.99^{abc}	4.17^{a}	3.80	76.74^{a}	779.32^{a}	2.91^{a}	2.23^{a}	75.76^{a}	13.37^{c}	83.50^{a}	6.12^{a}
±SEM		0.06	0.04	0.05	0.04	0.98	8.27	0.05	0.04	1.40	0.58	0.79	0.11
P value:													
Treatment		0.0001	0.0141	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0007	0.0001	0.0001	0.0001
Month		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0164	0.0001	0.0001
Interaction		0.0003	0.0054	0.0266	0.0033	0.0038	0.0042	0.0001	0.0001	0.0072	0.0075	0.0001	0.0014

Values with different superscripts within the same column are significantly different (P < 0.05). VOL = Volume; VIG= Vigor; MM= Mass motility; PM= Progressive motility; SCO= Sperm concentration / ml; TSO= Total sperm output; MSO= Motile sperm output; SV= Sperm viability; AB= Abnormality; IA= Intact acrosome; SF= Seminal fructose

Table 8. Semen characteristics post-thawing of male buffalo as affected by *Echinacea purpurea* extract treatment**.**

Factors		Semen characteristics post-thawing		
		PM (%)	SV (%)	IA (%)
Treatment effect				
Control		18.78^{b}	31.62^{b}	67.91^{b}
Treatment		36.25^{a}	35.25^{a}	71.51^{a}
±SEM		1.40	0.38	0.65
Month effect				
1st		21.20^{b}	24.60^{d}	64.37^{d}
2nd		24.96^{b}	30.36^{c}	67.72^{c}
3rd		30.65^{a}	37.35^{b}	70.49^{b}
4th		33.25^{a}	41.42^{a}	76.27^{a}
±SEM		1.99	0.53	0.91
Interaction effect				
Control	1st	13.98^{d}	23.25^{g}	63.54^{e}
	2nd	15.07^{d}	28.84^{e}	65.91^{de}
	3rd	20.99^{cd}	34.99^{c}	69.11^{cd}
	4th	25.09^{c}	39.39^{b}	73.09^{b}
Treatment	1st	28.42^{bc}	25.95^{f}	65.20^{e}
	2nd	34.85^{ab}	31.88^{d}	69.54^{bcd}
	3rd	40.32^{a}	39.70^{b}	71.88^{bc}
	4th	41.40^{a}	43.45^{a}	79.45^{a}
±SEM		2.81	0.75	1.29
P value:				
Treatment		<0.0001	<0.0001	0.0002
Month		0.0007	<0.0001	<0.0001
Interaction		0.0030	0.0007	0.0062

Values with different superscripts within the same column are significantly different (P < 0.05).

PM= Progressive motility; SV= Sperm viability; IA= Intact acrosome**.**

the untreated group, whereas, the treated group achieved the lowest value than the non-treated group (81.80 & 114.59 sec. vs. 92.03 & 121.61 sec. in RT & LP, respectively). RT values recorded in the present study ranged from (124.29 to 43.30 sec.) in the treated groups and (102.11 to 51.67 sec.) in the control group. The variation in RT of Egyptian bulls was confirmed by many studies, being 65.5 sec (Khalil et al. 2010). The shortest RT (43.30 sec) for ejaculation was recorded for the EPE group in the 1st month, while the longest was in the control group (126.04 sec) in the 4th month of experiment. There was a substantial (P<0.01) difference in the RT between the treated and control groups. LP also showed exactly similar trend as that of RT. Also, El-Azrak et al. (2017) found that cinnamon oil administration of ram increased significantly (P<0.05) libido and semen quality.

Effect of EPE on physical semen characteristics

The current investigation was performed to give clarity on the impact of EP supplementation on physical semen characteristics and seminal fructose levels in male buffalo (Table 7). The present results revealed that the least-square means of all semen characteristics and seminal fructose concentration were affected positively by EPE supplementation during all experimental months. Ejaculate volume was significantly (P < 0.001) lower in the control group than the treated one, and its values increased with advanced time of EPE supplementation. Herdis, (2017) mentioned that the ejaculation volume is associated with the sexual desire, which is affected by TST. Lower semen pH was observed in the supplemented group compared to untreated group, but the lower and highest values were within the normal range, generally, semen pH varied from 6.81 to 7.10. This means that the buffalo semen pH was on the acidic side but very close to neutrality. Garner and Hafez (2000) mentioned that the normal range of pH in bull semen is about 6.4 to 7.8, and semen pH has an impact on some disorders and physiological states. According to the present investigation, the prostate gland's enhanced acidic production in semen may be the cause of the lower semen pH (McDonald, 2003). Nishant et al. (2006) revealed significant (P< 0.01) lower semen pH of crossbred cattle within the normal range in Zn-treated groups compared to the control group. Santosoa et al. (2021) studied the effect of fresh sperm motilities (higher or lower) on semen characteristics of Pasundan bull cattle, and they found that pH values were reduced in the higher group (P< 0.05) compared to lower one. According to Zhou et al. (2015), the pH of semen can have a direct impact on spermatozoa. Sperm viability was more affected by acidic environmental circumstances than by alkaline ones. Thus, raising the pH of bull semen raises its viability and longevity. The vigor characteristic was influenced significantly (P< 0.001) with the EPE treatment. The present study's findings on mass motility and sperm vigor support the reference data (Vale, 2002), which indicates that bull ejaculates should have values above 3. Sperm motility (%) in both groups differed significantly (P< 0.001), indicating higher

sperm motility in the EPE-supplemented group. Kathiravan et al. (2008) and Verstegen et al. (2002) reported that progressive motility is one of the most significant features linked with the fertilizing potential, and sperm motility is considered one of several factors that affect the passage of the sperm through the female reproductive tract, whereas velocity parameters have been positively correlated with the in vitro fertility potential of bull spermatozoa.

Sperm concentration was higher ($P< 0.001$) in the treated group compared to the control, suggested a more favorable impact of EPE supplementation on sperm production. This effect may be due to the positive effect of EPE on the seminiferous tubules to function normally. Also, data revealed that total sperm output was significantly ($P< 0.001$) higher in the treated group than the untreated group. Saputra et al. (2017) mentioned that Testicular size and semen collection frequency have an impact on sperm concentration. Furthermore, in the Bali bulls, there was a positive association observed between the scrotal circumference and the amount, concentration, and motility of semen. The EPE supplemented group had the greatest mean viability (%), which was considerably ($P<0.001$) greater than the control group. Sperm viability is useful for predicting the sperm fertilizing ability in buffalo (Koonjaenak et al., 2007). Mahmoud et al. (2013) found the correlation between buffalo semen quality and conception rate. They found that semen volume and sperm concentration showed significant ($P< 0.05$) differences between buffalo bulls, whereas, live and motility sperm did not show any significant differences between them. Abnormalities sperm (%) was decreased significantly ($P< 0.001$) with the EPE treatment. Sperm morphology (both normal and abnormal) is entirely dependent on the spermatogenesis stage, which is controlled by Sertoli cells (McDonald, 2003). Abnormality percentages were influenced significantly ($P< 0.001$) by the EPE supplementation, which, some factors such as the semen processing methods, environmental condition, reaction time, and human error can influence sperm defect levels. The tabulated data indicated that the acrosome integrity was significantly ($P< 0.001$) different among the experimental groups, with the EPE-supplemented group showing a lower value compared to the control group. DNA integrity is of high importance to the spermatozoa cell. Some researchers contend that in contrast to sperm characteristics like motility, sperm DNA integrity is a more objective indicator of sperm activity. Damage caused by free radicals may result in DNA damage (Twigg et al., 1998; Rajesh et al., 2002).

The current study findings are also in agreement to Kumar (1993) in buffalo bull semen. The improvements in all studied semen characteristics may be attributed to the decreasing of oxidative stress like MDA and the increasing of TAC concentrations and antioxidant enzymes activity such as GPX, SOD, and CAT with EPE treatment (Table 5). Bilodeau et al. (2001) and Shamsi et al. (2010) revealed that MDA level and activity of SOD and GPX play a critical role in protecting spermatozoa from oxidative damage, therefore maintaining and improving fertility; blood SOD has a positive correlation with the sperm count and a negative correlation with the abnormal and dead spermatozoa, and GPX protects the sperm against peroxidative damage. In the present study, the improvement of serum antioxidant GPX level may be due to the active metabolites of herbs. Yun et al. (2016) found that boar's sperm concentrations and testosterone values were increased significantly in the treated group by ginger extract in comparison with the untreated one. These findings were explained by a decrease in oxidative stress levels, which prevent the steroidogenic enzymes needed to synthesise testosterone, or by the possibility that ginger extracts may directly influence Leydig cells to increase the production of testosterone. Santosoa et al. (2021) cleared that The criteria of sperm colour, consistency, motility, and concentration have a relationship since sperm colour is dictated by sperm density or concentration, and it is reflected in the mass motility and consistency of the semen.

In a sexual process, it can be noticed that semen quality parameters are highly associated with male fertility. The observed data revealed that In male buffalo, EPE supplementation increased the quantity and quality of semen. Since it acts as a transport and food supply for spermatozoa, seminal plasma is a crucial element in the majority of natural mating activities. Because it helps determine sperm concentration, measuring it as semen volume is essential to semen analysis. The potential of EPE to stimulate the epididymis and create semen may be the reason for the noted considerable increase in semen output in the treated group when compared to the control. The treatment group's much higher sperm concentration than the control group's shows how well EPE supplementation supports spermatogenesis, which improves fertilization capacity. Due to higher semen antioxidant enzyme activity, the percentage of viable spermatozoa in the current study showed a positive correlation with EPE supplements as compared to the control group. Additionally, the percentage of abnormal spermatozoa and treatment has a negative correlation. Abnormal spermatozoa can lead to reduced fertility and complicate ova fertilization. Findings indicated that supplementing with EPE decreased the proportion of sperm cells with deviant morphology, indicating that EPE improved the morphology of spermatozoa in male buffalo.

Effect of EPE on seminal fructose concentration

Seminal fructose level was significantly (P< 0.001) higher in the EPE-treated group than in the control group (Table 7); differences due to treatment, and months of the experimental period were highly significant (P<0.001). Fructose concentration in seminal plasma has been used as an indicator of the secretory activity of accessory sexual glands (Salisbury et al. 1978). This result may be attributed to the higher level of testosterone in the blood stimulating the secretory activity of the seminal glands (Corah, 1981). Barakat (2004) mentioned that initial fructose concentration improves the endocrinological output of hormones and has a positive relationship with growth and maturation.

Effect of EPE on pregnancy rate

Total number of buffalo-cows artificially inseminated using post-thawing semen were 10 per each experimental groups and total number of conceived animals were 7 (70%) in control group and 8 (80%) in treated group. These obtained data indicated that buffaloes inseminated with diluted semen produced from EPE-treated bulls showed a relatively higher pregnancy rate and higher non-return rates compared to buffaloes inseminated with diluted semen produced from untreated bulls. These results support that increasing the treatment period with *Echinacea* maintains sperm metabolism, function, and quality. Ahmed et al. (2020) reported that green tea extract supplementation to buffalo bulls enhanced significantly the fertility rate of spermatozoa by 34.21% in the treated group than that found in the control group.

Mahmoud et al. (2013) found a positive correlation between pregnancy rate and live sperm percentages in buffalo bulls. Barile et al. (1999), Chohan et al. (1992), Anzar et al. (2003), and Akhter et al. (2007) found that the pregnancy rates in buffalo were 45.2, 33.0, 30.0, and 60%, respectively, after artificial insemination. So, Vale (1997) considered a conception rate of more than 50% following AI using frozen-thawed semen in buffalo. On the contrary, Barros et al. (2007) and El-Sisy et al. (2010) reported no significant relationship between spermatozoa parameters, vitality, and pregnancy rates in buffalo.

Conclusion

The present findings concluded that *Echinacea* purpurea extract can be used as a growth promoter and is a potential immunomodulatory treatment that has a strong therapeutic benefit for boosting the immune system's suppression in Egyptian male buffalo. Also, it tended to improve enhancing hematological, biochemical, antioxidant markers, sexual desire, semen characteristics, and pregnancy rate.

Ethics statement

The Animal Ethics Committee's criteria for the care and use of experimental animals were followed during every step of the study process. The experimental protocol was approved by the ARC-IACUC committee (IACUC protocol number: ARC/AHRI/112/24).

Declaration of interest

There is no conflict of interest for any of the authors of this study.

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