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PROTECTIVE ROLE OF VITAMIN –TPGS TO OVERCOME OXIDATIVE STRESS INDUCED BY DIPPING OF SHEEP WITH CYPERMETHRIN

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Abstract

The sheep area unit sometimes swaybacked or treated with pesticides annually so as to stop parasites which will ruin the fleece and doubtless prove fatal. Cypermethrin is a synthetic cypermethrin widely utilized in sheep dip formulations, its high toxicity to the animal and aquatic environment with high concentrations. The object of this study to improve and support the antioxidant enzymes of the body as well as immune system against the oxidative stress result from pyretheroid.

Sixty sheep from One filed in Talyah city was divided into three groups: twenty sheep treated with subcutaneous S/C with 7mg/kg of vitamin E before for 3 days of suffering to dipping with cypermethrine; twenty sheep treated with orally 7 mg/kg of Vitamin E –TPGS before for 3 days of suffering to dipping; twenty animal still without treatment as control negative as well as blood sample was taken before given treatment. At the third animal was dipping with cypermethrine at concentration 0.001 of river water by using metallic tank at a size of 1000 litters. The serum blood level of norepinephrine and superoxide dismutase (SOD) was estimated and some physiological parameters. Our result showed that orally TPGS is more potent than ordinary vitamin E to reduce oxidative stress by normal return level of norepinephrine and elevating SOD, as well as improvement heart rate and regulate respiratory rate.

Key words: Vitamin E- TPGS, SOD, NE, Insecticide.

Introduction

Insecticides are substances utilize to kill insects (Stephenson *et al.*, 2006). Tick-borne diseases are widely distributed among animal populations and are responsible for significant economic losses (Hamzah and Hasso, 2019). Insecticides are employed in medicine, agriculture and industry. Commonly most insecticides have the scope to significantly convert ecosystems; numerous are extremely toxic to humans and/or animals; some be intensified as they diffusion over the chain of food (Dirwal *et al.*, 2019). Insecticides can be classified into two main categories : insecticides act systemically, which have body accumulation such as organochlorine and organophosphorus that depend on binding with lipid in the tissues and topical application insecticides, which have no body accumulated effect, less absorbable more preferable. The main ticks types identified in Iraq includes : *Rhipicephalus turanicus, Hyalomma anatolicum* and *Hyalomma turanicum* were identified in Iraqi areas (Hamzah and Hasso, 2019).

One the other hand, the modern classification of insecticide which involve three types. The first natural substance, such as pyrethrum, nicotine and neem extracts, derived by plants (Quarcoo *et al.*, 2010). Second group Inorganic insecticides, which are metals (Hamzah *et al.*, 2019). Third class its involve organic insecticides, which are organic chemical compounds. The mechanism of action involve how the pesticide destroyed or remove a pest. The mechanism of action its important model to

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identified whether an insecticide may be harmful to unrelated species, such as mammals, fish and birds. Pyrethroid pesticides has activity similar to natural compound of pyrethrum. These semi synthetic compounds are non-persistent in the ecosystem, sodium channel modulators and are moderate toxic than carbamates due to no related to inhibition of cholinetrase. Pyretheroids in this group are often applied against household pests due to highly safety to human. There are two different class of pyrethroid, the first contain cyano like Cypermethrine and Deltametherine, while second group contain non-cyano pyretheroid as Permethrine and Cismetherin (Holifield, 2004). In fact the main source of poisoning involve ingestion of feeds or water containing the pesticides and dermal absorption after topical application for control external parasite as well as by wound in human (Field *et al.*, 2017). Pyrethroids are axonic excitotoxins, the toxic action of which are patented *via* block the shutting of the voltage-gated channels of sodium in the axonal membranes. When the pyethroid holds the gate in their open state, the re-polarize of nerves inhibited, leading the axonal membrane constantly depolarized, thereby paralyzing the organism. Soderlund *et al.,* (2002) TPGS may be a artificial amphiphile that undergoes accelerator cleavage to deliver the antioxidant lipophilic properities, α-tocopherol (vitamin E) to cell membranes (Yan *et al.*, 2007). TPGS is derived and synthesized from the antioxidant with lipid-soluble properties, α-tocopherol (vitamin E) by grafting to a polythene glycol (PEG) oligomer via a succinate diester linker (Zhang *et al.*, 2012). TPGS is wide applied medically as a vitamin E formulation. it's generally considered Safe drug, excellent absorbable when given orally at semi-permanent doses at (5-17.8) mg/kg/day or up to one 0.1g/kg/day for individuals with minor uptake (Karrar *et al*., 2019). Further, TPGS a thousand (1000 denoting the PEG chain molecular weight) has been get to FDA approval as a drug solubilizer in, parenteral oral, sc, nasal and rectal/ vaginal therapies, TPGS has additionally appear to enhancement as a solublizer for drug delivery of respiratory system as inhalators (Dirwal *et al.*, 2019). Vitamin E-TPGS is commonly used as vitamin E with prosperities as a water-soluble formulation. (Rachmawati *et al.*, 2017). TPGS in drug delivery in modern six annually, which depend on the importance to has ability the inhibitory of P-gp and other main properties. We digested the medical applications of TPGS confirmed nitric oxide (NO) donor, prodrugs and polymers delivering therapeutic compound and to overcoming multidrug-resistant by enhancing therapeutic drug permeation (Umar, 2016; Rachmawati *et al.*, 2017).

Material and Methods

Chemical

Vitamin E and Vitamin E TPGS NF Grade were purchased from medical express chemistry (MEC, USA), Cyprmetherine, Indian company.

Animals filed

Sixty of sheep from One filed in Talyah city was divided into three groups: twenty sheep treated with sub cutaneous S/C with 7mg/kg of vitamin E before for 3 day of suffering to dipping with cypermethrine; twenty sheep treated with orally 7 mg/kg of vitamin E-TPGS before for 3 day of suffering to dipping; twenty animal still without treatment as control negative as well as blood sample was taken before given treatment. At the third animal was dipping with cypermethrine at concentration 0.001 of river water by using metallic tank at size of 1000 litter. The blood samples were collected from the jugular vein of sheep using gel tube after one day of dipping. All samples were transferred using cooling boxes, then centerfugate at 6000 rpm for collecting serum and freeze drier until day of Eliza analysis of norepinephrine and superoxide dismutase (SOD).

Antioxidant enzymes and norepinephrine determination

The enzymes were used in this assay includes sheep SOD, sheep with EKRAT-0650 and EKRAT-0634 and Sl 00070Sp) respectively, using ELISA kit by method of Sandwich-ELISA were determined according to (Jasim and Hasan, 2019) as following :

1. All reagents were Prepared prior beginning assay procedure. It is suggested that everyone Standards and Samples are further in like the small ELISA Strip plate.
2. 50µl of each SOD and epinephrine standards was added to standard wells.
3. Testing samples 10µl of serum were added to sample diluent 40µl of each kit to assay sample wells while the blank well doesn't add anything.
4. HRP-conjugate reagent 100µl of was added to well plate and then coated with an adhesive strip and incubated for at least one hour at 37°C.
5. Each well was perfused and washed, recurring the operation four times for a total of five washes. Following the last wash, any residue was eliminated.
6. 50µl of each SOD and epinephrine chromogen sol A and 50µl of chromogen sol B was pippeted to each well. Suavity was mixed and incubated at 37°C for 15 minutes. then Safekeeping source of light.

Table 1: Effect of vitamin E and TPGS on physiological parameter after dipping with cypermethrine in sheep.

Signs	Negative control		Positive control		VET E treated sheep		VET E TPGS treated sheep	
	No.	(Mean ±SE)	No.	(Mean ±SE)	No.	(Mean ±SE)	No.	(Mean ±SE)
Temperature (°C)	20	(39.26 ±0.14)A	20	(39.54 ±0.17)A	20	(39.38 ±0.09)A	20	(39.14 ±0.13)A
Pulse rate (beats/minute)		(78±1.53)B		(100 ±1.87)A		(85±1.29)B		(82±0.95)B
Respiratory rate (rate/minute)		(29.78±1.03)B		(38.33±0.61)A		(33.33±1.15)B		(30.67±0.88)B

The value represent mean±SE; N=20 for each group; Different capital letters indicate significant (P<0.05) among groups.

7. 50µl of each SOD and epinephrine of Stop solution was pippeted in to all wells. The colour blue transform to yellow in all wells.
8. The final steps scan the plate at Optical Density (O.D.) 450 nm employing a microtiter plate reader moreover as color stable for quarter-hour.

Calculations

The standard curve was premeditated for the absorbance versus the concentration of the standards. To calculate every sample concentration of SOD and norepinephrine, 1st the absorbance worth was entered on the coordinate axis and extended a horizontal line to the quality curve, the purpose of intersection was found, a vertical line was extended to the coordinate axis and therefore the corresponding sample concentrations were scanned to every SOD and norepinephrine.

Results

Effect of TPGS on physiological function of sheep

The present study in the table 1: reported an enhanced significantly (P<0.05) the levels of pulse rate and heart rate in the positive control group that not received any treatment and dipping with cypermethrin. On the other hand sheep that received Vitamin E and TPGS showed a significant reduction at (p<0.05) in pulse rate and heart rate and there is no significant at (p<0.05) when compared with the negative control group.

Role of norepinephrine and SOD in oxidative stress

The our data in table 2: showed a significantly reduction of SOD (P<0.05) in the positive control group that not received any treatment and dipping with cypermethrin accompany with elevating of serum norepinephrine. On the other hand sheep that received Vitamin E and TPGS orally received sheep showed a significant reduction at (p≥0.05) in serum norepinephrine and there is a significant elevation of serum SOD at (p<0.05) as well as no a significant difference when compared to the negative control group. On the other aspect TPGS orally received sheep appear highly a significant at (p<0.05) in serum NE when compared with sheep received intramuscular Vitamin E.

Table 2: Effect of vitamin E and TPGS on norepinephrine and superoxide dismutase parameter after dipping with cypermethrine in sheep.

Groups	T1	T2	T3
Pre- dipping/ NE	648 ± 17.5A	659±15.1A	645 ± 17.3A
After dipping /NE	749 ± 33.8A	566±80B	475 ± 75C
Pre- dipping/ NE	1.87±0.19A	1.97±0.34A	1.17±0.44A
After dipping /NE	0.87 ± 0.26C	1.14 ± 0.29B	1.89 ± 0.42A

The value represent mean±SE; N=20 for each group; Different capital letters indicate significant (P<0.05) among groups.

Discussion

The present study showed that an enhancement levels of pulse rate and heart rate in positive control group that not received any treatment and dipping with cypermethrin Moreover Vitamin E and TPGS showed a significant reduction at (p<0.05) in pulse rate and heart rate and there is no significant at (p<0.05) when compared with negative control group. The dipping process state involves comfort and alert so that elevating of levels of stress hormones during this process, therefore plasma concentrations of vasopressin, met-enkephalin, cortisol, noradrenaline, adrenaline, beta-endorphin, release at high level to resist stress (Ahbabi *et al*., 2015; Chrustek *et al*., 2018).

Cypermethrin appear to have an inverse effect on the body immune system, fertility, cardiology and liver biotransformation thus activity of body enzymes. cypermethrin induces inflammation, liver damage and disrupt the antioxidant activity enzymes and balance in body tissues (Chrustek *et al.*, 2018). Suzan, (2012) reported that cypermethrin administrated pigeon leading to vacuolation of myocardial muscle cells accompanied by foci of fat cells between cardiac muscles cells. Moreover lungs tissue recorded pathological changes involve congestion, selling with emphysema as well as foamy alveolar macrophages. The oxidative stress induced by dipping with irritant and toxic material may be lead to elevating release of norepinephrine from nerve ending as well from adrenal medulla. Some research evaluated that cypermethrin induce oxidative stress after applied dermally as well as an excess in catalase activity and superoxide dismutase (SOD) activity, glutathione peroxidase (GPX) and blood glutathione serum levels (Raina *et al.*, 2009). The body of mammals has two

defenses mechanism to resist oxidative stress such include enzymatic as superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), glutathione peroxidase (GSH-Px) and non-enzymatic like reduced glutathione (GSH), ascorbic acid and vitamin E (Athar, 2002).

Cypermethrin are hepatically metabolized by cytochrome P450 oxidative pathways generating (ROS) reactive oxygen species that attack body yeliding free radicals (Gassner *et al.*, 1997). Oxidative stress hold the utility of the obtainable mitochondrial electron to produce molecular oxygen, generate in over production of superoxide in most body tissues. These superoxide anions are transformed to water and hydrogen peroxide (Fridovich, 1995). Our result showed that TPGS orally received sheep appear highly a significant reduction of serum NE at ($p<0.05$) when compared with sheep received intramuscular Vitamin E. The antioxidant ability of vitamin E-TPGS is depend on hydrolysis by in cytoplasmic by esterases that generating free α-tocopherol, which then accumulation at the cell membrane and *via* eradicate and protects from lipid peroxidation that lead to damage of membranes (Yan *et al.*, 2007). Kirmizis and Chatzidimitriou, (2009) confirmed that Oxidized TPGS active half tocoferol is reduced once more to its active state by the soluble physiological reducer, ascorbate, to create an eternal cycle. recent report about medical effects of vitamin E on cardiopulomonary disease has evaluating and given high conflicting in the obtained results. The accepted recent theory, explain that oxidative of LDL-cholesterol raise leak in coronary and aortic artery that generate to aorta atherosclerosis and cardiovascular contravention, so vitamin E role as an antioxidant would limit cholesterol convert to LDL that easily oxidized to elevating risk of cardiovascular disease (Miki *et al.*, 1987; Kirmizis and Chatzidimitriou, 2009; Yang *et al.*, 2018).

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