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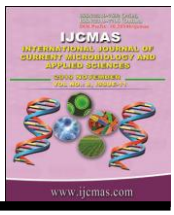

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Protective Role of Ashwagandha and Selenium against Chlorpyrifos (CPF) Induced Haemato-Biochemical and Hepatic Alterations in Wistar Rats

Vellanki Manvitha[1*], A. Gopala Reddy[2], B. Anil Kumar[1], M. Jeevanalatha[3] and G. Priyanka[1]

[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad-500030, India
[2]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Korutla, Jagtial-505326, India
[3]Department of Veterinary Pathology, College of Veterinary Science, Mamnoor, Warangal-506166, India

**Corresponding author*

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A B S T R A C T

The present experiment was detailed about the protective effect of Ashwagandha and selenium on chlorpyrifos (CPF) induced changes in haemato-biochemical parameters and liver histology in rats. 42 male albino *Wistar* rats were procured and divided into 7 groups consisting of 6 in each. Group 1- Normal Control. Group 2 - Chlorpyrifos(@ 2.5 mg/kg body weight/p.o./day). Group 3 – Ashwagandha (@ 100 mg/kg body weight/p.o./day). Group 4 – Selenium (@ 10 µg/kg body weight/p.o./day). Group 5 – Chlorpyrifos (@ 12.5 mg/kg body weight/p.o./day) + Ashwagandha (@ 100 mg/kg body weight/p.o./day). Group 6 – Chlorpyrifos (@ 12.5 mg/kg body weight/p.o./day) + Selenium (@ 10 µg/kg body weight/p.o./day). Group 7 – Chlorpyrifos(@ 12.5 mg/kg body weight/p.o./day) + Ashwagandha (@ 100 mg/kg body weight/p.o./day) + Selenium (@ 10 µg/kg body weight/p.o./day). Group 7 rats revealed a significant ($P<0.05$) increase in the mean values of total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb) concentration and packed cell volume (PCV) and a significant ($P<0.05$) decrease in aspartate amino transferase (AST) and alanine transaminase (ALT) and rejuvenated hepatic parenchyma when compared to group 2 rats. These results suggested that the administration of Ashwagandha and selenium offered remarkable synergistic protective action against CPF induced alterations in haemato-biochemistry and histology.

Introduction

Organophosphorus pesticides (OPs) are commonly used in agricultural, industrial and domestic settings (Darwiche *et al.*, 2018). Chlorpyrifos {O,O-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothionate-CPF} is a broad spectrum organophosphate insecticide,

commonly used in domestic and industrial applications Globally to kill a wide variety of insects (Olsvik *et al.,* 2015) and is currently in use in more than 100 countries Worldwide (Alavanja *et al.,* 2013).

The widespread use of pesticides in agriculture can lead to soil, water and air contamination resulting in adverse effects on inhabiting non-target organisms (Kartheek and David, 2016). The mechanism of CPF toxicity involves acetylcholinesterase (AChE) inhibition and induction of oxidative stress through production of reactive oxygen species (ROS) which causes damage to macromolecules such as lipids, proteins, and DNA (Ambali *et al.,* 2010). CPF is metabolized in hepatocytes by cytochrome P450 2B6 and converted to CPF oxon (oxygen analogue), which represents the major toxic CPF metabolite (Tanvir *et al.,* 2016). CPF causes multiple organ dysfunctions (liver and heart) due to hypoxia and inadequate tissue perfusion (Kumar *et al.,* 2010).

Ashwagandha is the most familiar ayurvedic medicine and it's root extract has found to alter the oxidative stress markers of the body, reduce the lipid peroxidation (LPO) and increase the superoxide dismutase (SOD) and catalase (CAT) activities, thus carrying free radical scavenging property (Sabiba *et al.,* 2013). Selenium is involved in the synthesis of glutathione peroxidase (reducing peroxides) and thioredoxin reductases (regenerating antioxidant systems) (Barciela *et al.,* 2008).

There is scanty literature available on synergistic action of microelement and herbal combination for their greater antioxidant potentiality.

The aim of this experiment was to investigate the protective effect of ashwagandha and selenium given in combination on haematological, biochemical and histopathological parameters after repeated exposure to CPF in *Wistar* rats.

Materials and Methods

In the present study, a total of 42 male albino *Wistar* rats weighing 150-180 grams were procured from Jeeva Life Sciences Pvt. Limited, Hyderabad. The rats were housed in solid bottom polypropylene cages at the lab animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Hyderabad and were maintained at ambient temperature (20-22^{0}C) throughout the course of experiment. Sterile husk was used as standard bedding material. All the rats were provided with standard pellet diet obtained from National Institute of Nutrition (NIN), Hyderabad and *ad libitum* water throughout the experimental period. Rats were randomly divided into 7 groups consisting of 6 in each group. Group 1 served as normal control whereas group 2 served as CPF toxic control (@ 12.5 mg/kg b.w./p.o./day). Groups 3 and 4 were administered with Ashwagandha @ 100 mg/kg body weight/p.o./day and Selenium @ 10µg/kg body weight/p.o./day, respectively and served as non-toxic controls. Group 5 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + Ashwagandha @ 100 mg/kg body weight/p.o./day. Group 6 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + Selenium @ 10µg/kg body weight/p.o./day. Group 7 was administered with CPF @ 12.5 mg/kg body weight/p.o./day + Ashwagandha @ 100 mg/kg body weight/p.o./day + Selenium @ 10µg/kg body weight/p.o./day. The experiment was carried out according to the guidelines and prior approval of Institutional Animals Ethics Committee (IAEC-No. III/2019-05/IAEC/C.V.Sc., Hyd, Dated 17/04/2019).

Drugs and chemicals

Chlorpyrifos (Chlorpyrifos 20% EC) was

procured from Insecticides India Ltd. Root powder of Ashwagandha was obtained from KSM-66 Ashwagandha. Selenium as sodium selenite was procured from Sisco Research Laboratories Pvt. Ltd., Taloja, Maharashtra, India. Stains for the histopathological study of liver were obtained from Qualigens Pvt. Ltd., Mumbai.

Haematology and biochemistry

Prior to blood collection, the selected experimental rats were put to fast for 12 hours. Blood collection was carried out at fortnight intervals for haematological and sero-biochemical analysis after initiation of the drug administration till the end of experiment. Approximately, 2-3ml of blood was collected through retro-orbital plexus with the help of capillary tube into an anticoagulant coated vacutainer {K_3-EDTA tube, 13mm x 75 mm, 4mL (Rapid Diagnostics Pvt. Ltd., Delhi)} for analysis of all haematological parameters and into serum vacutainers to analyse biochemical parameters. The whole blood was used for estimation of Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Haemoglobin (Hb) concentration and Packed Cell Volume (PCV) by using automatic whole blood analyser (Huma count, med source ozone biomedical Pvt. Ltd., Faridabad, Haryana). Blood collected in serum vacutainers was centrifuged at 3000 RPM for 15 min, and serum was separated and stored at -80 ^{0}C till analysis. Kits for AST and ALT estimation were procured from ERBA diagnostics Ltd, Surat, India.

Histopathology

On the 28th day, after blood collection, rats were euthanized by carbon dioxide exposure and tissue pieces of liver were collected from the rats and fixed in 10% neutral buffered formalin (NBF) for histopathology. The small representative pieces of fixed tissues were cut and subjected to overnight washing under running tap water. The tissues were then dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin at 55-56°C. The paraffin blocks were cut into thin sections of 5 micron thickness by microtome. The cut sections were lifted on grease free glass slides precoated with Mayer's egg albumin and were kept in incubator overnight at 37°C for drying. The slides were stained with routine Haematoxylin and Eosin (H and E) stain (Clayden, 1962; Culling, 1974) and the stained sections were mounted with DPX mountant and kept ready for microscopic examination (Fig. 1–7).

Data obtained (haematological and biochemical parameters) was subjected to statistical analysis by applying one-way ANOVA and using statistical package for social sciences (SPSS) version 25.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P<0.05$ (Snedecor and Cochran, 1994).

Results and Discussion

Haematology

The mean values of TEC (millions/µL) in different groups (1, 2, 3, 4, 5, 6 and 7) were ranged from 5.82±0.02 to 9.28±0.07 on 14thday and 5.08±0.05 to 9.30±0.02 on 28th day of experiment. Significantly ($P<0.05$) decreased values were observed in group 2 rats when compared to all other groups on 14th and 28th day of experiment. The treatment group 7 showed significant ($p<0.05$) improvement in the TEC values when compared to the treatment groups 5 and 6 (Table 1).

The mean values of TLC (thousands/µL) in different groups (1, 2, 3, 4, 5, 6 and 7) were ranged from 11.50±0.12 to 15.57±0.08 on

14th day and 10.51±0.13 to 15.58±0.05 on 28th day of experiment. Group 2 rats showed a significant (P<0.05) decrease in the TLC values when compared to all other group rats on 14th and 28th day of the experiment. The treatment group 7 showed significant (p<0.05) improvement in the TLC when compared to treatment groups 5 and 6 (Table 1).

The concentration of Hb (g/dl) in different groups (1, 2, 3, 4, 5, 6 and 7) ranged from 11.57±0.04 to 14.29±0.30 on 14th day and 10.17±0.05 to 14.40±0.32 on 28th day of experiment. The concentration of Hb (g/dl) in group 2 was significantly (p<0.05) lower when compared to all other groups on 14th and 28th day. The treatment group 7 showed significant (p<0.05) improvement in the Hb when compared to groups 5 and 6 on 14th and 28th day and the values were insignificant between groups (1 and 7) of group 7 was comparable on 28th day (Table 2).

The PCV (%) mean values in group 2 were significantly (p<0.05) lower compared to the control and treatment groups on 14th and 28th day. The values of groups 5, 6 and 7 were comparable without any significant difference and were comparable to group 1 (Table 2).

The activity of AST (IU/L) in group 2 (86.23±1.85 and 96.06±1.06) was significantly (p<0.05) higher when compared to group 1 (62.76±2.30 and 63.11±2.43), while group 5 (78.84±1.88 and 73.49±1.09), group 6 (79.34±1.85 and 73.99±1.39) and group 7 (75.43±1.00 and 69.55±0.83) showed significantly (p<0.05) lower values in comparison to group 2 on 14th and 28th day of experiment. The values of groups 5, 6 and 7 were comparable without any significant difference (Table 3).

The activity of ALT (IU/L) in group 2 (55.95±1.14 and 67.09±1.07) was significantly (p<0.05) higher compared to group 1 (34.03±1.43 and 33.83±1.44) whereas treatment group 5 (49.04±1.01 and 43.91±0.95), group 6 (48.54±1.09 and 43.71±0.96) and group 7 (46.94±0.83 and 41.67±0.77) exhibited significantly (p<0.05) lower values in comparison to group 2 on 14th and 28th day of experiment. The mean values of groups 5, 6 and 7 were comparable without any significant difference (Table 3).

The sections of the liver from group 2 showed thickened and dilated portal vein (PV) with congestion, haemorrhages in sinusoids, mild proliferation of Kupffer cells, narrowed sinusoids and degenerative changes of hepatocytes (Figure 2), whereas the treated groups showed dilation and mild congestion of central vein and focal areas of reduced sinusoidal space (Figure 5) in group 5, mild proliferation of Kupffer cells and mild dilatation of sinusoids (Figure 6) in group 6 and normal radiating appearance of hepatic cords with mild dilation of central vein (CV) (Figure 7) in group 7.

A significant change in haematological parameters in the present study may be due to dysfunction of haemopoietic system. A decreased TEC and Hb concentration might be attributed to the increased lipoperoxidation in the CPF group, which was reflected by significant increase in malondialdehyde (MDA) levels leading to haemolytic anaemia.

These findings are in agreement with the previous report of Mossa (2004). Decrease in the PCV may be due to the increased rate of breakdown of RBCs. Decrease in TLC may be due to free radical-induced oxidative damage and these observations are in accordance with Albasher *et al.,* (2019).

The increase in the activity of AST and ALT may be due to liver dysfunction and alteration in the permeability of liver membrane leading to their leakage into blood stream.

Table.1 Total erythrocyte count (millions/µL) and total leucocyte count (thousands/µL) in different groups

GROUP	TEC(Millions/µL)		TLC(Thousands/ µL)	
	DAY 14	DAY 28	DAY 14	DAY 28
G1	9.15±0.06 ^{d}	9.12±0.04 ^{d}	15.55±0.12 ^{d}	15.48±0.10 ^{d}
G2	5.82±0.02 ^{a}	5.08±0.05 ^{a}	11.50±0.12 ^{a}	10.51±0.13 ^{a}
G3	9.28±0.07 ^{d}	9.30±0.02 ^{d}	15.55±0.11 ^{d}	15.56±0.11 ^{d}
G4	9.22±0.05 ^{d}	9.25±0.08 ^{d}	15.57±0.08 ^{d}	15.58±0.05 ^{d}
G5	6.78±0.18 ^{b}	6.84±0.10 ^{b}	12.57±0.06 ^{b}	13.02±0.12 ^{b}
G6	6.51±0.12 ^{b}	6.74±0.18 ^{b}	12.59±0.07 ^{b}	13.09±0.16 ^{b}
G7	7.16±0.09 ^{c}	7.77±0.14 ^{c}	13.40±0.08 ^{c}	13.96±0.11 ^{c}

Values are Mean±SE (n=6); One-way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*).

Table.2 Haemoglobin concentration (g%) and packed cell volume (%) in different groups

GROUP	Hb (g/dl)		PCV (%)	
	DAY 14	DAY 28	DAY 14	DAY 28
G1	14.05±0.09 ^{d}	14.15±0.10 ^{c}	41.21±1.14 ^{c}	41.11±0.82 ^{bc}
G2	11.57±0.04 ^{a}	10.17±0.05 ^{a}	34.09±0.87 ^{a}	33.46±1.03 ^{a}
G3	14.29±0.30 ^{d}	14.40±0.32 ^{c}	41.69±1.04 ^{c}	42.06±1.03 ^{c}
G4	14.14±0.07 ^{d}	14.27±0.39 ^{c}	41.78±0.97 ^{c}	41.98±1.32 ^{c}
G5	12.23±0.11 ^{b}	12.84±0.13 ^{b}	37.57±1.21 ^{b}	37.72±1.48 ^{b}
G6	12.13±0.12 ^{b}	12.52±0.26 ^{b}	37.87±0.93 ^{b}	37.89±1.49 ^{b}
G7	13.29±0.19 ^{c}	14.01±0.32 ^{c}	38.87±0.89 ^{bc}	39.20±0.95 ^{bc}

Values are Mean±SE (n=6); One-way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*).

Table.3 Aspartate aminotranferase (IU/L) and alanine transaminase (IU/L) in different groups

GROUP	AST (IU/L)		ALT (IU/L)	
	DAY 14	DAY 28	DAY 14	DAY 28
G1	62.76±2.30 ^{a}	63.11±2.43 ^{a}	34.03±1.43 ^{a}	33.83±1.44 ^{a}
G2	86.23±1.85 ^{c}	96.06±1.06 ^{c}	55.95±1.14 ^{c}	67.09±1.07 ^{c}
G3	62.26±2.32 ^{a}	61.93±2.35 ^{a}	33.73±1.42 ^{a}	33.49±1.48 ^{a}
G4	62.28±2.40 ^{a}	61.95±2.27 ^{a}	33.57±1.28 ^{a}	33.35±1.52 ^{a}
G5	78.84±1.88 ^{b}	73.49±1.09 ^{b}	49.04±1.01 ^{b}	43.91±0.95 ^{b}
G6	79.34±1.85 ^{b}	73.99±1.39 ^{b}	48.54±1.09 ^{b}	43.71±0.96 ^{b}
G7	75.43±1.00 ^{b}	69.55±0.83 ^{b}	46.94±0.83 ^{b}	41.67±0.77 ^{b}

Values are Mean±SE (n=6); One-way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*).

Fig.1 Microphotograph of liver showing normal architecture of lobule with radiating appearance of hepatic cords (Group 1): H&E X 100µm

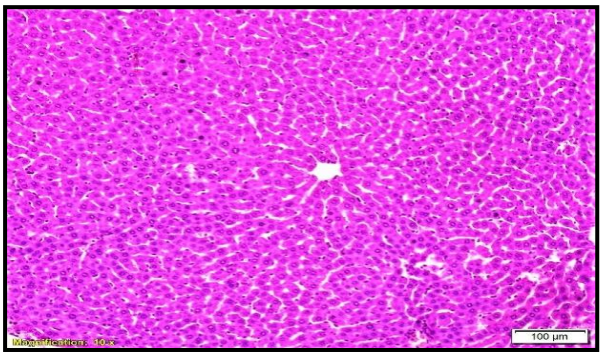


Fig.2 Microphotograph of liver showing thickened and dilated portal vein with congestion, haemorrhages in sinusoidal spaces, mild proliferation of Kupffer cells, reduced sinusoidal space with degenerative changes of hepatocytes and moderate distribution of dark round cells (Group 2): H&E X 200µm

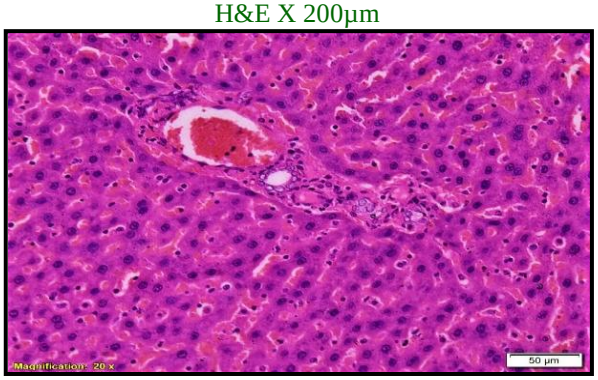


Fig.3 Microphotograph of liver showing normal architecture of liver lobules with radiating appearance of hepatic cords. (Group3): H&E X 100µm

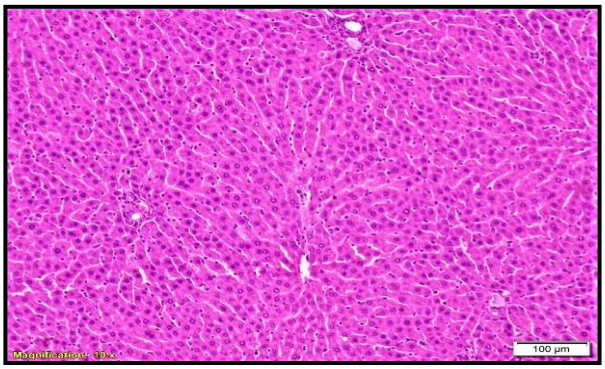


Fig.4 Microphotograph of liver showing normal radiating appearance of hepatic cords within liver lobule. (Group 4): H&E X 200µm

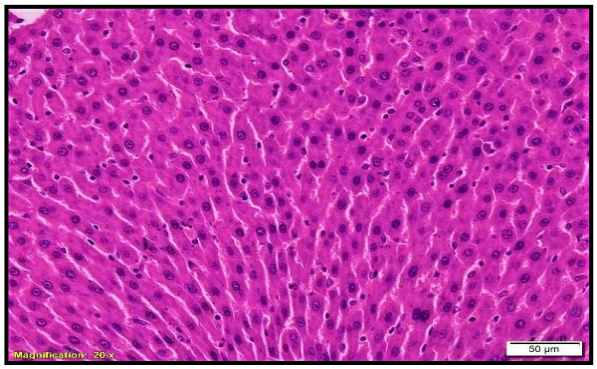


Fig.5 Microphotograph of liver showing dilation and mild congestion of central vein and focal areas with reduced sinusoidal space. (Group 5): H&E X 100µm

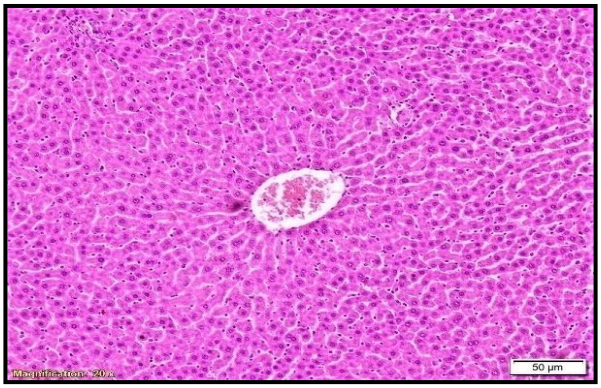


Fig.6 Microphotograph of liver showing mild congestion of central vein, mild proliferation of Kupffer cells and mild dilatation of sinusoidal spaces (Group 6): H&E X 200µm

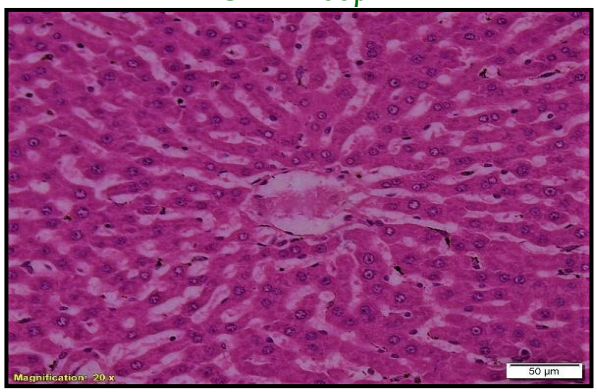
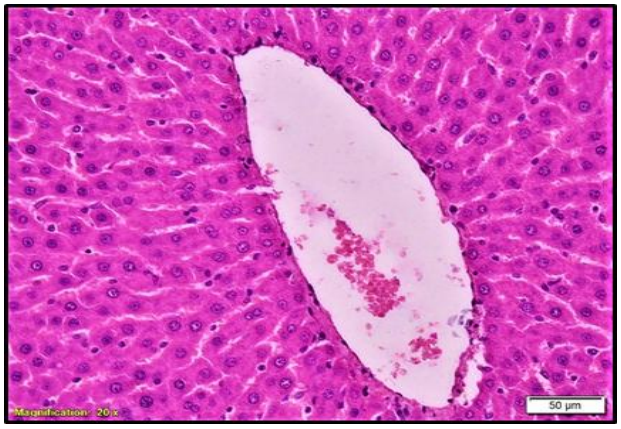


Fig.7 Microphotograph of liver showing normal radiating appearance of hepatic cords with mild dilation of central vein (Group 7): H&E X 200µm



These observations are similar to the earlier studies of Khan *et al.,* (2009); Krishna and Ramachandran (2009) and Wang *et al.,* (2009). These results are further substantiated by marked histopathological changes in the liver like portal vein congestion and haemorrhages in sinusoidal spaces.

In the present study, protective effect of ashwagandha could be due to its antioxidant defensive mechanism by scavenging free radicals and these results are in harmony with the earlier studies of Harikrishnan *et al.,* (2008) and Sujatha *et al.,* (2010). Selenium alleviated CPF induced liver damage in the present study, which could be due to its antioxidant properties as it is a constituent of selenoenzyme-GSH-Pxand these findings are in agreement with the previous studies of Kaushal *et al.,* (2011) and Abdel-Rahman *et al.,* (2017).

In group 7, a significant increase in the mean values of TEC, TLC, Hb and PCV, significant decrease in the values of AST and ALT and restoration of architectural details in liver tissues were observed when compared to group 2 which might be due to combined antioxidant defence mechanism of ashwagandha and selenium against chlorpyrifos induced oxidative damage of blood cells and liver tissues.

In conclusion, the present study clearly demonstrated that both Ashwagandha and selenium synergistically attenuate CPF induced haematological, biochemical and histopathological alterations in liver, possibly *via* antioxidant defence mechanism.

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