Original Research

Haemato-Biochemical Studies of Acephate Induced Toxicity and its Amelioration by *Picrorhiza kurroa* in Female Wistar Rats

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Abstract

An experimental trial on subacute toxico-pathological studies of Acephate and its amelioration by Picrorhiza kurroa was attempted in thirty female Wistar rats for 28 days through haemato-biochemical assessment. The experimental rats were divided into 5 groups each comprising of 6 rats. The group I and III were kept as a control and plant control, respectively. The rats of group II were Acephate toxicated, the rats of group IV and V were toxicated with Acephate and treated with Picrorhiza kurroa. Significant reduction in Hb, TEC, PCV, TLC values and non-significant differences in DLC were observed. There was significant increase in mean values of AST, ALT, ALP and BUN whereas mean STP, cholesterol, sodium, potassium and Acetylcholinesterase values were significantly decreased in group II and IV. These altered mean values could be due to Picrorhiza kurroa.

Key words: Acephate, Haemato-biochemical, *Picrorhiza kurroa*, *Wistar* Rats

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Introduction

Organophosphorus (OP's) results in phosphorylation of serine hydroxyl residue on acetylcholine esterase enzyme, which results in the accumulation of acetylcholine. This leads to cholinergic features, which can be classified into central and peripheral. Peripheral events includes vomiting, diarrhoea, miosis, muscle fasciculations, urinary incontinence and bronchoconstriction. Central effects includes respiratory depression and delirium (Carey *et al.*, 2013). One of the devastating cholinergic features of organophosphate poisoning is respiratory failure. Recently, at Yawatmal district of Vidarbha region of Maharashtra State, (India) around 50 farmers/farm labours have been died and nearly 800 have been hospitalized due to pesticide poisoning (Hindustan Times, Yawatmal, 13th Oct., 2017). About 20 percent of Indian food products, contain pesticide residues above tolerable level when compared with global

information which is 2 per cent only. Totally, there are about 200 organophosphorus ester insecticides in the market formulated into literally thousands of products. One among the top 10 organophosphate insecticides sold throughout the world is Acephate. The chemical name of Acephate is O, S dimethyl acetyl phosphoramidothioate with structural formula C4H10NO3PS. The medicinal importance of *P. kurroa* is due to its pharmacological properties like hepatoprotective, antioxidant (particularly in liver), antiallergic, antiasthmatic, anticancerous and immunomodulatory (Pandit *et al.*, 2013). It is an important medicinal plant used in traditional as well as modern medicine for the treatment of liver disorders, fever, asthma and jaundice (Bhandari *et al.*, 2009). Considering these facts, the present experimentation entitled, "Haemato-biochemical studies of Acephate induced toxicity and it's amelioration with *Picrorhiza kurroa* in female *Wistar* rats" was conducted with the following objective-

To assess sub-acute oral toxicity of Acephate and its amelioration with *Picrorhiza kurroa* in female *Wistar* rats through haemato-biochemical studies.

Material and Methods

Female *Wistar* Rats

Thirty (30) female *Wistar* rats of 6-8 weeks age having 140-160 g body weight were procured from M/S Worckdht Research Center, MIDC, Aurangabad, (MS) 431006.

Collection of Acephate Pesticide

Acephate (O, S dimethyl acetyl phosphoramidothioate) was procured from Department of Pesticide, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapith, Parbhani (MS).

Feeding

Rats were provided with standard pellet feed procured from M/S L.R. Pharmaceutical Company, Parbhani, Maharashtra, India.

Biochemical Kits

The kits for all biochemical [serum aspartate aminotransferase (AST), alanine aminotransferas (ALT), *alkaline phosphatase* (ALP), serum cholesterol, Blood urea nitrogen, serum total protein, blood glucose, sodium, potassium and serum acetylcholinesterase] estimations were purchased from M/S Ambica Diagnostics, MIDC, Parbhani (MS).

Preparation of Acephate Doses

Dose of Acephate was calculated as $1/20^{th}$ of LD_{50} (1127mg/kg) which was in powder form and mixed with propylene glycol as a vehicle and fed daily through oral gavage to the experimental rats.

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Procurement of *Picrorhiza kurroa* Plant Material and as Extract Preparation

Dried powder of a plant of *Picrorhiza kurroa* (kutki) was procured from the local market of Parbhani.

Extraction of Plant with Hot Water (Handa *et al.*, 2008)

250 g dried plant powder of *Picrorhiza kurroa* mixed with 500 mL distilled water

↓

Prepared mixture was boiled for 30 min, cool it and filtered with Whatman filter paper No. 42

↓

Extract was kept into conical flask and pluged with cotton swab and then that liquid extract was kept at 2-4^0 C till its use

Experimental Design

Table 1: Details of experimental groups of rat

Groups	Treatment	No. of Animals	Route
I	Control group with vehicle- *ad libitum* feed and water daily for 28 days	6	Normal feeding and watering
II	Acephate @ 56.35 mg/kg with vehicle	6	By oral gavage
III	*Picrorhiza kurroa* aqueous extract @ 50 mg/kg body weight	6	By oral gavage
IV	Acephate @ 56.35 mg/kg + *Picrorhiza kurroa* aqueous extract @ 25 mg/kg body weight	6	By oral gavage
V	Acephate @ 56.35 mg/kg + *Picrorhiza kurroa* aqueous extract @ 50 mg/kg body weight	6	By oral gavage
	Total	30	

Haematological Parameters

The blood samples were collected through retro-orbital plexus into sterilized EDTA vials for haematological investigations such as Hb, PCV, TEC, TLC, DLC & Clotting time from rats of all the groups during 0, 14th and 28th day of study.

Biochemical Studies

On 0, 14th and 28th day of experiment, the blood samples were collected into sterilized test tubes without anticoagulant and used for separation of serum. Biochemical parameters such as ALT, AST, alkaline phosphatase, serum cholesterol, blood glucose, blood urea nitrogen, serum total protein, AchE, sodium and potassium were analyzed on 0, 14th and 28th day of study by using reagent kits procured from M/S Ambica Diagnostics, MIDC, Parbhani on Semi-Automatic Biochemical Analyser.

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Statistical Analysis

The data generated from various parameters were statistically analysed by Completely Randomized Design (CRD) to know the statistical differences between means of various parameters at different intervals in each group as per the method described by Snedecor and Cochran (1994).

Results and Discussion

Haematological Studies

Table 2 and 3 shows the mean values of haemato-biochemical parameters at different intervals of study.

Table 2: (Mean ± S.E.) values of Haematological parameters in experimental rats at different intervals of study

Parameters	Days	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	CD
Haemoglobin Conc. (g/dL)	14	15.15^{a}±0.33	10.88^{b}±0.42	14.05^{a}±0.62	11.80^{b}±0.49	14.80^{a}±0.56	**1.457**
	28	14.58^{a}±0.56	10.71^{b}±0.39	14.10^{a}±0.79	12.01^{b}±0.61	14.55^{a}±0.59	**1.778**
TEC(millions /µL)	14	7.93^{a}±0.43	6.18^{b}±0.29	7.80^{a}±0.32	6.55^{b}±0.24	7.95^{a}±0.23	**0.919**
	28	8.08^{a}±0.33	5.90^{b}±0.16	7.43^{a}±0.29	6.39^{b}±0.21	7.44^{a}±0.29	**0.786**
PCV (%)	14	47.33^{a}±1.42	35.16^{b}±1.22	44.33^{a}±1.20	39.33^{b}±0.71	44.86^{a}±1.62	**3.718**
	28	46.50^{a}±1.94	35.50^{b}±1.05	44.25^{a}±1.73	39.33^{b}±1.17	44.08^{a}±1.66	**4.518**
TLC (thousand/ µL)	14	9.90^{a}±0.43	6.79^{b}±0.23	9.28^{a}±0.56	7.05^{b}±0.26	9.22^{a}±0.33	**1.126**
	28	9.96^{a}±0.47	6.83^{b}±0.36	9.52^{a}±0.28	7.31^{b}±0.37	9.47^{a}±0.29	**1.06**
DLC	14	25.66±2.26	27.83±2.49	25.33±1.97	25.66±1.58	26.50±2.27	**NS**
Neutrophil (%)	28	23.00±2.30	23.50±1.33	23.16±2.40	23.16±0.70	25.66±2.10	**NS**
Eosinophil (%)	14	1.16±0.30	1.16±0.40	1.16±0.30	1.50±0.34	1.16±0.30	**NS**
	28	1.00±0.36	1.50±0.42	1.33±0.49	1.16±0.54	1.00±0.36	**NS**
Lymphocyte (%)	14	71.66±2.17	69.50±2.44	69.00±2.50	73.50±1.80	70.16±1.97	**NS**
	28	75.00±2.16	72.66±1.72	72.66±3.02	77.16±1.55	72.00±2.26	**NS**
Monocyte (%)	14	1.50±0.42	1.33±0.49	1.33±0.49	2.33±0.42	2.16±0.47	**NS**
	28	1.33±0.61	2.50±0.88	2.83±0.60	1.83±0.47	1.66±0.49	**NS**
Blood Clotting Time (Sec)	14	54.16±3.52	52.33±2.34	52.33±4.56	51.50±2.44	49.33±3.11	**NS**
	28	56.00^{b}±2.59	75.50^{a}±5.61	53.50^{b}±2.04	73.33^{a}±2.77	56.83^{b}±5.44	**11.64**

Superscripts are to be read column wise for mean comparison; ***Mean with the similar superscripts in column do not differ significantly (P < 0.05)

The haematological estimations such as Haemoglobin (Hb), Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Total Leukocyte Count (TLC) in rats of group II and IV were found to be significantly decreased at 14th and 28th day of experiment. Improvement in these female haematological parameters mean values in group V rats were comparable with mean values of control group. The mean values of DLC were non-significant throughout the experiment. Blood clotting time was delayed at 28th day of study in group II and IV rats. All these values were comparable each other including control group. The mean values obtained in rats of group V could be due to ameliorative effect of *Picrorhiza kurroa* against toxicity of Acephate. Acephate might be attributing factor for decrease in Hb concentration due to tissue damage and oxidative

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stress (Thapar *et al*., 2002 and Jasuja *et al*.,2013) and was also due to inhibition of Na^+/K^+ ATPase activity in cells leading to enhanced free radical production (Siraj *et al*., 2010). Acephate induced cell death, oxidative stress and apoptosis might be responsible for reduction in mean TEC values in toxicated rats (Tripathi *et al*., 2007 and Mashali *et al*., 2005).

Table 3: (Mean ± S.E.) values of biochemical parameters in experimental rats at different intervals of study

Parameters	Days	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	CD
ALT (IU/L)	14	30.19^{b}±1.28	41.18^{a}±1.54	30.51^{b}±0.91	40.77^{a}±1.40	28.55^{b}±1.22	**3.764**
	28	29.46^{b}±1.69	41.15^{a}±2.26	30.90^{b}±1.14	40.24^{a}±0.99	29.37^{b}±0.58	**4.253**
AST (IU/L)	14	63.11^{b}±2.81	91.66^{a}±0.59	70.10^{b}±4.94	91.11^{a}±0.82	63.16^{b}±2.66	**8.292**
	28	74.20^{b}±1.80	98.11^{a}±0.57	73.91^{b}±1.12	95.13^{a}±0.50	74.18^{b}±2.49	**4.388**
ALP (IU/L)	14	173.48^{b}±15.09	270.65^{a}±5.08	173.29^{b}±14.10	266.26^{a}±6.38	171.34^{b}±9.96	**31.715**
	28	206.80^{b}±9.58	275.06^{a}±2.32	206.41^{b}±4.25	272.43^{a}±5.80	201.45^{b}±10.91	**21.34**
Serum Cholesterol (mg/dL)	14	82.51^{a}±2.46	43.17^{b}±0.43	82.06^{a}±4.66	45.01^{b}±0.26	82.56^{a}±4.97	**9.469**
	28	84.38^{a}±3.43	41.22^{b}±0.80	81.98^{a}±2.86	43.10^{b}±0.44	84.31^{a}±1.69	**6.407**
TP (g/dL)	14	6.50^{a}±0.25	3.36^{c}±0.22	6.38^{a}±0.16	4.03^{b}±0.14	6.43^{a}±0.12	**0.54**
	28	6.43^{a}±0.15	2.75^{c}±0.13	6.40^{a}±0.15	4.71^{b}±0.24	6.23^{a}±0.12	**0.489**
Blood Glucose (mg/dL)	14	117.66^{b}±4.63	152.68^{a}±1.40	116.80^{b}±3.60	149.33^{a}±3.39	117.21^{b}±2.09	**9.421**
	28	127.33^{b}±2.49	162.86^{a}±1.64	128.83^{b}±2.50	156.48^{a}±5.63	126.46^{b}±3.81	**10.219**
BUN (mg/dL)	14	21.60^{b}±0.56	28.25^{a}±0.73	21.48^{b}±0.89	27.73^{a}±1.25	21.55^{b}±1.01	**2.693**
	28	22.49^{b}±0.52	30.62^{a}±0.95	22.41^{b}±1.19	29.04^{a}±1.33	22.24^{b}±0.49	**2.807**
AchE (µmol/L)	14	1.22±0.09	1.90±0.55	1.66±0.17	1.46±0.11	1.87±0.25	**NS**
	28	1.50^{a}±0.26	0.80^{b}±0.20	1.59^{a}±0.15	0.86^{b}±0.04	0.97^{b}±0.15	**0.522**
Sodium (mEq/dL)	14^{t}	144.10^{a}±1.01	136.33^{b}±0.76	143.16^{a}±1.07	137.00^{b}±0.96	144.66^{a}±1.05	**2.857**
	28	143.00^{a}±0.70	131.66^{b}±1.24	142.66^{a}±1.63	134.33^{b}±1.56	142.66^{a}±1.36	**3.75**
Potassium (mEq/dL)	14	6.32^{a}±0.18	4.02^{b}±0.16	6.28^{a}±0.31	4.12^{b}±0.20	6.31^{a}±0.14	**0.615**
	28	6.11^{a}±0.18	3.77^{b}±0.32	6.05^{a}±0.17	4.07^{b}±0.24	6.04^{a}±0.21	**0.684**

Superscripts are to be read column wise for mean comparison; *Mean with the similar superscripts in column do not differ significantly (P < 0.05)

The reduction in TLC have also been recorded by Manna & Bhattacharya (2004), Mishra (2014) in their studies and opined that it could be due to immunotoxic effect which destroys the lymphoid tissue (Tripathi, 2007). The hypercoagulability seen in OPs compound poisoning is probably due to a massive release of catecholamines from the adrenals (Petrianu *et al*., 1999). The hepatoprotective action of *Picrorhiza kurroa* may be attributed to its ability to inhibit the generation of oxygen anions and to scavenge free radicals which helps in restoration of hematological parameters (Deshmukh *et al*., 2015).

Biochemical Studies

Significant elevation in serum ALT, AST and ALP levels in group II and IV was due to induced hepatopathy on 14^{th} and 28^{th} day of experiment. The mean values of serum ALT, AST and ALP in group III and V remained comparable with mean values of control group. The mean values obtained in group V was suggestive of ameliorative effect of aqueous extract of *Picrorhiza kurroa* against Acephate induced toxicity.

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Liver is a vital organ playing principle role in metabolism and detoxification. Hypoxia and toxicants affects the functional capabilities of liver. Hepatic disorder in Acephate toxicity is substantially reported to possibly disturb the balance of endogenous compounds such as, amino acids and fatty acids (Y. Xu *et al.*, 2011 and Berlanga *et al.*, 2014). The mean values of serum cholesterol and serum total protein were decreased significantly at 14th and 28th day in group II and IV and the mean values were improved in group V and found to be comparable with control group. The observations in group V at 14th and 28th day of study is suggestive of hepatoprotective role of aqueous extract of *Picrorhiza kurroa* on liver. The fall in serum total protein level could be due to Acephate stress, or general toxic action. The decreased levels of total plasma protein in Acephate toxicity might be due to hepatotoxicity, nephropathy and malabsorption due to gastroentropathy (Tripathi, 2007). There was significant difference in mean values of BUN at 14th as well 28th day of study in group II and IV. However, group V rats showed improved levels of BUN at 14th as well 28th day of experiment. These mean BUN values in female rats of group III remained statistically on par with respective BUN values of control group. Increase in BUN level could be attributed to Acephate induced renal injury. Also, Acephate toxicity may lead to cause abnormal metabolism of substances including glucose, nucleic acid and protein (Yurong *et al.*, 2013). There was significant increase in blood glucose level in group II and IV when compared with respective control group mean values. However, the mean values of blood glucose in group III and V remained comparable with the mean values of control group. Hyperglycemia was observed in female rats exposed to Acephate in the present experimental study could be due to an increase in hepatic glucose 6 phosphate activity or inhibition of insulin secretion from β cells. Insecticides stimulate catecholamines that are known to inhibit insulin secretion by activation of alpha receptor of pancreas (Mishra, 2014).

Estimation of mean values of serum Acetylcholinesterase in group II and IV showed significant decline at 28th day of experiment. However, these mean values of Acetylcholinesterase of group V remained comparable with mean values of group I and III indicating the ameliorative effect of *Picrorhiza kurroa* in minimizing the neurotoxic effects. The primary target site for the OPs compound is the acetylcholine. The OPs react and phosphorylate the serine hydroxyl group within the active site of enzyme and blocks the degradation of the neurotransmitter acetylcholine (Carey *et al.*, 2013). There was a significant reduction in serum sodium and potassium levels in group II and IV than the respective control group, however, these values were regained and similar to the mean values of group V is indicating the protective role of aqueous extract of *Picrorhiza kurroa*. The decrease in sodium levels could be due to enhanced lipid peroxidation by free radicals in OPs toxicated rats, and also might be due to consequential effect of reduction in potassium levels (Siraj *et al.*, 2010). Recovery and restoration of the enzymes in group V rats is evocative of ameliorative action of *P. kurroa* which could be able to restoration of damaged hepatocellular membrane and preserving structural integrity of liver, thus prevented enzymes leakage into circulation treated (Bhar

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et al., 2005, Singh *et al.*, 2005 and Talmale *et al.*, 2010) and was also due to proven action as an antioxidant, antidiabetic, anticholestic, immunomodulatory and nephroprotective properties (Salma *et al.*, 2017).

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