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TOXICOLOGICAL STUDY OF PLANT HORMONE NAPHTHALENE ACETIC ACID IN SWISS ALBINO MICE

F. Yeasmin[1], M. H. Morshed[1*], M. Ibrahim[2]and M. Azizul Islam[3]

[1]*Department of Chemistry, Khulna University of Engineering and Technology, Khulna-9203, Bangladesh*
[2]*BCSIR Laboratories, Binodpur Bazar, Rajshahi-6206, Bangladesh*
[3]*Department of Chemistry, Rajshahi University, Rajshahi, Bangladesh*

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ABSTRACT

The aim of the present work was to investigate the sub acute toxicity of plant hormone naphthalene acetic acid (NAA) on Swiss albino mice. The studies include the gross general observation such as changes in body weight, hematological profiles [total count of red blood cells (RBC) and white blood cells (WBC), differential count of WBC, platelet count, hemoglobin (Hb) %], biochemical parameters of blood [serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), creatinine] and histopathology of the liver, kidney, lung, spleen, heart and brain of both control and experimental groups. Administration of hormone (NAA) at a dose of 300 μg mice^{-1} day^{-1} for consecutive 14 days, showed no significant change of hematological and biochemical parameters. No abnormalities were also found in the histopathology of the liver, kidney, lung, spleen, heart and brain in the experimental group of animals following same dose when compared with control group. This preliminary toxicological study suggests that the plant hormone naphthaleneacetic acid (NAA) may be used safely for agricultural purposes and as an external preservative.

1. INTRODUCTION

The plant hormones (auxin) regulate the amount, type and direction of plant growth. They are found in all members of the plant kingdom. Auxin affects numerous plant processes, e.g., cell division and elongation, autumnal loss of leaves and the formation of buds, roots, flowers and fruits (Audesirk *et al.,* 1986). They are widely used commercially to produce more vigorous growth, to promote flowering and fruiting and also root formation in plants not easily propagated by stem cuttings, to retard fruit drop and to produce seedless varieties (e.g., of tomatoes) by parthenogenetic fruiting (Audesirk *et al.,* 1986; Folkes *et al.,* 1999). Naphthalene acetic acid (NAA) is also a synthetic auxin which is commonly employed to induce the formation of adventitious roots in cutting and to reduce fruit drop in commercial crops (Folkes *et al.,* 1999). NAA has also been reported to have antifungal property against some plant fungi (Folkes *et al.,* 2002; Folkes *et al.,* 2003). Antibacterial and cytotoxic characteristics were also reported for the compound NAA (Greco *et al.,* 2000; Pal *et al.,* 2001). The application of NAA as an external preservative has also been reported for different cultivars of mango (Raven *et al.,* 2003; Rossiter *et al.,* 2002). So far we know, subacute toxic effects of NAA have not been investigated that limits its application. So it is thought worthwhile to investigate the different hematological and biochemical profiles with a view to assess its safety profile which may be helpful for its application.

2. MATERIALS AND METHODS

For the purpose of study, Swiss albino mice (12 nos, male) of two weeks old, weighing 22.0-28.1 g were collected from ICDDRB, Mohakhali, Dhaka. The mice were kept in properly numbered iron cages individually in a clean animal house with an optimal room temperature (25-30°C) and were given standard laboratory diet and allowed to drink water ad libitum (Yue *et al.,* 2000). The animals were maintained in this way for 15 days before drug administration and continued up to the end of the experiment. The weight of the individual mice was taken and were grouped into two. The group B (6 mice, average weight 24.03 g) was used for experiment while the group A (6 mice, average weight 24.23 g) was used as control. The plant hormone NAA in a pure grade was made of Fluka Company, Germany, was collected and maintained at 4°C. The compound (NAA) was dissolved in distilled water using tween-20 as co-solvent, so that 0.3 ml contained 300 μg of the hormone. The mice in group A and B were injected intraperitoneally with vehicle (300 ml isotonic saline) and compound (NAA) 300 μg mice^{-1} day^{-1} respectively for 14 consecutive days. On the 15th day blood was collected from external jugular vein under mild ether anaesthesia for the estimation of hematological and biochemical parameters. Then all the mice were sacrificed and lung, kidney, liver, spleen, heart and brain were excised for histological study. During the whole experimental period their behavior, central nervous system (CNS) excitation, CNS depression, reflexes, muscular weakness, salivation, diarrhea and food intake were observed. Biochemical parameters included SGOT, SGPT, serum alkaline phosphatase (SALP), urea and serum creatinine were determined by using the procedures reported (Rethman *et al.*, 1957; Fawcell and Favreau, 1960; Coulombe and Favreau, 1963).

* Corresponding Author: hasanchem03@yahoo.com

3. RESULTS AND DISCUSSION

Table 1 shows the change in body weights of all the mice (both group A and B). The mice of group A and B were being treated with vehicle (isotonic saline) and the compound (NAA) respectively, showed no signs of tremor, convulsions and reflex abnormalities. The body weights of all the mice (both group A and B) were increased after treatment. Moreover no muscular numbness of the hind and fore legs, salivation or diarrhea was observed. The food intake per day was also found normal. So from the results it is decided that hormone (NAA) has no effect on normal growth.

Table 1: Effect of Compound (NAA) on body weight of mice

Group	Dose (i.p.) $\mu g\ mice^{-1}\ day^{-1}$	Body weight (g) before hormone treatment n = 6, $M_1 \pm SD_1$	Body weight (g) after hormone treatment n = 6, $M_2 \pm SD_2$	%change
A	300 µl vehicle	24.23±1.705	31.02±1.733	+28.02(S)
B	300 µg NAA	24.03±2.183	27.52±3.967	+15.52(S)

M_1 and M_2 = Sample mean value, SD_1 and SD_2 = Standard deviations, n = Number of mice, + = Increase, S = Significant, Group A = Control mice, Group B = Experimental mice, NAA = Naphthaleneacetic acid. vehicle = isotonic saline

Table 2: Hematological profiles (TC of RBC, TC of WBC, DC of WBC, platelet count and Hb%) of group A (control) and group B (experimental) mice (M ±SD)

Hematological parameters	Mice treated with vehicle Group-A (control) 14^{th} day	Mice treated with IAA Group-B (experimental) 14^{th} day
Total RBC count (million cu. mm^{-1})	3.93±0.163	3.75±0.138
Total WBC count (thousand μl^{-1})	3.00±0.147	2.80±0.210
Differential count of WBC		
a) Neutrophil (cells/µL)	85.83±1.722	86.83±3.545
b) Lymphocyte (cells/µL)	7.00±1.265	7.50±1.049
c) Monocyte (cells/µL)	5.50±0.548	4.50±1.378
d) Eosinophil (cells/µL)	1.17±0.752	0.67±0.516
Platelet count (cu. mm^{-1})	243333.30±35950.26	230000.00±34496.38
Haemoglobingm (%)	9.82±0.833	10.53±0.922

Table 3: Effect of NAA on biochemical parameters in mice after intraperitoneal administration of 300 $\mu g\ mice^{-1}\ day^{-1}$

Biochemical Parameters	Group A n=6, $M_1 \pm SD_1$	Group B n=6, $M_2 \pm SD_2$	% change	t_c value	t_s value	Remark
SGPT (UL^{-1})	28.33±1.633	16.17±1.722	-42.92	+9.952	2.57	MI
SGOT (UL^{-1})	44.50±1.871	60.67±3.266	+36.34	-14.590		MD
SALP (UL^{-1})	236.33±3.829	122.50±3.271	-48.17	+108.811		HI
Urea (mmol L^{-1})	19.13±0.393	13.17±1.633	-31.16	+7.637		MI
Serum creatinine (mg dl^{-1})	1.00±0.110	0.88±0.117	-12.00	+1.472		NS
ESR (mm in 1^{st}hr)	6.83±0.753	8.33±0.817	+21.96	-6.708		MD

M_1 and M_2 = Sample mean value, SD_1 and SD_2 = Standard deviations, n = Number of mice, + = Increase, - = Decrease, NS = Not Significant, MD = Moderately decreased, MI = Moderately increased, HI = Highly increased, t_c = Calculated t value, t_s = t value at 5% level of significance, Group A = Control mice, Group B = Experimental mice, IAA = Indoleacetic acid, SGPT = Serum glutamate pyruvate transaminase, SGOT = Serum glutamate oxaloacetate transaminase, SALP = Serum alkaline phospatase, ESR = Erythrocyte sedimentation rate

Table 2 shows hematological profiles that were studied on control group of mice and 14 days of treatment. Each time the value of the parameters in each mouse was changed slightly. However the parameters remained within the normal range. Table 3 shows biochemical parameters of control group and treated groups of mice. However the parameters (SGPT, SGOT, SALP, Urea, Serum creatinine and ESR) remained within the normal range. This

indicates that the compound (NAA) has no adverse effects on liver and kidney function.

After the 14th day of drug treatment the animals of both control and experimental groups were sacrificed and the organs such as liver, kidney, lung, spleen, heart and brain were isolated and histopathological examinations were done. No abnormality was observed between the control and the drug treated mice when the tissue slides were examined under microscope. This indicates that the compound (NAA) has no effects on cellular structure, i. e., the hormone does not cause degeneration of cells of these organs (Table 4).

Table 4: Histopathological studies after treatment with compound (NAA) at a dose level of 300 μg mice^{-1} day^{-1} for 14 consecutive days

Group	Dose (i. p) μg mice^{-1} day^{-1}	Histopathological changes observed					
		Liver	Kidney	Lung	Spleen	Heart	Brain
A	300 μl vehicle	NAD	NAD	NAD	NAD	NAD	NAD
B	300 μg NAA	NAD	NAD	NAD	NAD	NAD	NAD

NAD = No abnormality detected, Group A = Control mice, Group B = Experimental mice, NAA = Naphthalene acetic acid, vehicle = isotonic saline

4. CONCLUSION

As a part of our continuous search for plant hormone naphthalene acetic acid (NAA) and its significant antimicrobial screening was reported. The present work is the continuation of this antimicrobial screening (Morshed *et al.,* 2005). The results of our present study demonstrate that the compound possesses no adverse effect on Swiss albino mice at a dose of 300 μg mice^{-1} day^{-1}. Thus the findings of this investigation and previous investigation would give valuable support to use this plant hormone safely in agricultural purposes and as an external preservative.

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