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Assessment of the Effects of *Scoparia dulcis* Extract on Certain Biochemical Parameters in Male Albino Wistar Rats Exposed to 2,4-D Herbicide

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Abstract

The effects of *Scoparia dulcis* ethanolic extract on certain biochemical parameters in male albino wistar rats exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) is the aspect this research work set to contribute. 2,4-D herbicide is used worldwide in agricultural practices as a selective herbicide. It has been shown that it can produce a wide range of adverse health effects on living organisms. *Scoparia dulcis* (*S. dulcis*) on the other hand is a medicinal plant used widely as a remedy for treating ailments such as kidney stones, hypertension, diabetes and many others. Albino wistar rats were exposed to 2,4-D herbicide daily by force feeding. 19.44 mg/kg body weight of 2,4-D and co-administered of 27.78 mg/kg and 38.89 mg/kg, respectively, body weight of *Scoparia dulcis* ethanolic extract was done consecutively for 28 days. 4 rats in each group were sacrificed and blood tissues were collected for biochemical assays. The Weekly changes in the mean values body weight of the rats exposed to 2,4-D herbicide were relative compared to the control. The results of the experiments showed changes in serum testosterone, luteinizing hormone (LH), total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) levels; an indication of 2,4-D adverse effects on biochemical activities. However, co-administered of the *Scoparia dulcis* extract produced a significant ($p < 0.05$) reversal in the 2,4-D induced hyperlipidemia and reproductive toxicity, which cleared showed an absorption capability of *Scoparia dulcis*. These results therefore suggested that *Scoparia dulcis* plant could be used as an antidote since it proved to have counteractive ability on agrochemical biotoxicity.

Keywords: 2,4-Dichlorophenoxyacetic Acid, Albino Wistar Rat, *Scoparia dulcis*, Testosterone, Luteinizing Hormone, Total Cholesterol, Triglycerides and High-Density Lipoprotein

1. Introduction

Medicinal plants have played significant role since the existence of human beings. In the early days of man, human beings depend on herbs as sources of medicines. For many years, medicinal plants have been utilized to treat health ailments due to the secondary metabolites they contained. Thus medicinal plants are used throughout the world by herbalists depending on the secondary metabolites which are usually responsible for the biological activities of plant species [1,2]. Traditional medicine is

practice worldwide: in China, India, Japan, Pakistan, Sri Lanka, Thailand and others. Thailand herbalists make use of *Caesalpiniaceae*, *Fabaceae*, and *Mimosaceae* (legumes) as medicinal herbs, whereas traditional doctors in Nigeria, South China, Brazil and Paraguay used *Scoparia dulcis* (*S. dulcis*) as medicinal plant [1,3].

Scoparia dulcis also known as sweet-broom or licorice weed that belongs to *Scophulariaceae* family is found mostly in tropical and subtropical regions (Figure 1). Chinese traditional herbalists used *S. dulcis* to treat fever, sore throat, enteritis and malaria, because of the stomachic, diuretic, antitussive, heat clearing and toxic absorbing effect it possess. The pharmacological properties of *S.dulcis* such as hypoglycemic, tumor-fighting and anti viral activities have been supported by scientific research findings [1,3,4]. It has been reported that the plant was historically utilized for treatment of hypertension and diabetes mellitus in Taiwan and India. It is used to treat health problems like tuberculosis, cough, skin rashes, ulcers, cancer and among others. In Nigeria it is utilized for the management of diabetes; surprisingly as a love charm (chewed) to gain favour from people are of affluent [5].

Figure 1. Pictorial of *Scoparia dulcis.*

Furthermore, *S. dulcis* was found to contain high level of antioxidant that defends the body from damage caused by free radicals. The herb is effective in the management of diabetes due to the presence of hypoglycemic component it contained [6,7]. The phytochemical screening has shown that the pharmacological effects of *S. dulcis* is as a result of the chemical substituent such as coumarin, tarpenoids, phenols, flavonoids, saponins, tannins, terpenoids, amino acids and catecholamines. *Scoparia dulcis* was also known to contain other chemical components viz: scoparic acid A and B, scopadulcic acid A and B, scopadulciol and scopadulin, which have been proved to be additional medicinal effect of the plant [8-10].

Though S*coparia dulcis* has been world-wide reported to have pharmacological properties; some of such properties evaluated include antidiabetic, antitumor, antiviral, jaundice, stomach problems, skin diseases, piles and many others. However, there is lack of adequate scientific facts on the assessment of effects of *Scoparia dulcis* ethanol extract on certain hormonal indices and lipid fractions in male albino wistar rats exposed to 2,4-D herbicide.

2. Materials and Methods

All chemicals used are of analytical grade reagents (supplied by either Merck or QualiKems) and were used as supplied.

2.1. Plant Material and Processing

The fresh herb was collected from Kumbur Aga forest in Mbakume Gwer-East, Benue state, Nigeria by Shekinah Ehi Ninga. The plant was identified and authenticated by Joshua Waya, a botanist in the Department of Biological Sciences, Benue State University Makurdi, Nigeria. The plant was thoroughly air-dried for 10 days and pulverized using mortar and pestle. The pulverized sample was

soaked in 90 % absolute ethanol to obtain the ethanolic extract.

2.1.2. Animal and Pre-treatment

Thirty (30) male albino wistar rats were purchased at animal house in College of Health Sciences, Benue State University, Makurdi, Nigeria. The weight of the rats was estimated to weigh between 160 g and 200 g. The animals were randomly distributed into five (5) wooden cages of six (6) rats per group. They were fed with pelletized feeds (Agro feed Ltd, Makurdi) and allowed access to tap water ad libitum. The rats were orally exposed to 2,4-D herbicide and co-administered with the *Scoparia dulcis* ethanolic extract once and daily for 28 days. At the end of the 28 days, 4 rats in each group were sacrificed and the relevant tissues were collected for analysis. Table 1 portrayed group distribution and pre-treatment.

Table 1: Animal Distributions and Treatment

Group	Treatment	No. of rats
1	Control (Distilled water)	6
2	27.78 mg/kg body weight of *S. dulcis* Extract only	6
3	19.44 mg/kg body weight of 2,4-D only	6
4	27.78 mg/kg body weight of *S. dulcis* Extract + 19.44mg/kg body weight of 2,4-D	6
5	38.89 mg/kg body weight of *S. dulcis* Extract + 19.44mg/kg body weight of 2,4-D	6

2.1.3. 2,4-D Herbicide and *S. dulcis* Ethanolic Extract Administration

Five (5) mL syringe needle was used to measure 0.2 mL of 2,4-D herbicide for daily administration per rat for group 3, 4 and 5, respectively. 0.2 mL *Scoparia dulcis* extract was administered to group 2 and 4, whereas 0.3 mL of the extract was administered to group 5. The herbicide was kept away from sunlight or heat throughout the period of experiments.

2.2. Determination of Acute Toxicity (LD_{50}) of 2,4-Dichlorophenoxyacetic Acid

Acute toxicity test of 2,4-D herbicide was based on Iorkes' method (1993).

2.3. Determination of Weight Increase and Growth Rate

The total body weight of each rat was measured using a chemical balance, before and after the experimental period. The mean body weight for each group was weighed as a total body weight. Weight changed was expressed as percentage weight increase (I) and percentage growth rate (II), respectively. Percentage weight increase (% WI) and percentage rate growth (% GR), respectively was calculated from the formula:

I. Percentage weight increase (% WI) was calculated from the formula

$$\% WI = \frac{FBW - IBW}{IBW} \times 100$$

II. Percentage rate growth (% GR) was calculated from the formula

$$\% GR = \frac{FBW - IBW}{Y} \times 100$$

Where; IBW = initial body Weight, FBW = final Body weight, Y = Number of exposure days

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2.4. Specimen Collection and Serum Preparation

The rats from each group were anesthetized with chloroform in an anesthetic chamber for 24 hours after the last administration. The animals were sacrificed using cervical dislocation methods. Blood sample of each rat was obtained by cardiac puncture with the aid of 2 mL syringe and needle. The blood samples were collected into separate heparinized rostock plain vacuum tube. The samples were centrifuged at 2000 rpm for 20 minutes. The serum obtained was collected into a dry sample bottles and kept in refrigerator prior for biochemical assay.

2.4.1. Estimation of Serum Testosterone

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 25 µL of standards, specimen and control was added into a micro plate well. 50 µL of rabbit anti-testosterone was added into the well followed by 100 µL of testosterone-horseradish peroxidase conjugate reagent. The mixture was thoroughly mixed for 30 seconds and incubated at 37 ^{o}C for 90 minutes. The incubated mixture was removed by flicking the plate contents into a waste container. The microtiter well was rinsed 5 times with distilled water and dabbed on a paper towel to remove all residual water droplets. 100 µL of 3,3', 5,5'-tetramethylbenzidine (TMB) substrate was added to the well and mixed gently for 10 seconds. It was incubated at ambient temperature for 20 minutes. The reaction was stopped by adding 100 µL of stop solution to the well and mixed gently for 30 seconds. The colour was observed to change completely from blue to yellow and the absorbance was read at 450 nm with a microtiter well reader within 15 minutes.

2.4.2. Estimation of Serum Luteinizing Hormone (LH)

The method employed was microwell immunoassay (ELISA) using analytical grade reagents. 50 uL of standards, specimen and control was added into a microplate well. 100 µL of Testosterone-Horseradish Peroxidase conjugate was added into the well and thoroughly mixed for 30 seconds. It was then incubated at 22 ^{o}C for 60 minutes. The well was washed 5 times with distilled water. 100 µL of TMB substrate was added to the well and mixed gently for 5 seconds. It was then incubated at ambient temperature for 20 minutes. The reaction was stopped by adding 100 µL of stop solution to the well and mixed gently for 30 seconds. The reading was taken at 450 nm with a microtiter well reader within 15 minutes.

2.4.3. Serum Lipid Assay

The serum total cholesterol was determined by the enzymatic colorimetric method, while the serum high density lipoprotein was determined by the dextran sulphate magnesium (II) precipitation method; glycerol phosphate oxidase enzymatic method was used to determine the serum triglyceride.

2.5. Statistical Analysis

Results were presented as mean standard deviation (SD) and statistically analyzed using one-way analysis of variance (ANOVA) with SPSS (statistical package for social science) window statistical software program (version 29.0). Student‘t’ test was used for pair-wise comparison and difference were considered significant at confidence level of $p < 0.05$ (95 %).

3. Results and Discussion

3.1. Acute Toxicity Test of *Scoparia dulcis* Ethanolic Extract

The results of acute toxicity test of *Scoparia dulcis* ethanolic extract were presented in Table 2. Acute toxicity results of *Scoparia dulcis* ethanolic extract indicated that the extract did not produced noticeable change in behaviour of rats up to the dose of 500 – 2500 mg/kg after 48 hours.

Table 2: Acute Toxicity Test (LD_{50}) of *scoparia dulcis* on albino wistar rats

Phase	Dose (mg/kg)	Number of Death
1	500	0/3
	1000	0/3
	2500	0/3
2	3000	1/3
	3500	2/3
	4000	3/3
	Tween-80	0/3

$$LD_{50} = \sqrt{D_0 \times D_{100}} = \sqrt{2500 \times 3000} = 2738.61 \text{ mg/kg}$$

At dosage of 3000 mg/kg, 3500 mg/kg and 4000 mg/kg, the number of death recorded was one, two and three, respectively; indicating that at those doses the toxicity is lethal (Table 2). The calculated LD_{50} for the extract was 2738.61 mg/kg of body weight of rats. This implies that the extract is practically nontoxic at doses from 500 – 2500 mg/kg. This finding is in accordance with the research conducted by Moniruzzaman and co-workers (2015), on sedative and hypnotic activity of *Scoparia dulcis* linn extract on mice [11]. Another research work carried out by Zikang and his co-worker (2021), is clearly in support to our findings [3]. Furthermore, Tween-80 mg/kg is the dosage at which *Scoparia dulcis* effect is placebo; meaning the treatment that seems to be real but without pharmacological actions or benefits. In the real sense placebo is usually a treatment or drug undergoing clinical trials to avoid experimenter's bias [13,14].

3.2. Body Weight of Albino Wistar Rats

The results of weekly change in body weight of rats exposed to 2,4-D herbicide for four weeks and co-administered with *Scoparia dulcis* ethanolic extract were presented in Table 3. The mean values of body weight change of male albino wistar rats at the end of 28 days has shown that there is a significant ($p < 0.05$) difference in group 2 and 3 compared to the values for control.

Table 3: Change in body weight of rats exposed to 2,4-D herbicide and treated with *scoparia dulcis* ethanolic extract in 28 days.

Group	Week 1	Week 2	Week 3	Week 4
1	160.40± 1.10	195.35 ± 0.01	226.06 ± 0.25	248.26 ± 1.31
2	165.40 ± 1.25	189.00 ± 1.25	206.85 ± 1.41	267.93 ± 0.62
3	165.78 ± 1.18	203.60 ± 1.25	215.95 ± 1.83	226.75 ± 0.11
4	201.58 ± 0.47	220.63 ± 1.67	234.95 ± 1.47	241.85 ± 0.51
5	162.48 ± 1.51	214.04 ± 1.93	221.56 ± 2.50	246.00 ± 2.00

Values are expressed as mean ± S.D, n = 4 and $p < 0.05$

The noticeable weight change in group 2 and 3 may be attributed to alteration in the physiological

activities. We also observed that rats (group 3) under 2,4-D herbicide exposure have shown severe symptoms of toxicity in form of ataxia and muscular weakness. This implies that the endocrine system responsible for regulating various physiological processes is disrupted [12]. However, co-administered of *Scopoaria dulcis* in group 4 and 5 has shown body weight increment more than group 3 with no significant ($p < 0.05$) difference from the control. This means oral administration of *S. dulcis* ethanolic extract has significant remodeling of biological activities; as result of week four (4) has portrayed. The finding has agreed with the literature which established that *S. dulcis* herb has the capability of heat clearing and toxic absorbing effect [3].

3.3. Serum Testosterone, Luteinizing Hormone and Lipid Assay

The indicators for 2,4-D herbicide toxicity on reproductive and cardiovascular systems were observed on the serum testosterone, LH, TC, TG and HDL, and the results were presented in Table 4.

Table 4: Results of serum testosterone, LH hormonal and lipid profile of rats exposed to 2,4-D and co-administered with the extract

Group	Testosterone (nS/mL)	LH (IU/L)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)
1	0.8 ± 1.31	0.7 ± 1.01	2.01 ± 1.01	1.41 ± 0.02	1.54 ± 0.33
2	2.0 ± 1.10	0.5 ± 1.12	1.99 ± 0.05	1.43 ± 0.08	1.01 ± 0.21
3	0.3 ± 1.32	0.1 ± 1.21	2.71 ± 0.06	1.83 ± 0.04	0.45 ± 0.02
4	1.4 ± 1.41	0.4 ± 0.51	2.35 ± 0.20	1.37 ± 0.03	1.27 ± 0.01
5	1.7 ± 1.22	0.6 ± 1.20	1.86 ± 0.10	1.41 ± 0.17	1.03 ± 0.02

Values are expressed as mean ± S.D, n = 4 and $p < 0.05$

Rats (group 3) exposed to 2,4-D herbicide showed significant decreased in the serum levels of testosterone and LH values compared to values recorded for the control (group 1). Reduction in the serum testosterone and LH clearly demonstrated the possible inhibitory effect of 2,4-D herbicide on biosynthesis of testosterone and gonadotropin-releasing hormone that induces pituitary gland and secrete LH [15]. Table 4 has shown an increased in TC and TG but low HDL cholesterol compared to control group. Increased in TC and TG clearly showed that 2,4-D herbicide has an adverse effect on lipid metabolism and possible cardiotoxicity. The increased could be possibly attributed to effects of the herbicide on permeability of the liver membrane and blockage of liver bile ducts causing a cessation or reduction of cholesterol secretion into duodenum [16,17]. Besides, HDL cholesterol which aids the removal of cholesterol from the bloodstream by transporting it back to the liver for oxidation and elimination; hence low HDL could linked to high TC and TG, respectively [18]. Meanwhile low HDL level noticed in group 3 clearly suggested alteration in endocrine system that linked to lipid and lipoprotein metabolic disruptions [19]. Our finding is in accordance with the studies conducted by Navdep Kaur and co-workers on animals exposed to organophosphate insecticides at low-dosage. Their findings demonstrate that exposure to organophosphate insecticide prevent insulin-induced suppression of free fatty acid release from adipose cells and increased blood glucose; it increased plasma triglycerides and LDL cholesterol but decreased HDL cholesterol and caused oxidative stress-induced apoptosis of pancreatic beta-cells [20]. Our result also agreed with Wei Wang and his team researchers, which also showed that endocrine disruption, reproductive disorders and genetic alterations is linked to 2,4-D herbicide exposure [21]. Our research study has also agreed with the literature which established that, a rise in testosterone concentration in male rats has been linked to a low HDL cholesterol concentration [22]. The results of Table 4 also demonstrated that group 4 and 5 co-administered with *Scoparia dulcis* significantly remodeled TC, TG and HDL cholesterols. The reversal in serum lipid parameters in group 4 and 5 were close to values of the control; is an indication of *Scoparia dulcis*' ability to reduce or absorb adverse

effect of toxins [3].

4. Conclusion

The study has shown that the body weights of male albino wistar rats exposed to 2,4-dichlorophenoxyacetic acid were weekly changed compared to the control; due to mark down physiological activities indicating toxicity or enzymatic metabolic dysfunction effect of 2,4-D herbicide. Acute toxicity studies of the plant extract indicated that the plant is safe at dosage up to 2500 mg/kg body weight of test models. The results of the wistar rats experiments showed that 28 days of oral exposed to 2,4-D herbicide and concomitant administered of *Scoparia dulcis* extract have profound remodeling effect on physiological and biochemical functions as indicated in the reversal of testosterone, LH,TC, TG and HDL values relative compared to group 3 exposed to 2,4-D herbicide. The results of the study also showed that *Scoparia dulcis* ethanolic extract contains some active phytochemical ingredients that protect against 2,4-D herbicide mediated oxidative injury in male albino wistar rats. Therefore, *Scoparia dulcis* is confirmed as an antidote to counteract the effects of agrochemical biotoxicity on certain biochemical parameters.

Abbreviations

ANOVA: Analysis of Variance
FBW: Final Body Weight
GR: Growth Rate
HDL: High-Density Lipoprotein
IBW: Initial Body Weight
LD_{50}: Lethal Dose
LDL: Low- Density Lipoprotein
LH: Luteinizing Hormone
TMB: Tetramethylbenzidine
TC: Total Cholesterol
TG: Triglycerides
WI: Weight Increase

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Conflict of Interests

The authors declare that they have no competing interests regarding the publication of this paper.

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