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Effects of Extract of *Spilanthes uliginosa* (SW) on Total Protein, Albumin, Total and Conjugated Bilirubin in Ethylene Glycol Exposed Albino Rats

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ABSTRACT

Effects of leaf extract of *Spilanthes uliginosa* (SW) on total protein, albumin, total and conjugated bilirubin levels in ethylene glycol exposed albino rats were studied. The animals were divided into 3 groups namely; A, B and C and groups A and B were subdivided into 2; A_I, A_2, B_1 and B_2. All the groups and subgroups contained 6 albino rats each and were given water and food *ad libitum*. Groups A_1 and B_1 with A_2 and B_2 were treated with 1.85 mg kg^{-1} and 3.70 mg kg^{-1} of 90% ethylene glycol intraperitoneally once, respectively. Groups B_1 and B_2 were also given 1 mL of the plant extract, respectively by oral incubation for 21 consecutive days while group C received only the water and food. The average body weights of the treated groups decreased significantly at $p<0.05$ compared to control. The result showed no significant change at $p<0.05$ in total protein levels and significant decrease in albumin, total and direct bilirubin levels compare to control. The result obtained suggests toxicity of ethylene glycol and the ability of the plant extract to affect the levels of the analyzed parameters.

Key words: Liver, pollutant, hepatotoxicity, tradomedicine

INTRODUCTION

Traditional medicine refers to health practices, approaches, knowledge and belief incorporating plants, animals and minerals based medicine, spiritual therapies, manual, techniques and exercises applied either singularly or in combination to treat, diagnose and prevent illness or maintain well being (Sunday *et al*., 2014a; WHO., 2005). Traditional African Medicine (TAM) is our socio-economic and socio-cultural heritage, surviving over 80% of the African population (Sunday *et al*., 2014b; Elujoba *et al*., 2005). Although, it has come a very long way from times of our ancestors but little or no significance progress on its development and utilization has taken place due to colonial suppression on our land, foreign religions in particular, absolute lack of patriotism and political will of our government and on the other hand, the care-free attitudes of most African medical scientists of all categories (Elujoba, 2005). Traditional African medicine is based on the use of plants and plant extracts, hence the term herbalism being used to describe it. A common misconception about herbalism and the use of natural products in general is that natural equals safe (Elujoba *et al*., 2005).

Medicinal plants are plants which one or more of their organ contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Fabricant and Farnsworth, 2001; WHO., 2003). A number of plants have being used in

traditional medicine for many years. Plants have developed the ability to synthesize chemical substances that help them defend themselves against attack from a wide variety of predators such as insects, fungi and herbivorous mammals (Lai and Roy, 2004).

Spilanthes uliginosa (Sw) is an annual herb that belongs to a family asteraceae. It is a herbaceous plant that grows up to 30 cm high with opposite leaves, terminal, stalked and flower heads. It sends up gold and red flower inflorescences and its flowers are small, yellow and solitary on terminal peduncles. It is found mainly in a swampy and damp sites and roadsides. The plant is known as toothache plant because of the effect on numbing toothache. It stimulates the salivary glands to produce more saliva and may serve as a simple tonic for healthy gums and oral flora. It is an effective antiparasitic and has been used as a native remedy against malaria (Uraku *et al*., 2015). Thus, it has natural antibacterial and antifungal actions as well as boosts the production of leukocyte and antiviral interferon suggesting that the plant may be useful in enhancing immune system function and it is often use topically to treat bacterial infection of the skin, wounds and fungal infections like ringworm (Uraku and Ogbanshi, 2015). In Cameroun, the plant is used to cure fly bites and promote rapid sore healing while in Ivory Coast, leaf extract mixed with little tobacco and salt is taken to stave off a threatening abortion.

Ethylene glycol (ethane-1, 2-diol) is an organic compound widely used as an automotive antifreeze and precursor to polymers. Ethylene glycol in its pure form is colourless, odourless, syrupy and sweet tasting liquid. Ethylene glycol itself is not toxic but when it gets into the human system, it oxidized to glycotic acid, which is in turn oxidized to oxalic acid, that is toxic (Bobbitt *et al*., 1986).

The use of ethylene glycol as an antifreezer has made it a public health hazard and one of the most common pollutants of our environment. As a result of this, a good percentage of the populance come in contact with it daily, especially those that live near places where refrigerators are repaired. An overdose of ethylene glycol can damage the brain, central nervous system, heart, lung, liver and kidney (ATSDR., 1997). The effects may be severe enough to cause profound shock, organ failure and death (Melnick, 2004; Sieber *et al*., 2000).

This work is aimed at determining the effects of *Spilanthes uliginosa* (Sw) on total protein albumin, total and conjugated bilirubin in ethylene glycol exposed albino rats.

MATERIALS AND METHODS

Collection and preparation of *Spilanthes uliginosa* (Sw) leaf extract: The plant of *Spilanthes uliginosa* (Sw) was collected from Ogboji-Agoutu Ezzagu in Inyaba Development Centre of Ebonyi State, Nigeria. The plant was identified by Dr (Mrs) Nnamani, K. of the Department of Applied Biology of Ebonyi State University, Abakaliki. The leaf of the plant was removed from plant stalk, rinsed with clean water and shade dried to a constant weight. The dried plant sample was ground to fine powder with grinding machine, packaged in glass jars and stored at 4°C until analysis.

Extraction of plant materials: Exactly 144 g of powder samples of *Spilanthes uliginosa* was soaked at 35°C in 400 mL of distilled water and stood for 24 h. The preparation was filtered with a clean white handkerchief into a graduated beaker and exposed to mild heat at 40°C in water bath to remove the water until solid crude extracts were obtained. The obtained crude extracts were dissolved in normal saline and administered to experimental animals at different concentrations based on body weights.

Experimental animals: Thirty albino rats aged 2 months weighing 150-250 g of both sexes were obtained from Chris King Animal Farm of Nnamdi Azikiwe University Awka, Anambra State and transferred to Animal House of Department of Biochemistry, Ebonyi State University, Abakalki. The animals were housed in metal cages under controlled conditions and acclimatized for 7 days under standard environment conditions and fed *ad-libitum* on their normal diets.

Experimental design: Thirty albino rats were used for the study. The animals were grouped into 5 groups namely; A1, A2, B1, B2 and C of 6 albino rats each. Groups A_1 and B_1 with A_2 and B_2 were treated with 1.85 mg kg^{-1} and 3.70 mg kg^{-1} of 90% ethylene glycol intraperitoneally once, respectively. Groups B_1 and B_2 were also given 1 mL kg^{-1} of the plant extract, respectively by oral incubation for 21 consecutive days. Group C received only the water and food and such served as negative control. All groups were given water and fed *ad libitum*. On the 20 sec day, the albino rats were starved overnight, sacrificed and blood collected for various biochemical analysis.

Preparation of serum: Fasting blood was collected from each animal into a sterile, plain tube, and then it was centrifuged at 1,200×g for 5 min at room temperature to obtain the serum sample, which was stored frozen at -20°C until analyzed.

Determination of selected biochemical parameters: Determination of total protein, albumin, total and conjugated bilirubin were done according to the method described by Tietz (2000) and modified by Uraku (2015).

Statistical analysis: The results obtained were expressed as Mean±S.D. of 6 rats in each group. All the average body weights were subjected to statistical analysis using ANOVA. Differences between means were regarded significant at $p<0.05$.

RESULTS

Results of the effects of extract on the mean body weight of animals: The result of mean body weight of animals treated with leaf extract of *Spilanthes uliginosa* (Sw) are showed in Table 1. The mean body weight of the animals in group C (negative control) showed a significant weight gain at $p<0.05$ on the 20 sec day while other groups; A_1, A_2, B_1 and B_2 showed significant weight loss at $p<0.05$ on the last day.

Results of the effect of extract on serum total protein, albumin, total and conjugated bilirubin levels of the animals: The results of total protein, albumin, total and conjugated bilirubin levels are shown in Table 2. The results showed that there were significant reduction ($p<0.05$) in the levels of total protein and albumin in groups A_1 and A_2 (positive control) compare to group C (negative control) while the levels of total protein, albumin in extract treated groups showed a significant increase compared to positive control. There was a significant increase ($p>0.05$) in levels of total bilirubin in group B_1 and B_2 compare to group C with only a significant

Table 1: Effects of the extract on the body weight of animals

Weight (g)	C	A1	A2	B1	B2
Initial weight	214.00±21.68^a	166.67±30.55^b	196.67±20.82^b	193.33±25.17^b	206.67±30.55^b
Final weight	253.58±18.58^b	129.33±30.11^a	143.67±15.82^a	133.00±31.58^a	146.00±24.56^a

Values are expressed as Mean±SD, n = 6 animals per group. Values in same column with different superscripts are significantly different from each other at $p<0.05$, C: Negative control group, A_1, A_2: Albumin positive control group, B_1, B_2: Bilirubin groups

Table 2: Effects of the extract on serum total protein, albumin, total and conjugated bilirubin levels of the animals

Groups	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Total bilirubin (μmol L^{-1})	Conjugated bilirubin (μmol L^{-1})
C	6.05±0.07^{b}	4.90±0.14^{c}	20.05±0.35^{c}	13.60±0.14^{d}
A1	5.60±0.00^{a}	3.10±0.14^{b}	26.15±0.21^{e}	17.90±0.00^{e}
A2	5.80±0.14^{a}	2.55±0.21^{a}	24.50±0.00^{d}	11.80±0.14^{c}
B1	6.80±0.14^{c}	4.60±0.14^{c}	16.75±0.21^{b}	9.10±0.42^{b}
B2	6.15±0.21^{b}	4.80±0.14^{c}	13.15±0.07^{a}	7.95±0.21^{a}

Values are expressed as Mean±SD, n = 6 animals per group. Values in same column with different superscripts are significantly different from each other at $p<0.05$, C: Negative control group, A_1, A_2: Albumin positive control group, B_1B_2: Bilirubin group

increase ($p>0.05$) in levels of conjugated bilirubin in group A_1 compare to group C while the extract treated groups showed reduction compared to positive control.

DISCUSSION

In this study, the effects of aqueous extract of *Spilanthes uliginosa* (Sw) on some selected biochemical parameters of albino rats exposed to ethylene glycol were investigated. The results of the average b.wt., showed a significant increase in the b.wt., of the unexposed animals (negative control group) compare to ethylene glycol exposed untreated animals (positive control) and experimental groups. The general observed decrease in average b.wt., of the animals could be attributed to stress and decrease in food and water intake. This result is in consonance with the reported of Haruna *et al*. (2013) and Uraku *et al*. (2015). Also, this result agreed with the report of Melnick (2004) who reported that the administration of ethylene glycol reduced growth in animals, increased kidney weight and alteration in serum clinical chemistry.

The animals treated with the extract showed a general increased level of total protein and albumin when compared to positive control and this observation indicated absolute protection of integrity of the liver. Reduction in serum total protein and albumin levels in the ethylene glycol exposed animals may have resulted from hepatic collapse in view of the fact that liver is the major source of most serum protein and their production are useful marker of normal task (Uraku, 2015). However, production of proteins is not only affected in the liver disease but also by dietary position, hormonal balance and osmotic pressure (Orhue *et al*., 2005). The observed increase in total protein levels in the treated animals may suggest that the plant extracts contained essential phytocompounds which help to restore the damaged liver. Apart from being a useful indicator of the integrity of glomerular membrane, albumin also is vital in determining the cruelty of disease (Adedapo *et al*., 2005). This result is in agreement with the report of the other researchers (Akanbi, 2013).

The increase in total and conjugated bilirubin levels observed in ethylene glycol exposed untreated group might be a sign of either over production of bilirubin as a result of extreme breakdown of hemoglobin or an impairment of the bilirubin excretion mechanism. Similarly, elevated total bilirubin could also be an end result of strict defects in transportation of bilirubin. Thus, the elevated levels of total bilirubin reported in this research could possibly suggest mild haemolysis and consequently may result in jaundice, as the disease state that occurs in venomous or contagious liver diseases. In the same way, the elevation of conjugated bilirubin levels shows that the conjugation process is intact but however, points to the inability to effectively excrete conjugated bilirubin. This may be attributed to influx of bilirubin into the blood stream as a result of biliary obstruction and abnormal function of the liver. Also, the increase in the total and conjugated bilirubin may not only indicate obstruction of the biliary duct but also

suggest an effect on the normal excretory function of the liver (Uraku, 2015). The significant reduction in the level of bilirubin in the serum of the experimental animals suggests hepatoprotective potential of the extracts against ethylene glycol liver damage. This study concluded that ethylene glycol is hepatotoxic while the extract of *Spilanthes uliginosa* (Sw) exact hepatoprotective potential.

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