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# PHYTOCHEMICAL SCREENING, ACUTE TOXICITY AND ANTI-INFLAMMATORY EFFECT OF *CHRYSOPHYLLUM WELWITSCHII* (ENGL.) LEAF EXTRACT IN RAT

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## ABSTRACT

The aqueous and alcoholic extracts contained flavonoids, tannins, alkaloids, sterols and terpenes, anthocyanins, saponins and cardiotonic glycosides. The water extract from the leaves of *Chrysophyllum welwitschii* (CHRYSOW) was evaluated for its acute toxicity in female rats. For the study of acute toxicity, oral doses of 300, 2000 and 5000 mg/kg of body weight was administered orally in rats. The results showed no signs of toxicity such as general behavior change; no morbidity and mortality. Both extracts of CHRYSOW were hardly toxic and their LD50 was higher than 5000mg/kg bw. The aqueous and ethanolic extracts of dried leaves of *Chrysophyllum welwitschii* Engl. was tested for anti-inflammatory action by carrageenan induced paw edema. The anti-inflammatory degree observed with aqueous extract revealed a higher anti-inflammatory activity than ethanolic extract. The aqueous extract in doses of 250 and 500 mg/kg of body weight showed 43.94 and 56.31% inhibition of paw edema, respectively at the end of 5 hours. The dose of 500 mg/kg of body weight was compared to that of standard dichlofenac sodium (56.92%). The anti-inflammatory activity may be due to presence of saponins, sterols and flavonoids in leaf extract.

**KEYWORDS**: *Chrysophyllum welwitschii*, Extracts, Anti-inflammatory effect, Acute toxicity, LD50.

## INTRODUCTION

Traditional medicine is basically with the use of medicinal plants in decoction, maceration and concoction preparations. Because of their values in treatment of diseases by choice in many developing countries, their worldwide use is lagging because of the lack of scientific bases to support their safety use. The affordability, reliability, availability and low toxicity of medicinal plants in therapeutic used has made then popular and acceptable by all religions, for implementation in medical health care all over the world (Akharaiyi F.C. 2011). Each plant is used to treat some pathologies. Among the medicine plants, we have *Chrysophyllum welwitschii* Engl. (Sapotaceae).

*Chrysophyllum welwitschii* is a woody scandent liana of scrub vegetation, height up to 20 m, with fluted trunk and grey bark. The bark contains white latex. Young shoots, buds and young petioles with ferruginous pubescence. Leaf is wide and thinly coriaceous and the apex is tapered. Flowers are very small and greenish, clustered in axils of current leaves or at old nodes. Fruit is shortly stalked and yellow when it's ripe. It is found especially in tropical vegetation that growths across the littoral of Liberia to Democratic Republic of Congo. This specimen generally exists in tropical Africa.

Traditionally, leaves of CHRYSOW were used to cure naso-pharyngeal affections, diarrhoea, dysentery and cough-medicine. Leaves and bark were used in treatment of arthritis and rheumatism but also sedative. Thus, the present study is based on phytochemical screening, acute toxicity and anti-inflammatory effect of CHRYSOW leaf extract in albinos' rat.

## MATERIALS AND METHODS

**Plant Material:** The leaves of *Chrysophyllum welwitschii* Engl, were collected from the southern region of Côte d'Ivoire. The specimen voucher number 10 874 was identified and authentified by Professor **Aké ASSI**, National Floristic Center, for future reference.

**Preparation of Extract:** The leaves of CHRYSOW were dried for three weeks and then ground to powder with an electric grinder (IKA Labotechnik). The extraction of secondary metabolites from the powder CHRYSOW was performed using solvents of increasing polarity. 200 grams of leaves powder of *C. welwitschii* was macerated under vibrator for 24 hours in 2 liters of hexane. The macerated obtained was then filtered twice on white cotton and once on Whatman filter paper N°3. Then the vegetable residue was dried at 37°C in an oven for 2 hours and re-extracted under the same conditions with ethyl acetate. These operations continued with ethanol and water. The filtrate obtained in 70% ethanol

was evaporated to dryness at reduced pressure at a temperature of 40°C using a rotary evaporator type Buchi 161 Water Bath. The aqueous filtrate was concentrated by evaporation at reduced pressure at a temperature of 50°C using a type Med Center Venticell.

**Experimental Animals:** The apparently healthy Wistar rats of female sex weighing about 110 -170 g, were purchased from Department of Toxicology, Unit of research and formation to Biologic and Pharmaceutical Sciences, University of Felix Houphouet Boigny, Côte d'Ivoire. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets (Vital feeds) and water *ad libitum*. They were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals.

**Acute toxicity study**

***Animal treatment:*** Albino female rats weighing 110-170g were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines (OECD, 2001). The animals were divided in 7 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The control group received distilled water vehicle. The groups 2, 3 and 4 received respectively aqueous extract of CHRYSOW in the doses of 300, 2000 and 5000 mg/kg of body weight orally. The groups 5, 6 and 7 received respectively alcohol extract of CHRYSOW in the doses of 300, 2000 and 5000 mg/kg body weight orally. The animals were observed for 5 minutes, every 30 minutes till 2 hours and then at 4, 8 and 24 hours after treatment. They were further observed daily for 7 days. After 14 days the rats were kept fasted for 16-18 hours and weighted.

***Organs weight:*** Euthanization of animals was done by exsanguination with anaesthesia on 14 days after treatment. The liver, kidneys, lung and heart were quickly removed soon after the animals' death, cleaned with saline and their wet weight was determined.

**Anti-inflammatory activity carrageenan induced hind paw oedema:** Carrageenan-induced rat paw oo is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The animals were fasted overnight before experimentation, but had been allowed free access to water. Albinos rats of either sex weighing 110-170grams were divided into 6 groups of 6 animals each. The dosage of the drugs administered to the different groups was as follows

Group I-Control (distilled water 2mL/100g bw), Groups-II and III- aqueous extract of *C. welwitschii* (250 mg/kg and 500 mg/kg bw respectively), Groups-IV and V - ethanol extract of *C. welwitschii* (250 mg/kg and 500 mg/kg bw respectively), Group VI- diclofenac sodium (25 mg/kg bw). All the drugs were administered orally. Diclofenac sodium served as the reference standard of anti-inflammatory drug. After 1 hour of the administration of drugs, 0.1 mL of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the right hind paw of the rat. The paw volumes of the rats were measured by the Digital Calliper (METRICA) at the end of 0mn, 1hr, 2 hr, 3hr, 4hr, 5hr. The percentage increase in paw oedema of the treated groups was compared with them and the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. The percentage inhibition was calculated using the following formula:

% Inhibition of oedema = (1-Vt/Vc) x100 (Saba et al., 2007; Charles et al., 2006). Where Vt was paw volume in test group animals and Vc was paw volume in control group animals.

**Phytochemical screening:** Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant under study were carried with extracts prepared using the standard procedures (Bagre et al., 2007, Békro et al., 2007; Hegnauer, 1973).

Briefly, ***sterols and polyterpenes*** were detected by the reaction of Liebermann-Buchard. 5 mL of each of the two extracts were evaporated on a sand bath. The residue was dissolved in 1 mL of hot acetic anhydride; we added 0.5 mL of concentrated sulphuric acid. The appearance at the interphase of a purple ring, turning blue to green indicated a positive reaction.

***Test for alkaloids*** were detected by Dragendorff and Bouchardat reagents. 6 mL of each plant extract of the two extracts were evaporated on a sand bath. The residue of each extract is taken up in 6 mL of alcohol (60°) and the alcoholic solution thus obtained was distributed in two tubes test. In the first tubes were added 2 drops Dragendorff reagents (aqueous solution of iodo-bismuth potassium). The appearance of a precipitate or an orange color indicates the presence of alkaloids. In the second tubes, are added 2 drops of Bouchardat reagent (aqueous solution of iodine-iodide). The appearance of a reddish-brown color indicates their presence.

***Identification of phenols.*** 2 mL of each extract was added a drop of alcohol solution of ferric chloride (2%). The appearance of a blackish-blue or darker or lighter green color indicated the presence of phenolic compounds.

***Test for tannins.***

*Detection of Catechin tannins.* 5 mL of each extract are evaporated. The dry residue was added 15 mL of reagent

Stiasny (10 mL of 40% formalin added 5 mL of hydrochloric acid (HCl) concentrate). The mixture was kept in a water bath at 80°C for 30 minutes. It is cooled under running water. The observation of large flake precipitate characterizes catechin tannins.

***Detection of Gallic tannins.*** The solution containing the flakes is filtered and the filtrate collected is then saturated with sodium acetate. To the mixture, 3 drops of ferric chloride 2%. The appearance of an intense black-blue color indicates the presence of gallic tannins.

***Identification of flavonoids.*** 2 mL of each plant extract are evaporated in a capsule, without carbonizing the residue. After cooling, the residue is taken up 5 mL of hydrochloric alcohol (obtained by mixing 10 mL of 96° ethanol,10 mL of distilled water and 10 mL of concentrated hydrochloric acid) diluted 2 times in a test tubes. It is added 2 to 3 magnesium shavings (exotherm). This gives a pink-orange or purple. The addition of 3 drops isoamyl alcohol intensifies a pink-orange or purple, indicating the presence of flavonoids. The control is performed with the alcoholic solution of quercetin.

***Test for saponosides.*** 10 mL of each plant extract were put into a test tube of 160 mm height and 16 mm in diameter. This was stirred vigorously test tube for 10 seconds. The foam height is measured after 10 minutes resting. A height of more than 1 cm of foam, indicates the presence of saponins. The saponins may also be demonstrated by the persistence of the foam.

***Identification of quinones.*** Identification of quinones was used Borntraeger reagent (ammonia diluted 2 times) that allows the detection quinone substances. Evaporated to dryness in a capsule 2 mL of each plant extract. The residue was mixed in 5 mL of hydrochloric acid (HCl) diluted 1/5. The solution is in a boiling water bath for half an hour in a test tube. Then cooled in a cold water stream and the hydrolyzate is extracted with 20 mL of chloroform in a test tube. The chloroform phase was collected in a test tube and mixed with 1/2 mL of dilute ammonia 2 times. The appearance of a color ranging from red to purple indicates the presence of quinones.

***Identification of polyterpenes and sterols.*** Liebermann the reagent was used for this demonstration. 5 mL of plant extract were dried under rotary evaporator. The residue was dissolved hot in 1 mL of acetic anhydride and collected in a test tube. Along the tube, allowed to flow with a 1/2 mL of concentrated sulphuric acid. The appearance at the interphase with a purple or purple ring, turning blue to green; indicating the presence of polyterpenes and sterols.

***Identification of coumarins.*** The test solution was obtained after maceration for 12 hours to 1/2 g of the previously obtained lyophilizate in 10 mL diclochlorométhane. The mixture was then filtered and the volume was made up to 10 mL. We have taken 1 mL of the filtrate above then evaporated to the solvent dryness and added to the residue 400 µL of hot water. The resulting mixture was then partitioned in two test tubes. In one we added 100 µl of $NH_4OH$ (25%). We have mixed and observed fluorescence under UV at 366 nm. The presence of coumarins is indicated by fluorescence in the test tube.

***Identification of cardiac glycosides.*** We introduced 2 g of the lyophilizate in a test tube and added 10 mL of 60% ethanol and 5 mL of a neutral solution of acetate lead 10%. The whole was heated to boiling and filtered after cooling. We waved the filtrate with 10 mL of chloroform ($CHCl_3$) avoiding emulsion formation. After decanting, the chloroform phase was removed with a pipette and then shared between 2 test tubes and evaporated to dryness over a boiling water bath. The residues were taken up with a 1/2 mL of isopropanol. In each of the two tubes, respectively we added 1.5 mL of Baljet reagent and 1.5 mL of reagent Kedde. Finally, we have introduced in each tube 2mL KOH 2% drops in ethanol at 80°. After 3 minutes of contact on a hot plate in the presence of cardiac glycosides, the following colorations was developed: Tube 1 (Baljet): orange coloration; Tube 2 (Kedde): purplish-red coloration.

***Test for anthocyanins.*** 2 mL of each extract was evaporated. After cooling, the residue was added 5 mL of hydrochloric acid and 1 mL of isoamyl alcohol. The solution was heated for 15 minutes in a water bath at 80°C for 30 minutes. The appearance of a cherry-red or purple characterizes the presence of anthocyanins.

**Statistical analysis**

The results were expressed as Mean ± SEM and statistically analysed by one-way analysis of variance (ANOVA) followed by Turkey's test, with level of significance set at $p<0.05$.

## RESULTS

**Phytochemical Screening**

Preliminary phytochemical investigation of aqueous and ethanolic extracts of CHRYSOW showed the presence of steroids, alkaloids, flavonoids, phenolic compounds, anthocyanins, sterols and terpenes, cardiotonic glycosides. But no presence of quinones, coumarins and tannins was observed. Aqueous extract contains saponins (**Table 1**).

**Table 1: Chemical composition of total extracts of leaves of *C. welwitschii***

| secondary metabolites | Extracts | | |
|---|---|---|---|
| | | Aqueous extract | alcohol extract |
| Alkaloïds | Dragendorff | ++ | ++ |
| | Bouchardat | ++ | ++ |
| Phenolic compounds | | ++ | ++ |
| Flavonoids | | ++ | ++ |
| Tannins | catechic | - | - |
| | gallic | - | - |
| Sterols et polyterpenes | | + | ++ |
| Anthocyanins | | + | ++ |
| Saponosides | | ++ | - |
| Cardiotonic glycosides | | ++ | ++ |
| Coumarins | | - | - |
| Quinones | | - | - |

(+) = presence; (++) : high presence; (-) : absence

**Acute toxicity Study**

***Animal behaviour***

Oral administration doses of total aqueous and ethanolic extracts of CHRYSOW, between 300 and 5000 mg/kg body weight did not cause mortality and morbidity in female rats for 14 days during observation time. However, the animals allowed us to observe the following events: after short agitation, rats regroup and trend to immobilize. In addition, we observed accelerated respiratory rate (tachypnea) and difficult locomotion for dose 5000mg/kg of body weight.

***Body and organ weights:*** The body weight analysis, assessed for 14 days of observation showed that CHRYSOW did not induce significant changes in female animals. No significant differences existed in the relative weights of the isolated organs of aqueous and ethanol treated and control animals (**table 2**).

**Table 2: aqueous and alcoholic extracts effects of CHRYSOW on body and internal organs of female rats**

| Extract | Day 0 Initial body weight (g) | Day 14 Final body weight (g) | Liver weight (g) | Heart weight(g) | Lungs weight (g) | Kidney weight(g) |
|---|---|---|---|---|---|---|
| G1 | 122.7±2,30[a] | 133,90±1,94[a] | 7,09±0,54[a] | 0,59±0,01[a] | 1.38±0,02[a] | 0,51±0,04[a] |
| G2 | 116 ,00±5,10[a] | 123,60±4,84[a] | 5,67±0,26[a] | 0.59±0.01[a] | 1,28±0,00[a] | 0.57±0,02[a] |
| G3 | 122.50±3,10[a] | 135,20±3,90[a] | 5,28±0,54[a] | 0,58±0,03[a] | 1,33±0,04[a] | 0.58±0,01[a] |
| G4 | 115.2±1,50[a] | 129,7±0,75[a] | 7,03±0,39[a] | 0.62±0.04[a] | 1,24±0,05[a] | 0,51±0,03[a] |
| G5 | 110,00±9,35[a] | 120,20±6,03[a] | 7,06±0,30[a] | 0,55±0,02[a] | 1,03±0,04[a] | 0,43±0,01[a] |
| G6 | 108,7±6,44[a] | 148,40±6,24[a] | 6,80±0,20[a] | 0,57±0,02[a] | 1,04±0,04[a] | 0,45±0,00[a] |
| G7 | 121,30±1,11[a] | 149,50±9,25[a] | 7,54±0,68[a] | 0,59±0,00[a] | 1,17±0,12[a] | 0,50±0,04[a] |

*The values of parameters were expressed as Mean ± S.E.M. for three rats (n=3). In the same line values, the same letters are not significantly different (p< 0.05).*

*G1 = control group*

*G2 = groups received aqueous extract (300mg/kg body weight)*

*G3 = groups received aqueous extract (2000mg/kg body weight)*

*G4 = groups received aqueous extract (5000mg/kg body weight)*

*G5 = groups received ethanolic extract (300mg/kg body weight)*

*G6 = groups received ethanolic extract (2000mg/kg body weight)*

*G7 = groups received ethanolic extract (5000mg/kg body weight)*

**Anti-inflammatory activity of CHRYSOW**

The results of anti-inflammatory activity of CHRYSOW against carrageenan induced paw oedema have been shown in **Table 3**. No adverse effect or mortality was detected in albino rats up to 250 and 500 mg/kg body weight, orally of aqueous and ethanolic extracts of leaves of CHRYSOW. There was a gradual increase in oedema paw volume of rats in the positive control group (DFS 25). However, in the test groups, aqueous extract with doses of 250 and 500 mg/kg bw showed a significant reduction in the oedema paw of volume. There was weak reduction of thickness of oedema paw in case of rats treated with ethanolic extract (EE 250 and EE 500). The higher effect was found in the group received 500 mg/kg bw compared to group received 250 mg/kg bw of aqueous extract. The dichlorofenac sodium as reference drug (25 mg/kg orally) produced a no significant reduction of thickness of oedema paw comparable to aqueous extract with 500 mg/kg bw. Aqueous extract (EA 500) and dichlorofenac sodium (DFS 25) respectively exhibited 0.166±0.05 and 0.155±0.003 mm of circumference of oedema paw after 5 hours carrageenan administration. Thus the percentage of inhibition was shown in the figure 1. The percentage inhibition of the paw volume in the group of animals

treated with CHRYSOW aqueous extract 250 mg/kg bw was 43.94% and for the 500 mg/kg was 56.31% at 5 hours. It showed that aqueous extract (500 mg/kg bw) have significant (P <0.05) anti-inflammatory effect and the results were compared with dichlofenac sodium (DFS 25) which showed reduction of 59.92 % at the same time.

**Table 3: Effect of CHRYSOW extract and carrageenan induced paw oedema in rats**

| Treatment | 0 hour | 1 hour | 2 hours | 3 hours | 4 hours | 5 hours |
|---|---|---|---|---|---|---|
| Control | 0,145±0,010 | 0,211±0,011 | 0,260±0,008$^{a}$ | 0,310±0,004$^{a}$ | 0,345±0,007$^{a}$ | 0,380±0,005$^{a}$ |
| DFS 25 | 0,155±0,004 | 0,174±0,003 | 0,178±0,004$^{b}$ | 0,161±0,005$^{c}$ | 0,161±0,01$^{c}$ | 0,155±0,003$^{d}$ |
| EA 250 | 0,159±0,012 | 0,191±0,004 | 0,231±0,008$^{a}$ | 0,227±0,05$^{b}$ | 0,220±0,004$^{b}$ | 0,213±0,005$^{c}$ |
| EA 500 | 0,145±0,012 | 0,185±0,005 | 0,200±0,016$^{b}$ | 0,183±0,006$^{c}$ | 0,166±0,006$^{c}$ | 0,166±0,005$^{d}$ |
| EE 250 | 0,134±0,005 | 0,202±0,004 | 0,252±0,004$^{a}$ | 0,276±0,06$^{a}$ | 0,290±0,004$^{a}$ | 0,331±0,007$^{b}$ |
| EE 500 | 0,152±0,016 | 0,192±0,005 | 0,246±0,005$^{a}$ | 0,241±0,07$^{a}$ | 0,273±0,01$^{a}$ | 0,303±0,011$^{b}$ |

*DFS 250 = dichlorofenac sodium with 25 mg/kg bw; EA 250 = aqueous extract with 250 mg/kg bw; EA 500 = aqueous extract with 500 mg/kg bw; EE 250 = ethanolic extract with 250 mg/kg bw; EE 500 = ethanolic extract with 500 mg/kg bw. The values of parameters were expressed as Mean ± S.E.M. for 6 rats (n=6). In the same column values, the same letters were not significantly different (P< 0.05).*

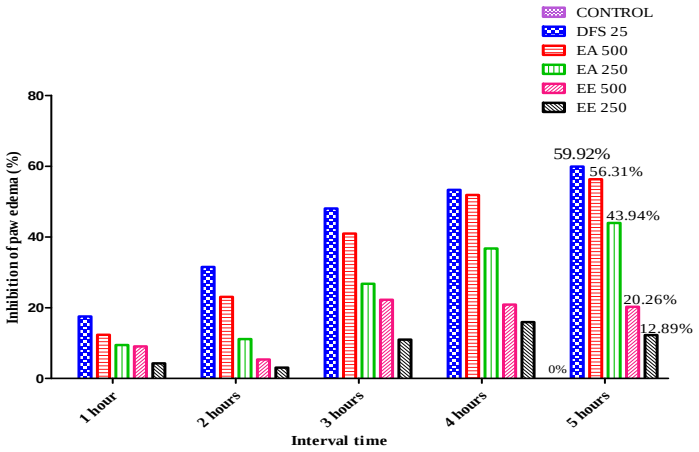


**Figure 1**: percentage inhibition of paw edema

## DISCUSSION

The screening of the leaf of CHRYSOW showed the presence of polyphenols; flavonoids, anthocyanins; cardiac glycosides; sterols; polyterpenes and alkaloids. However, the alcoholic extract contained over polyterpenes, sterols and anthocyanins which would use in therapeutic treatment. But both total extracts did not contain tannins, coumarins and quinones. The saponins were observed in the aqueous extract and not in the ethanol extract.

According to the relationship table of clinical signs of intoxication and organs of systems (Laroche et al., 1986; Hachette et al., 1989), it might infer that the crude aqueous and ethanolic extracts of dose of 5000 mg/kg of bw of CHRYSOW have an effect on the respiratory system. The impaired locomotion, observed in the open field test 3hours after administration without altering the muscle strength, indicating some toxicity of doses, orally administered. The literature demonstrated that the reduction of the spontaneous locomotor activity reflects a reduced excitability, originated from disturbances of central nervous system (Ozturk et al., 1996; Perez et al., 1998). These disturbances may be due to saponins and alkaloids. The saponins and alkaloids may have depression effects and decrease motor activity in experimental animals (Hussain et al., 1991; Fazly et al., 1997; Zirihi et al., 2006). Accelerated breathing and contortion flank muscles of these extracts could have an effect on the respiratory system. Saponins and alkaloids may be basis of breathing disturbance.

The body weight analysis, assessed 14 days of observation showed that *C. welwitschii* did not induce significant changes in female animals. The animal body weight is also an important factor to evaluate the toxicity of a substance (Jahn et al., 1997). The reduction in body weight and internal organs weight can be a simple and sensitive index of toxicity after exposure to a toxic substance (Raza et al., 2002; Teo et al., 2002). Changes in organ weight have long been accepted as indicators of test-induced changes, which are often associated with treatment-related effects (Sellers et al., 2007). In the present work, the extracts of CHRYSOW did not induce significant changes to the relative body weight of rats.

No significant difference of weight was not observed between the treated animals and the control; suggesting that crude aqueous and ethanol extracts of *C. welwitschi*i were not toxic for the essential organs.

The extracts of this plant administered orally at doses up to 5000 mg/kg bw caused no morbidity and lethality, which means that by this route the LD50 is greater than this dose. According to Global Harmonized Classification System (SCGH), substances whose dose of 5000mg/kg bw was not lethal of Category 5 or unclassified. Both extracts of CHRYSOW are hardly toxic orally and their LD50 is higher than 5000mg / kg bw.

The inflammation model of a carrageenan induced oedema is usually used to assess the activity of natural products in resisting the pathological changes associated with acute inflammation (Sawadogo et al., 2006; Wang et al., 2013). Inflammation induced by carrageenan is acute, non-immune, well researched, and highly reproducible. Cardinal signs of inflammation – oedema, hyperalgesia, and erythema – developed immediately after subcutaneous injections a result of the action of proinflammatory agents and reactive oxygen and nitrogen species (Pan et al., 2010). The development of Carrageenan induced inflammation was a biphasic event. First phase occurs within an hour of injection of phlogistic agent and was the first and the second phase was due to kinin like substances. The second phase is related to release of prostaglandins, bradykinins, the cyclooxygenase products and lipooxygenase products like substances (Brooks et al., 1991; Vinegar et al., 1969). Second phase is sensitive to both the clinically useful steroidal and nonsteroidal anti-inflammatory agent (Martini et al., 2004). These findings indicated that aqueous and ethanolic extracts of CHRYSOW reduced the carrageenan induced oedema to similar extent as the potent anti-inflammatory drug diclofenac Sodium. Moreover, these results provide further evidence that the extracts of CHRYSOW contained anti-inflammatory principle that may be useful in the treatment of the acute inflammatory conditions. The percentage of paw oedema was found to be better with the aqueous extract than the ethanolic extract.

The aqueous and ethanolic extracts were found to possess saponin, sterol and flavonoid (Alcatraz et al., 1998; Hima et al., 2014). The possible anti-inflammatory effect may be due to inhibition of cyclooxygenase enzyme which catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid. Although preliminary biological study has revealed that CHRYSOW possesses significant anti-inflammatory activity mechanisms underlying the observed pharmacological effects are not clear. The assessment of observed pharmacological effects with isolated individual chemical constituent merit further investigation for better understanding of the molecular mechanisms underlying anti-inflammatory activity of the CHRYSOW. However, the present study suggested that the aqueous extract of *Chrysophyllum welwitschii*, may be used as an herbal remedy for the management of inflammation.

## CONCLUSION

The effects of extracts on body and internal organs weights indicated that CHRYSOW was no toxic. The crude aqueous and ethanol extracts of *C. welwitschii* were not toxic effect on organs and body weights. Both extracts of *C. welwitschii* are hardly toxic orally and their LD50 was higher than 5000 mg/kg bw. The leaf extracts *Chrysophyllum welwitschii* showed an anti-inflammatory effect and their screening revealed the presence of saponins, sterols and flavonoids.

## REFERENCES

1. Akharaiyi F.C. (Antibacterial, phytochemical and antioxidant activities of Datura metel). In. J. PharmTech. Res, 2011; 3 (1): 479-483.
2. Bagre I., Bahi C., Gnahoue G., Djaman A.J. et Guede G.F. Composition phytochimique et évaluation in vitro de l'activité antifongique des extraits des feuilles de Morinda morindoides (baker) milne-redhead (rubiaceae) sur Aspergillus fumigatus et Candida albicans. J. Sci. Pharm. Biol. 2007; 8(1): 15-23
3. Békro Y.A., Békro J.A., Boua B.B., Tra B.F., et Ehilé E.E. Etude ethnobatanique et screning phytochimique de Caesalpinia benthamiana (Baill.) (Caesalpiniaceae). Rev. Sci. Nat. 2007; 4: 217-225.
4. Hegnauer R. Chemotaxonomie der Pflanzen, BikhäuserVerlag, Basel, Suttgart 1973; 6: 761.
5. OECD (Organization for Economic co-operaion and Development. OECD guidelines for the testing of chemicals /Section 4: Health Effects Test No. 423; Acute oral Toxicity –Acute Toxic Class method. OECD. 2001; Paris.
6. Saba M, Aravanan P, Illavarasan R. Anti-inflammatory activity of Lawsonia inermis leaves, Indian Drugs. 2007; 44(3): 224.
7. Charles Dorni AI, Vidyalakshmi KS, Hannah R, Vasanthi, Rajamanickam GV, and Dubey GP. Anti-inflammatory activity of Plumbago capensis, Pharmacognosy Magazine. 2006; 2(8): 239.
8. Laroche MJ, Fabiani F, Rousselet F. L'expertise toxicologique des médicaments. Edition Masson, Paris. 1986; 367.
9. Hachette JC .Toxicologie d'urgence: produits chimiques et industriels. Edition Masson, Paris. 1989; 189.
10. Ozturk, Y., Aydini, S., Beis, R., Baser, K.H.C., Berberoglu, H. Effect of Hypericum perforatum L. and Hypericum calximun L. extract on the central nervous system in mice. Phytomedicine. 1996; 3: 139-146.
11. Perez, R.M.G., Perez, J.A.L., Garcia, L.M.D., Sossa, H.M. Neuro-pharmacological activity of Solanum nigrum fruit. J. Ethnopharmacol. 1998; 62: 43-48.

12. Hussain, MM, Sokomba EN, Shok M. Pharmacological effects of Gardenia erubescens in mice, rats and cats. Int J Pharmacogn. 1991; 29: 94-100.
13. Fazly Bazzaz BS, Haririzadeh G, Imami SA, Rashed MH. Survey of Iranian plants for alkaloids, flavonoids, saponins, and tannins (Khorasan province). Int J Pharmacy. 1997; 35: 17-30.
14. Zirihi G. N. Études botanique, pharmacologique et phytochimique de quelques plantes médicinales antipaludiques et/ou immunogènes utilisées chez les Bété du Département d'Issia, dans l'ouest de la Côte d'Ivoire. Thèse de Doctorat d'Etat, Université de Cocody-Abidjan, UFR Biosciences. 2006; 126.
15. Jahn, A.I., Günzel, P.K.H. The value of spermatology in male reproductive toxicology: do spermatologic examinations in fertility studies provide new and additional information relevant for safety assessment? Reprod. Toxicol. 1997; 11: 171-178.
16. Raza, M., Al-Shabanah, O.A., El-Hadiyah, T.M., Al-Majed, A.A. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liverand kidney of Swiss albino mice. Sci. Pharm. 2002; 70: 135-145.
17. Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A., Khetani, V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague- Dawley rats. Toxicology. 2002; 179: 183-196.
18. Sellers, R.S., Morton, D., Michael, B., Roome, N., Johnson, J.K., Yano, B.L., Perry, R., Schafer, K. Society of toxicologic pathology position paper: organ weight recommendations for toxicology studies. Toxicol. Pathol. 2007; 35: 751-755.
19. Birnbaum and al. Differential toxicity of 1, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin TCDD in C57BL/6J mice congenic at the locus. Fund Appl Toxicol 1990; 15: 186-200.
20. Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP, Nacoulma OG: Anti-inflammatory, Analgesic ad Antipyretic Activities of Dicliptera verticillata. Intl J Pharmacol. 2006; 2: 435 - 438.
21. Wang BS, Huang GJ, Lu YH, Chang LW: Anti-inflammatory effects of an aqueous extract of Welsh onion green leaves in mice. Food Chem. 2013; 138: 751 - 756.
22. Pan MH, Lai CS, Ho CT: Anti-inflammatory activity of natural dietary flavonoids. Food Funct. 2010; 1: 15–31.
23. Brooks PM, Day RO. Non-steroidal anti-inflammatory drugs difference and similarities. N Engl J Med. 1991; 324: 1716-1725.
24. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema on rats. J. Pharmacol. EXP. Ther. 1969; 66: 96-10.
25. Martini N.D., Katerere D.R.P., Eloff J.N.: J. Ethnopharmacol. 2004; 93: 207.
26. Alcatraz, M.J. and M.J. Jimenez. Flavonoids as anti-inflammatory agents. Fitoterapia. 1998; 59: 25-38.
27. Hima Bindu M., Venkateswarlu Guddeti, Kala Praveen T., Sri Lakshmi Surekha P., Gayathri M., Prakash Allam VS. and Radha Ch. Evaluation of anti-inflammatory activity of musa paradisiaca (linn.) leaves extract in rats IJPCBS. 2014; 4(3): 753-757.