

Antioxidant, Anti-Proliferative and Bronchodilatory Activities Of *Euphorbia Hirta* Extracts

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ABSTRACT *Euphorbia hirta* is a plant commonly found in wild lands in tropical countries. It has various medicinal uses such as for conjunctivitis, ulcerated cornea, asthma, bronchitis, upper respiratory catarrh etc. In this study, crude n-hexane, chloroform, methanol and water extracts of the plant were screened for their antioxidant activities using an improved 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorisation assay method. The methanol and water extracts showed comparable antioxidant activities to those of green and black teas. On the other hand, the chloroform and n-hexane extracts did not show significant antioxidant activities. The extracts were also screened for anti-proliferative activities against normal mouse fibroblast cells (NIH/3T3). Results indicated that the methanol and chloroform extracts were less anti-proliferative than the water and hexane extracts. The effect of *Euphorbia hirta* aqueous extract on guinea pig trachea was studied. The water extract alone did not have any effect on the guinea pig trachea but it showed partial inhibition on the histamine induced tracheal smooth muscles contractions. Therefore, further studies may focus on water extract as it showed considerable antioxidant activity and partial inhibitory effect on histamine induced tracheal smooth muscle contractions.

(**Keywords:** Antioxidant activity; Bronchodilation; Anti-proliferative activity; *Euphorbia hirta*; Plant extracts.)

INTRODUCTION

Euphorbia hirta is also known as *Euphorbia pilulifera* comes from the *euphorbiaceae* (spurge family). *E. hirta* is native to Africa and Australia where it is known as Australian asthma herb and Queensland asthma weed¹ (Johnson et al., 1998). This plant is also very commonly found in tropical countries such as Malaysia. Flower extracts of *E. hirta* have been found to be anti-bacterial and non-cytotoxic and water and ethanol leaf extracts function as a diuretic² (Vijaya et al., 1995). Aqueous leaf extracts had been shown to decrease gastrointestinal motility in rats and mice³ (Hore et al., 2006) and whole plant extracts shown to have chemosuppressive activities on parasitaemia in mice infected with *P. berghei berghei*⁴ (Tona et al., 1999). This study aims to investigate the antioxidant and cytotoxic properties of *E. hirta* against normal cells as it has been widely used in traditional medicine for decades. Besides this, the bronchodilatory property was also studied as it had been used for asthma, bronchitis and upper respiratory catarrh^{3,5} (Hore et al., 2006; Burkill; 1966).

MATERIALS AND METHODS

Preparation of extracts

Euphorbia hirta used in this study had been authenticated by Roy Vickery, Department of Botany, The Natural History Museum, London. The whole plant material was washed and dried at 40°C. The dried plant material (8 g) was powdered and extracted sequentially with 200 mL each of n-hexane, chloroform, methanol and water using a soxhlet extractor. The solvents in the extracts were removed under reduced pressure using a rotary evaporator until consistent extract weights were obtained. The water extract was further freeze-dried to obtain a consistent weight. The yields of the n-hexane, chloroform, methanol and water extracts were 2%, 1%, 7% and 16%, respectively, from the dried material.

Antioxidant activity

The antioxidant activities of the extracts were assessed using an improved ABTS radical cation decolorisation assay⁶ (Re et al., 1999).

ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (7.0 mM, 715 µL) and potassium persulfate (8.6 mM, 285 µL) were mixed and allowed

to react in the dark at room temperature for 16 h to form ABTS^{•+} radical. The ABTS^{•+} solution was diluted with phosphate buffered saline (PBS) at pH 7.4, to an absorbance of 0.70 (\pm 0.20) measured at 734 nm. A 10 μ L aliquot of each extract solution was added to 990 μ L of the diluted ABTS^{•+} solution and the absorbance of the resulting solution at 734 nm was recorded at every minute after mixing up to 6 minutes. The final concentrations of the water and methanol extracts in the reaction mixture ranged between 1.25 to 10 μ g/mL. For the chloroform and hexane extracts, the assays were carried out at 10 μ g/mL (precipitation occurred at higher concentrations, whereas the response was insignificant at lower concentrations). The effects of Trolox standards (final concentration 2.5 to 25 μ M) were also determined in the assay. All determinations were carried out in triplicates. Appropriate solvent blank was included in each assay. The percentage inhibition of the absorbance at 734 nm was determined. The antioxidant capacity of each extract was determined at 6 minutes and expressed in Trolox equivalents (TE)/g dry matter (using the concentration-response curve of Trolox determined at 6 minutes).

Anti-proliferative activity against normal mouse fibroblast cells (NIH/3T3)

The assay is based on the ability of viable, metabolic active cells to cleave yellow tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to form a blue formazan product using the mitochondrial dehydrogenase. Dead cells or tissue culture medium do not participate in the reduction. The solubilised formazan product is analysed spectrophotometrically and the percentage of cell survival can then be quantified.

The weighed extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted to various concentrations with DMEM media containing 10% foetal bovine serum. These were filtered through 0.22 μ m membrane filters. The final concentration of DMSO in each diluted extract solution was not more than 0.3 % (v/v) (it has previously been shown that cell growth is not affected in media containing not more than 0.3 % (v/v) DMSO). The diluted extract solutions were added to the respective wells of 96-well flat-bottomed tissue culture plates (Nunc, Denmark) containing NIH/3T3 cells at 1×10^4 cells/well. The final concentrations of the extracts in each assay ranged from 6.25 to 300 μ g/mL. Triplicates were performed for each extract concentration in each assay and the assay was repeated. Wells containing cells only in culture

medium containing not more than 0.3% (v/v) DMSO were included as the negative controls. The plates were incubated in a humidified 5% CO₂ incubator at 37°C for 72 hours. At the end of the 72 hours incubation period, 20 μ L of MTT (5 mg/mL in PBS) was added to each well and the plates were further incubated at 37°C 5% CO₂ for 4 hours. The media in each well was then discarded and formazan crystals formed were dissolved with 80 μ L dimethyl sulfoxide. The absorbance at 550 nm in each well was obtained and the percentage of cell survival was calculated.

Bronchodilatory activity

The guinea pigs were housed in the animal holding facility where food and water were given *ad libitum*. All experimental procedures were approved by the ethics committee of International Medical University. Before the removal of tracheas, the guinea pigs were euthanised using chloroform. The tracheas were isolated from male guinea pigs weighing about 400g. Tracheas were cut spirally into two strips 20mm long and 4mm wide. The tracheal strips were mounted vertically in a 10mL organ bath containing Krebs buffer aerated with a mixture of 95% O₂ and 5% CO₂ at 37°C. The composition of Krebs buffer was as follow (in mM): NaCl 118, KCl 4.7, CaCl 2.4, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11. Changes in isometric tension were recorded with PowerlabTM. Before each experiment the strips were subjected to a tension of 2 g for about 2 hours until a stable baseline tension was obtained. Dose response curves for histamine (10⁻⁷ to 10⁻⁴M) were obtained in the absence or presence of water extracts (1, 5, 10 and 20 μ g/m), methanol extracts (20 μ g/mL), chloroform extracts (27 μ g/mL), and n-hexane extracts (28 μ g/mL). Dried solvent extracts were suspended in DMSO before adding into organ bath. All drug concentrations indicated are expressed as the final concentrations in the organ bath.

Student t-test was used for statistical analysis.

RESULTS

Antioxidant activity

The graphs of the percentage inhibition of absorbance at 734 nm versus time for the Trolox standards and extracts are shown in Figures 1(a) – 1(d). The concentration-response curve for the Trolox standards, water and methanol extracts are shown in Figures 2(a) - 2(c). The antioxidant capacity of each of the water and methanol extracts was determined by dividing the gradient of the concentration-response curve of the extract by the gradient of the plot for Trolox. The antioxidant capacities of the water and methanol extracts are 1437 μ mol Trolox Equivalent/g dry matter and 854 μ mol Trolox.

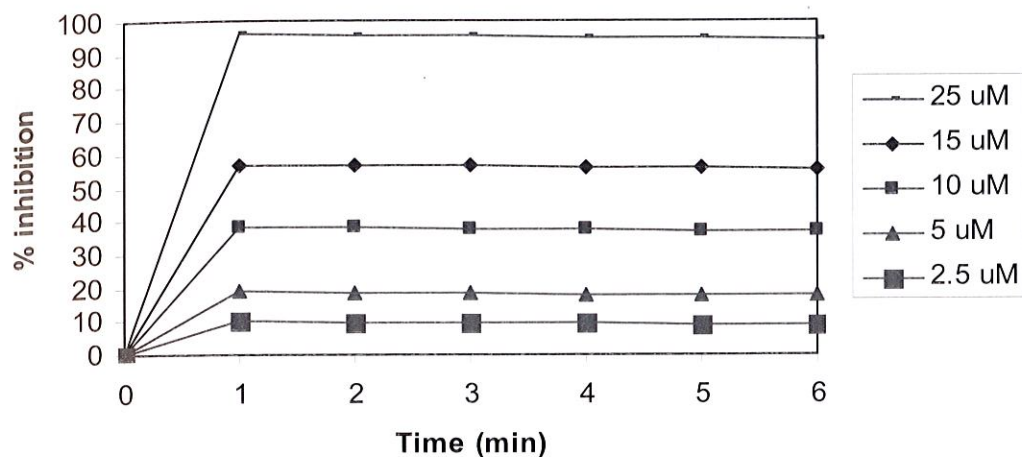


Figure 1(a): Time-dependent study of the percentage inhibition of the absorbance of ABTS⁺ by various concentrations of Trolox.

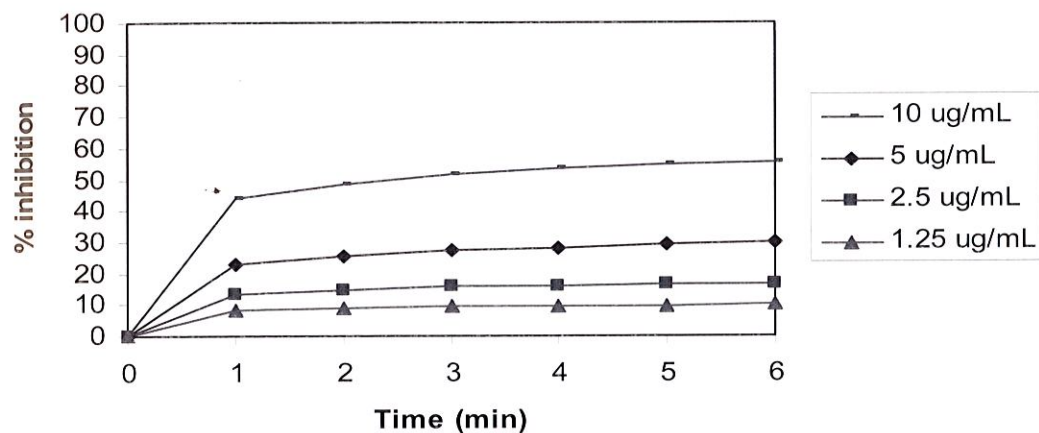


Figure 1(b): Time-dependent study of the percentage inhibition of the absorbance of ABTS⁺ by various concentrations of the aqueous extract of *Euphorbia hirta*.

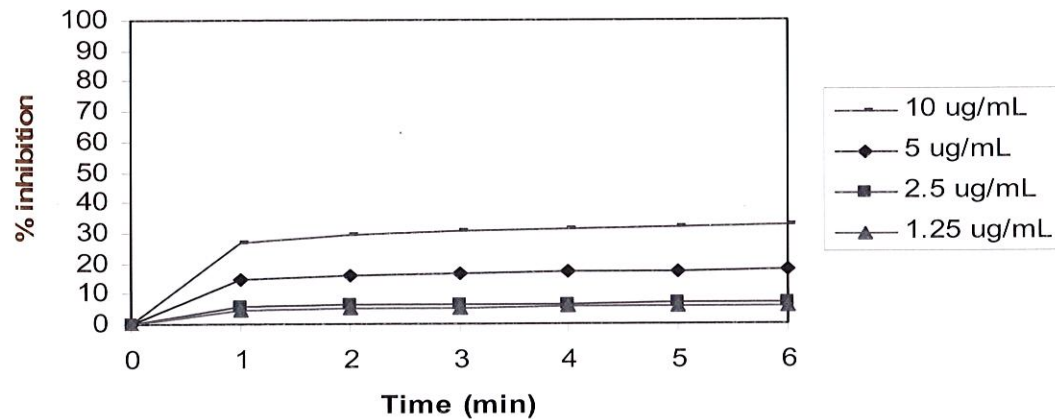


Figure 1(c): Time-dependent study of the percentage inhibition of the absorbance of ABTS⁺ by various concentrations of the methanol extract of *Euphorbia hirta*.

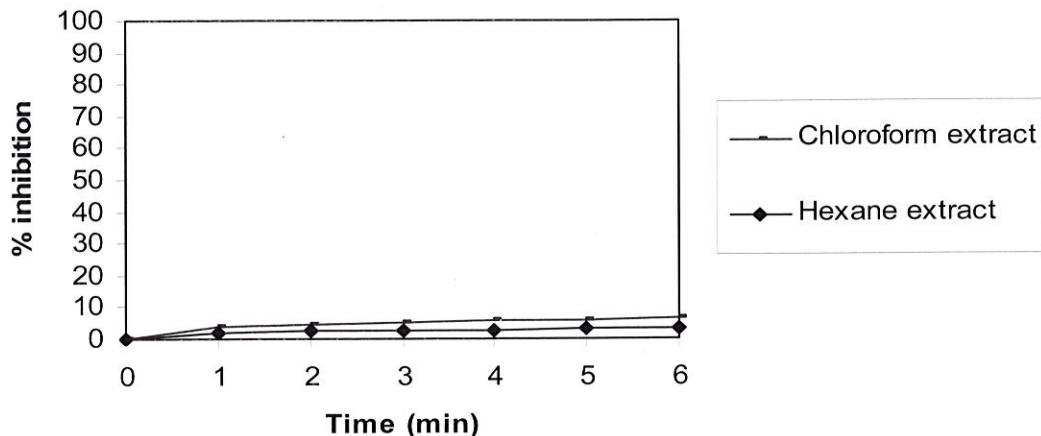


Figure 1(d): Time-dependent study of the percentage inhibition of the absorbance of $ABTS^{+}$ by $10 \mu\text{g/mL}$ of the chloroform and hexane extracts of *Euphorbia hirta*.

Equivalent/g dry matter, respectively. Meanwhile, the chloroform and n-hexane extracts at $10 \mu\text{g/mL}$ showed less than 10% inhibition of absorbance at 734 nm, indicating that their anti-oxidant activities were insignificant.

Anti-proliferative activity against normal mouse fibroblast cells

The dose-response curves for the extracts are shown in Figures 3(a) – 3(d). The IC_{50} values for all extracts

against normal mouse fibroblast cells are above $200 \mu\text{g/mL}$, with the methanol and chloroform extracts being less anti-proliferative than the water and hexane extracts. According to the National Cancer Institute, a crude extract should have an IC_{50} of less than $20 \mu\text{g/mL}$ for it to be considered cytotoxic⁷ (Geran et al., 1972). Therefore, these extracts are considered to be non-cytotoxic against normal mouse fibroblast cells.

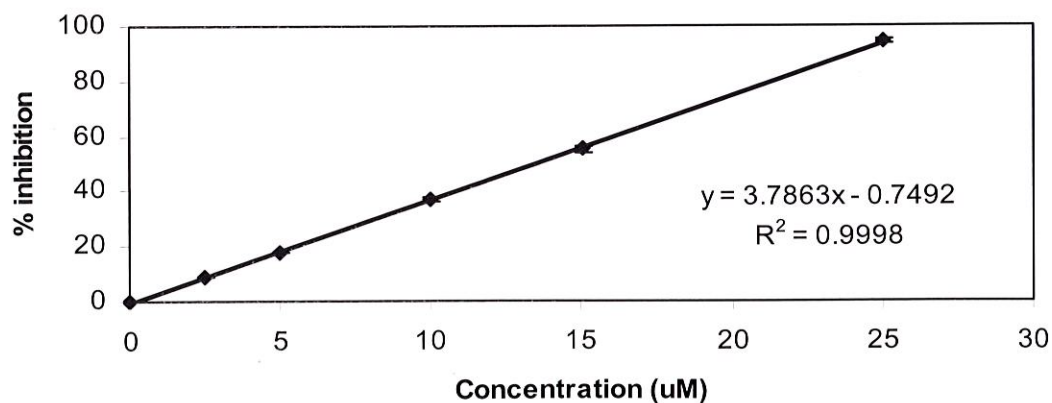


Figure 2(a): Concentration-response curve for the percentage inhibition of the absorbance of $ABTS^{+}$ as a function of concentration of standard Trolox solution.

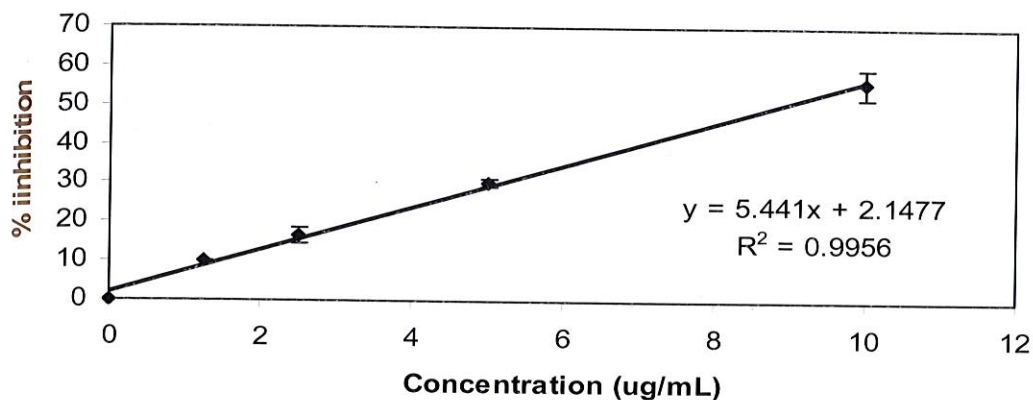


Figure 2(b): Concentration-response curve for the percentage inhibition of the absorbance of ABTS⁺ as a function of concentration of the aqueous extract of *Euphorbia hirta*.

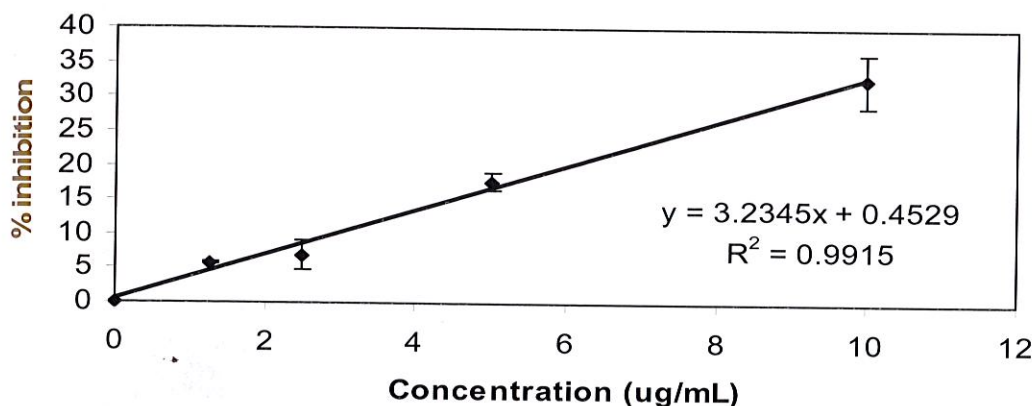


Figure 2(c): Concentration-response curve for the percentage inhibition of the absorbance of ABTS⁺ as a function of concentration of the methanol extract of *Euphorbia hirta*.

Bronchodilatory activity

There was significant inhibitory effect of water extract on histamine induced tracheal contraction (Figure 4). The minimum effective concentration of the water extract found was 5 µg/mL and there was no greater inhibition at 10 and 20 µg/mL. The EC₅₀ value for histamine was 1.7 x 10⁻⁶M (n=5) and the EC₅₀ values for histamine in the presence of 5 µg/mL and 10 µg/mL water extracts were 1.6 x 10⁻⁵M (n=4) and 6.2 x 10⁻⁶M (n=5) respectively. The results showed that the water extracts were able to shift the dose-response curve parallelly to the right. The EC₅₀ values of histamine in the presence of water extract were significantly larger than that of histamine alone (p<0.05). No inhibitory effect was observed at 1 µg/mL water extract (data not shown). Water extract alone did not cause relaxation in tracheas. All the other solvent extracts (chloroform extract, 27 µg/mL; hexane extract, 28 µg/mL and methanol extract, 20

µg/mL) tested showed no effect either on the tracheas alone or on histamine induced tracheal contraction.

DISCUSSIONS AND CONCLUSION

The water and methanol extracts of *Euphorbia hirta* showed antioxidant capacity comparable to those of commercial green and black teas with antioxidant capacity ranging from 235 µmol Trolox Equivalent/g dry matter to 1526 µmol Trolox Equivalent/g dry matter⁸ (Prior and Cao, 1999). This may be attributed to the presence of one of the active constituents in the plant, quercetin⁹(Tona et al., 2004). Quercetin is a flavonoid that has been shown to have antioxidant activity. The lack of cytotoxic activity of the extracts against normal mouse fibroblast cells provides some assurance for the safety of the plant which is commonly used by traditional medicine practitioners. However, further

in vivo experiments are needed to ascertain its toxicological effects.

The inhibitory action of the water extracts of *Euphorbia hirta* on histamine induced tracheal contraction provides evidence to its use as a traditional medicine to treat asthmatic patients who are hypersensitive to bronchoconstrictor agents such as histamine and $\text{PGF}_{2\alpha}$. The underlying mechanism may be due to its anti-inflammatory properties reported by Lanhers¹⁰ (1990). This can be studied by

investigating the bronchodilatory effect of water extracts of *Euphorbia hirta* on tracheas pretreated with indomethacin. Moreover, quercetin in *Euphorbia hirta* may play a role as quercetin has been shown to inhibit histamine release from basophils¹¹ (Ogasawara et al., 1986).

Further studies should focus on water extract as it showed considerable antioxidant activity and partial inhibitory effect on histamine induced tracheal smooth muscle contractions.

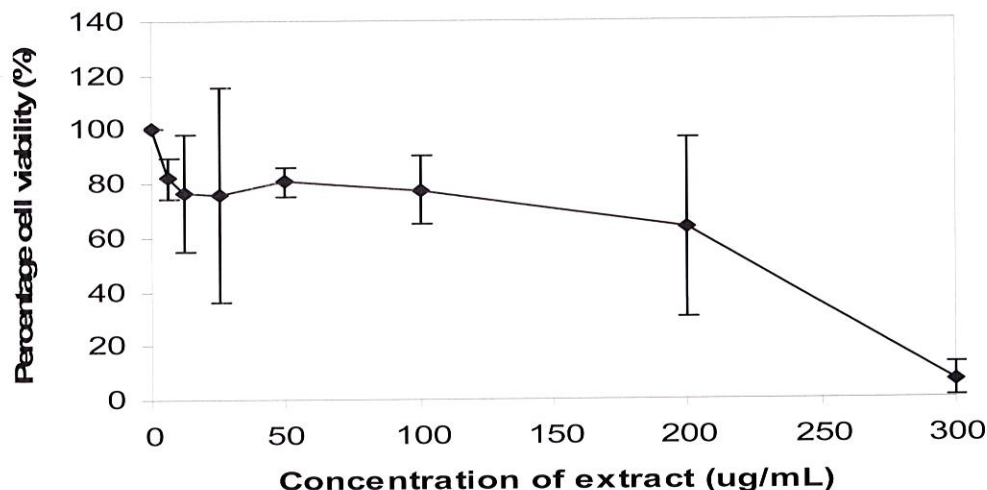


Figure 3(a): Percentage of cell viability of NIH/3T3 cells cultured in media containing 10% FBS in the presence of varying concentrations of the aqueous extract of *Euphorbia hirta*.

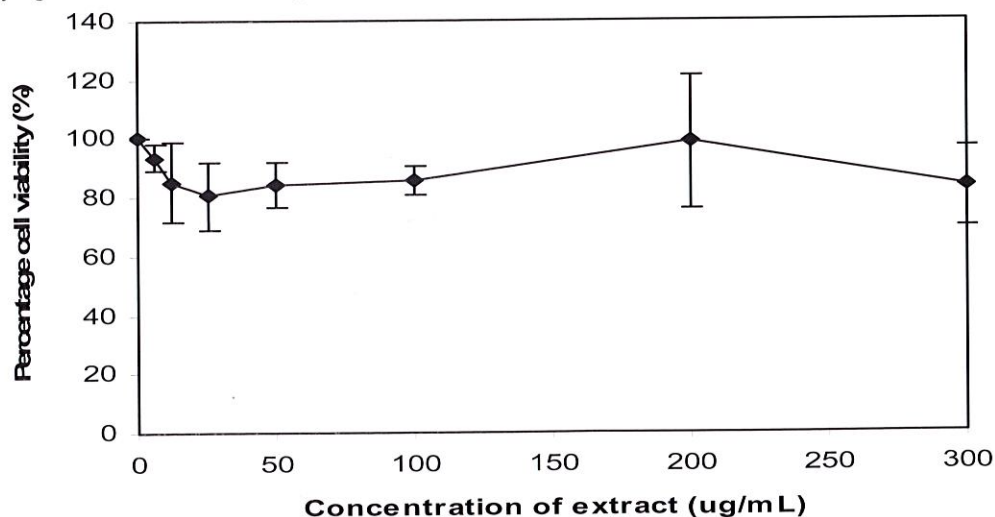


Figure 3(b): Percentage of cell viability of NIH/3T3 cells cultured in media containing 10% FBS in the presence of varying concentrations of the methanol extract of *Euphorbia hirta*.

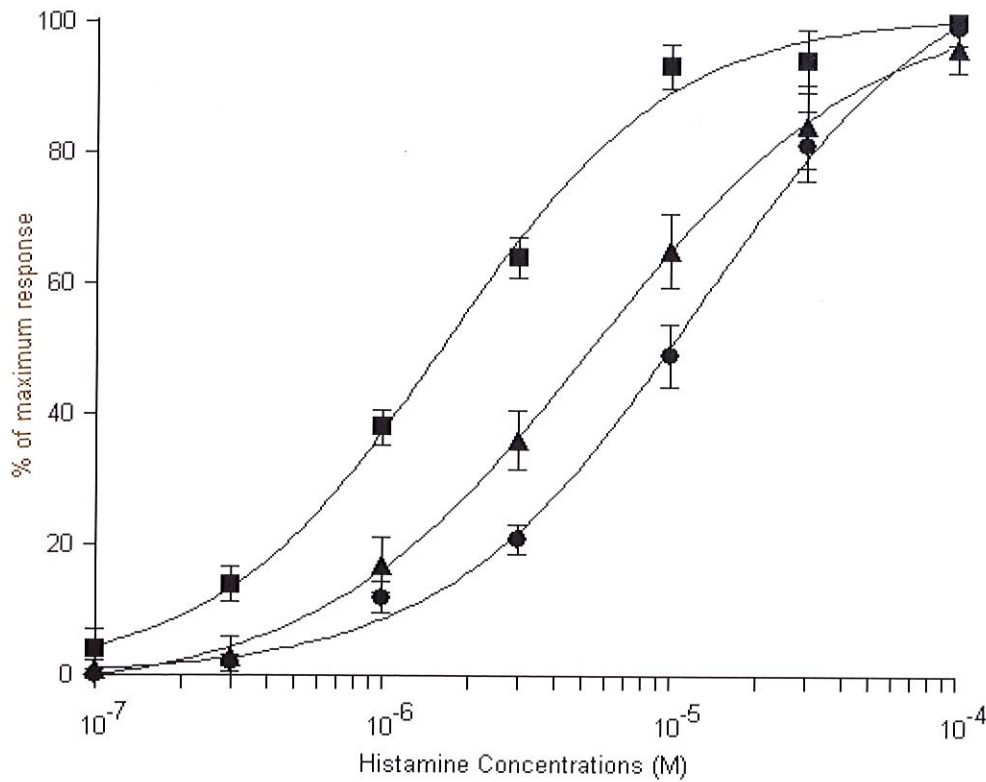


Figure 3(c): Percentage of cell viability of NIH/3T3 cells cultured in media containing 10% FBS in the presence of varying concentrations of the chloroform extract of *Euphorbia hirta*.

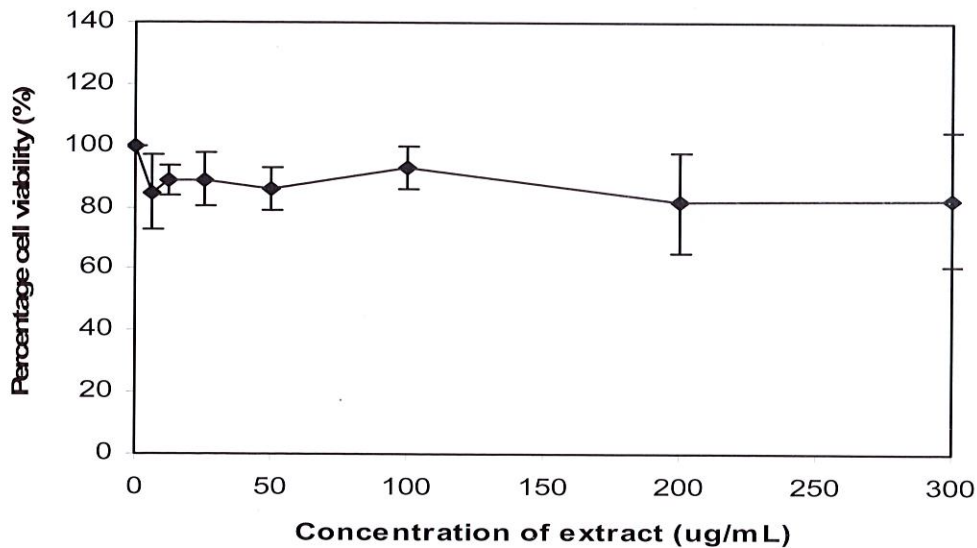


Figure 3(d): Percentage of cell viability of NIH/3T3 cells cultured in media containing 10% FBS in the presence of varying concentrations of the hexane extract of *Euphorbia hirta*.

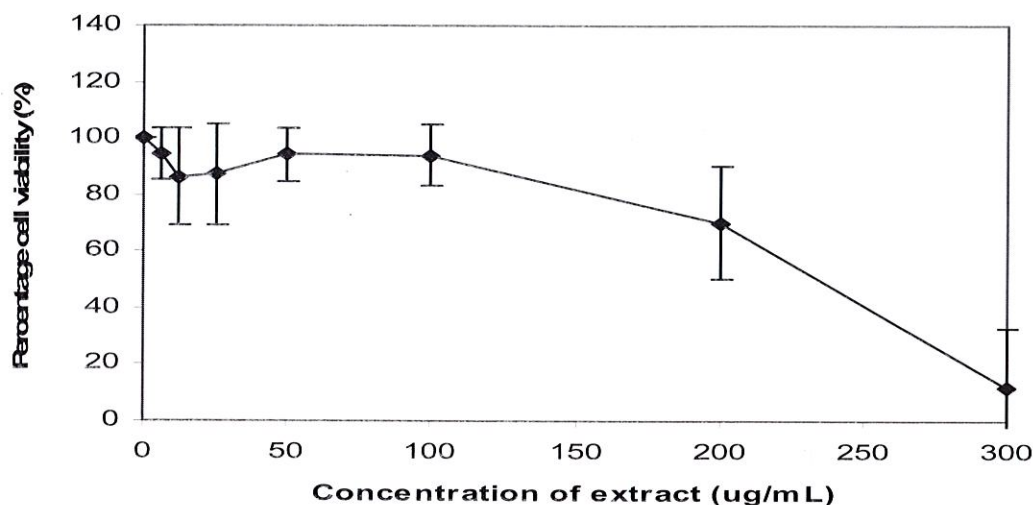


Figure 4: The results (s.e.m. \pm s.e.) showed the response of guinea pig ileum to histamine. Histamine alone (solid square), n=5. In the presence of 5mg/mL of aqueous extract of *Euphorbia hirta* (solid circle), n=4. In the presence of 10mg/mL of aqueous extract of *Euphorbia hirta* (solid triangle), n=5

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