Journal of Applied Pharmaceutical Science Vol. 3 (11), pp. 051-058, November, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.31110
ISSN 2231-3354

Toxicity Testing of Tartrazine using the Nematode *Caenorhabditis Elegans*, Brine Shrimp Larvae (*Artemia Salina*) and KGN Granulosa Cell Line

Imane Himri[1*], Abdelkarim Guaadaoui[2], Faiza Souna[1], Mohammed Bouakka[1], Ahmed Melhaoui[3], Abdelkader Hakkou[1], Ennouamane Saalaoui[1]

[1] Université Mohamed Ier, Faculté des Sciences, Laboratoire de Biochimie, Oujda-Maroc. [2] Université Mohamed Ier, Faculté Des Sciences, Laboratoire de Génétique et Biotechnologie, Oujda-Maroc. [3] Université Mohamed Ier, Faculté Des Sciences, Laboratoire de Chimie Organique, Macromoléculaire et Produits Naturels, Oujda-Maroc. [4] Université Mohamed Ier, Faculté de Médicine et de Pharmacie, Oujda-Maroc.

ARTICLE INFO

Article history:
Received on: 19/03/2013
Revised on: 05/04/2013
Accepted on: 23/11/2013
Available online: 29/11/2013

Key words:
Tartrazine; Sulphanilic acid; *Caenorhabditis elegans*; brine shrimp; KGN granulosa cell line.

ABSTRACT

Tartrazine is a yellow food colorant, widely used in food products, drugs and cosmetics. The acceptable daily intake (ADI) oh human is 0-7.5 mg/kg body weight. The objective of our study was to examine the toxicity of Tartrazine and its main metabolite Sulphanilic acid to the nematode Caenorhabditis elegans, Brine Shrimp larvae (Artemia Salina) and KGN granulosa cell line; in the aim to develop our knowledge about their toxicity effects. In this research, toxicity of Tartrazine and Sulphanilic acid were examined to the nematode Caenorhabditis elegans with Escherichia coli as a food source. Our results showed that from a 3 mM concentration of Tartrazine, and 1mM of Sulphanilic supplementation can disrupt the cell cycle nematode C. elegans even if it does not cause death. Different concentrations of Tartrazine and Sulphanilic acid (1, 2.5, 5, 7.5, 10, 25 50, 75, 100 µg/ml) were tested for their toxicity in a short term bioassay using Brine Shrimp (Artemia salina). The Brine Shrimp were hatched in artificial sea water and exposed to the Tartrazine and Sulphanilic acid after 48 hours. LC50 values were calculated after probit transformation of the resulting data. Tartrazine did not show any significant toxicity against Brine Shrimp but Sulphanilic acid was mildly toxic (LC50 value (µg/ml) of ~82.3 µg/ml). The Brine Shrimp assay proved to be a convenient and rapid system for toxicity assessment. The human KGN ovarian granulosa-like tumor cell culture line has been used as an in vitro system for determination of the effects of Tartrazine and sulphanilic acid, the result showed that Tartrazine and Sulphanilic acid were unaffected after 24 h of treatment exposure.

INTRODUCTION

Tartrazine, FD&C Yellow No. 5, C.I. No. 19140 is an orange-coloured, water soluble powder widely used in food products, drugs, cosmetics and pharmaceuticals, in a way to improve the esthetic quality of a food product. It has the chemical structure illustrated in Figure.1.

Moreover, this food colorant is used in cooking in many developing countries as a substitute for saffron (Mehedi *et al.,* 2009). The Acceptable Daily Intake (ADI) for humans is 0-7.5 mg kg^{-1} body weight (JECFA, 1965). Tartrazine is reduced in the organism to an aromatic amine which is highly sensitizing.

Its main metabolite identified to date is sulphanilic acid (Neuman *et al.,*1978)**.** It has been implicated as the food additive which is most often responsible for allergic reactions in specific human populations (Stenius & Lemola, (1976), Devlin and David (1992), JONES *et al.,*(1964)). Some countries such as Sweden, Switzerland and Norway have withdrawn Tartrazine on the ground of its anaphylactic potential (WÜTH RICH, 1993)**.** The study of the carcinogenetic and mutagenetic effects of Tartrazine was established by some authors which gives variable results (ROXON J. *et al.,*(1967b), PATTERSON, RM. and BUTLER, JS.*,* (1982), MAEKAWA *et al.,*(1987), BORZELLECA, JF. and HALLAGAN JB.*,* (1988), COLLINS, TFX., et al. (1990), COLLINS, TFX., *et al.,*(1992). The animal that has been widely utilized and accepted in toxicity testing is the nematode, *Caenorhabditis elegans*.

** Corresponding Author*
Université Mohamed Ier, Faculté des Sciences, Laboratoire de Biochimie, Oujda-Maroc. E-mail: imanehimri@gmail.com
Phone:+212629323982

The *C. elegans* is a free living nematode, present in soil and found in temperate regions of the world. The *C.elegans* was selected by Dr. Sydney Brenner in 1965, as an experimental model organism to study animal development and behaviour (Riddle, D. L., *et al.,*1997). In the wild, *C.elegans* feed on the micro-organisms that develop on decaying vegetable matter. In the laboratory *C.elegans* are easily cultured on agar medium plates with *Escherichia coli* as a food source.

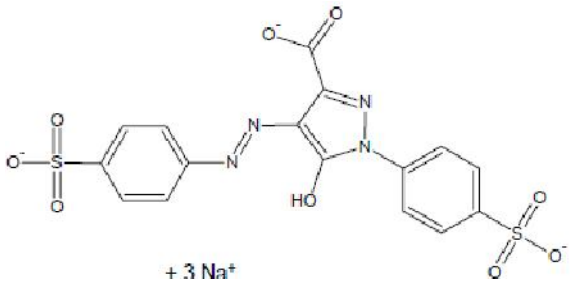


Fig. 1: Chemical structure of Tartrazine (trisodium salt of 3-carboxy-5-hydroxy- 1-(p-sulphophenyl)-4-(p sulphophenylazo) pyrazole.

The ease of laboratory cultivation, its small size, large brood size, short development time and well studied biology make the *C.elegans* an ideal model organism for biological studies (neuroscience, development, signal transduction, cell death, aging, and RNA interference) (Antoshechkin, I., and Sternberg, P. W. 2007). The transparency of the *C.elegans* allows for high quality microscopic images to be taken. For this reason, we used *C.elegans* as a suitable model to determine toxicity of Tartrazine.

The Brine Shrimp cytotoxicity assay was considered as an excellent method for preliminary investigations of toxicity, detection of fungal toxins, heavy metals, pesticides and cytotoxicity testing of dental materials, this technique is easily mastered, costs little, and utilizes small amount of test material. Since its introduction (Meyer N, *et al.,*1982), this in vivo test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents (Pisutthanan S, *et al.,*2004). The biological activity using the Brine Shrimp bioassay was recorded as a lethal concentration (LC 50) that kills 50% of the larvae within 24 hour.

The original KGN granulosa cell line was derived from a 63 year old woman with stage III ovarian cancer, histo-pathological analysis indicated granulosa cell carcinoma. The cell line exhibited stable, long-term proliferation (5 years) confirming carcinoma origin (Nishi, Y. *et al.,*2001). The human KGN ovarian granulosa-like tumor cell culture line has been used as an in vitro system for determination of the effects of Tartrazine and sulphanilic acid.

Food colorants are widely used in our food and little is known about their toxicity effects. Therefore, the aim of this investigation was to evaluate for the first time the effect of Tartrazine and his major metabolite sulphanilic acid on *C. elegans* growth, Brine Shrimp and KGN granulosa cell line culture.

MATERIALS AND METHODS

Chemicals

All chemicals used were of reagent to higher grade. Tartrazine (CAS 1934-21-0, Purity 86.7%) was purchased from Alfa Aesar (Germany).

In vitro Caenorhabditis elegans toxicity

Nematodes used in the present study were wild-type N2. *C. elegans* were grown in Petri dishes on nematode growth medium (NGM) and fed OP50 strain *Escherichia coli* according to a standard protocol (Brenner, S, *et al.,*1974). The components of NGM were agar, peptone, cholesterol, KH_2PO_4 and K_2HPO_4 buffer, NaCl and $MgSO_4$ (Stiernagle T. *et al.,*2006). *E. coli* was cultured overnight in sterilized Luria-Bertani (LB) medium ($5gL^{-1}$ of yeast extract, 10 g L^{-1} of tryptone, and 10 g L^{-1} NaCl). The test NGM plates were freshly prepared prior to use and nematodes were cultured at 20±1 °C. The eggs were used in toxicity testing. They were obtained by treating gravid mature adults with hypochlorite (Sulston, J. and Hodgkin, J. 1988), followed by rinsing with M9 buffer (Hitchcock D.R. , *et al.,*1998). Briefly, each test consisted of three concentrations (0.5 mM, 1 mM, and 3 mM) and a control, in which 10 eggs of *C. elegans* were transferred to culture plates containing 1mL of the test solution. The eggs were exposed at 20 ±1 °C and allowed to hatch on agar plates with *E. coli* OP50 as food source. Growth and development from eggs to adults were monitored after each 8 hours of continuous incubation until new eggs were seen in the uterus of individual worm, and after 72 h the numbers of live and dead worms were determined via visual inspection under a microscope. Nematodes were considered dead if no movement was observed in different stage even when tapping the nematodes on the head with a hair. The experiments were independently repeated at least three times.

Brine Shrimp toxicity assay

Tartrazine and sulphanilic acid were evaluated for lethality to Brine Shrimp larvae (Artemia salina Leach) according to according to the procedure described by Meyer et al. 1982 and Anderson et al. 1991. Brine Shrimp eggs (0.5 g) were hatched for 48 h in a conical flask containing 500 ml of artificial seawater made by dissolving a commercial marine salt in deionised water (19g of NaCl/ 500 ml of distilled water). The flasks were well aerated with the aid of an air pump; the air was placed in the bottom of the flasks to ensure complete hydration of the cysts. After 24 h and kept in a water bath at 29–30°C. A bright light source was left on and the nauplii hatched within 48 h. The freshly hatched free-swimming nauplii were used for the bioassay. Tartrazine and sulphanilic acid were dissolved in distilled water to obtain a concentration of 5 mg/ml. These were serially diluted and different concentrations were obtained (10 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 80 µg/ml, 85 µg/ml, 90 µg/ml, 95 µg/ml, 100 µg/ml). An aliquot of each concentration was transferred, in triplicate, into clean sterile universal vials with pipette, and aerated

seawater (5 ml) was added. Fifteen shrimp nauplii were transferred to each vial (45 shrimps per concentration). Seawater and Colchicine at different concentrations (5 µg/ml, 10 µg/ml, 50 µg/ml, 100 µg/ml), were used as negative and positive controls, respectively. After 24 h the numbers of survivors were counted and percentage of death calculated. The numbers of survivors were counted and percentages of deaths were calculated. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. The concentration that killed 50% of the nauplii (LC50 in µg/ml and Confidence Intervals 95%) were calculated from the regression equations obtained from the graphs using the statistical method of probit analysis with software ***STATISTICA***. The test is valid if the mortality in the control tubes does not exceed 10%. When this condition is fulfilled, we calculate the percentage of mortality corrected. The method used is that of Abbot (Meyer et al. 1982). Abbots formula was used to correct the values, i.e., P=Pi-C/1-Cn where P denotes the observed non-zero mortality rate and C represents the mortality rate of the control group.

The Brine Shrimp results are interpreted as follows:

LC50 <1.0 µg/ml – highly toxic;
LC50 1.0-10.0 µg /ml – toxic;
LC50 10.0-30.0 µg/ml – moderately toxic;
LC50 >30<100 µg/ml – mildly toxic,
And > 100µg/ml as non-toxic (M.J.MOSHI, et al. 2010).

KGN granulosa cell line Culture

The KGN granulosa cell line (Nishi, Y. et al.2001) was maintained in Dulbecco's modified Eagle's HAMS F12 (DMEM-F12) medium supplemented with 10% FBS, penicillin/streptomycin 5000 IU/ml and 5000 µg/ml respectively, Kanamycine 100 µg/ml, Fungizone 0,25 µg/ml. The KGN was maintained in 75 cm2 sterile tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO2 and sub-cultured every 2–3 days as required. When the cells were 80% confluent they were either passaged or used in experiments. Viable cell numbers were determined using the trypan blue exclusion assay on a haemocytometer (Freshney, R.I. 2005). The KGN (10,000 cells per well) was plated into sterile 96-well plates in 0.1 ml per well of complete cell culture medium for 24 h to allow cell adherence. Standard curve and microscopic observation of 0–25,000 cells per well was plated into clear 96-well plates (Figure 2, Figure 3).

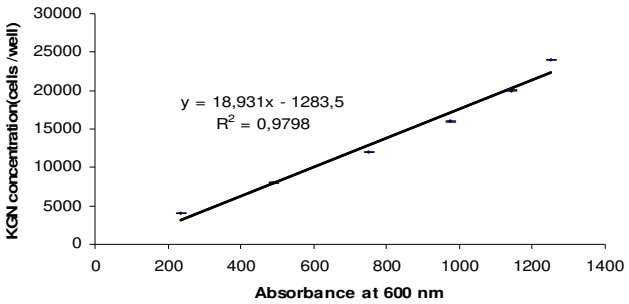


Fig. 2: Crystal Violet Standard Curve of KGN.

KGN (n=3) seeded at densities in the range of 0 to 4× 10^4 cells/well were allowed to adhere for 24 h in DMEM/F12 + 10% FBS and then processed for the Crystal Violet assay. Linear regression of mean absorbance (600nm) ± SEM in relation to KGN concentration (cells/well).

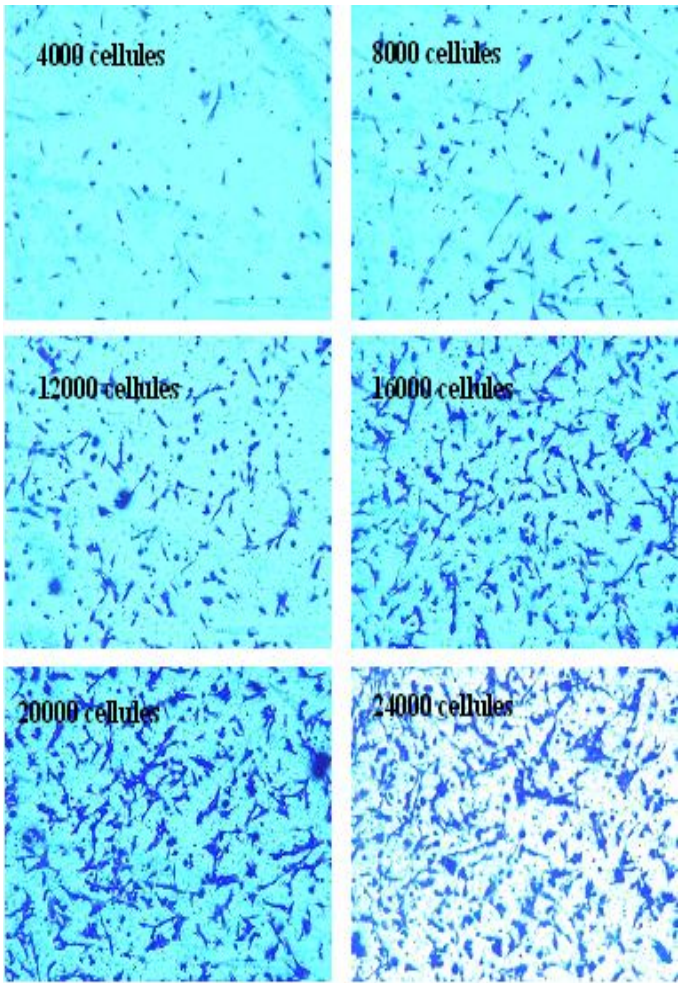


Fig. 3: Microscopic observation of different concentrations of KGN cells 0–25,000 cells per well was plated into clear 96-well plates.

After the initial cell adherence period, spent media (media deficient of nutrients and serum) were discarded and KGN cells were treated with Tartrazine and sulphanilic acid at different concentrations: 3, 6, 12, 25, 50 and 100 mM in a final volume of 0.1 ml per well in triplicate wells, for 24 h at 37 °C + 5% CO2. This experiment was repeated on three separate occasions.

After 24 h exposure to Tartrazine and to sulphanilic acid, media and all dead unattached cells were removed. The remaining adherent cells were rinsed with sterile 2× PBS and the crystal violet assay was performed (Siddiqui, E.J.et al., 2006). The crystal violet assay is a colorimetric assay in which only the nuclei of live cells take up the crystal violet stain (0.5%) (Siddiqui, E.J.et al., 2006).The absorbance in the wells of the crystal violet plates were read on an automatic spectrophotometer at 600 nm (mode 1). This assay was repeated on three separate occasions (n = 3).

Statistical analysis

Data are presented in tables or figures as the mean ± SEM. The statistical significance of the differences between control and experimental groups was evaluated by Student's *t*-test using GraphPad Instat 3.06.

RESULTS AND DISCUSSION

In vitro Caenorhabditis elegans toxicity

It has been predicted that between 2008 and this year (2012) as many as 10,000 new chemicals have evaluated at an approximate expense of $2.1 billion which include several million animals (Reach, 2003). Consequently, alternative animal models (R.T. Peterson, et al. 2008) and high throughput screening methods are being explored to improve toxicity characterization, increase efficiency, reduce cost, as well as refine, reduce, or replace animal use (P. Flecknell, 2002). One alternative animal model that has been widely utilized and accepted in toxicity testing is the nematode, Caenorhabditis elegans. A number of toxicity studies have been conducted which compared the behavioral and toxic effects of metallic salts (G.L. Anderson, et al. 2001), alcohol (P.G. Morgan, et al. 1995), organophosphates (R.D. Cole, et al. 2004), anesthetics and organic solvents (J.A. Humphrey, et al.2007) in *C. elegans* and similar effects were observed in mammals. The data collected from these studies suggest that the toxicological effects observed in the worms closely reflected the effects observed in mammalian models for most compounds tested (Sprando R.L., et al. 2009). For this reason, we decided to test the Tartrazine (TZ), which is used as food colorant in many developing countries, and its by-product Sulphanilic acid (AS) at different concentrations on the worm *C. elegans* in the aim to develop our knowledge about their toxicity effects. Figure 4 presents the overall assessment of nematode growth and development of the N2 population at different concentrations. The analysis of results shows that after 16h of incubation at 20±1°C the eggs start hatching and the larvae begin to mature and reach the adulthood after about 64 hours. From this result, we can confirm that Tartrazine and Sulphanilic acid does not cause the death of the nematode *C. elegans* even with a very high concentration (3 mM). However, different stage of development differs from one product to another and according to the concentrations (Figure. 5). Indeed, the monitoring of the *C. elegans* development for each test repeated three times give us an average development for each concentration in order to compare the effect of different concentrations on the growth of nematodes. The results we have obtained shows that Tartrazine at a concentration of 0.05mM and 1mM, and Sulphanilic acid at a concentration of 0.05mM (Graph (B), (C) and (E)) have no effect on the normal development of nematodes and have the same percentages exist in the control (Graph (A)). While from a 3mM concentration of Tartrazine, and 1mM of Sulphanilic acid we see a retardation of hatching of 20% on average for both products, and this percentage remains the same for Sulphanilic acid to 3mM (Graph (D), (F) and (G)). For the last three concentrations we also notice a disruption of the transition stage of young adulthood to adulthood. For the case of AS2 it gets to 100% of adults after 72 h, whereas we only reached 90% at the same time for TZ3 and AS3. It remains to note that the AS3 shows another retardation in the larval stage L3 with a percentage of 10% on average. We can not predict now what reasons for these transition retardations between different stages of development in *C. elegans*, but we can estimate that a high concentration of Tartrazine and Sulphanilic acid can disrupt the cell cycle of *C. elegans* even if it does not cause death, a further study may give us a clear view on this subject.

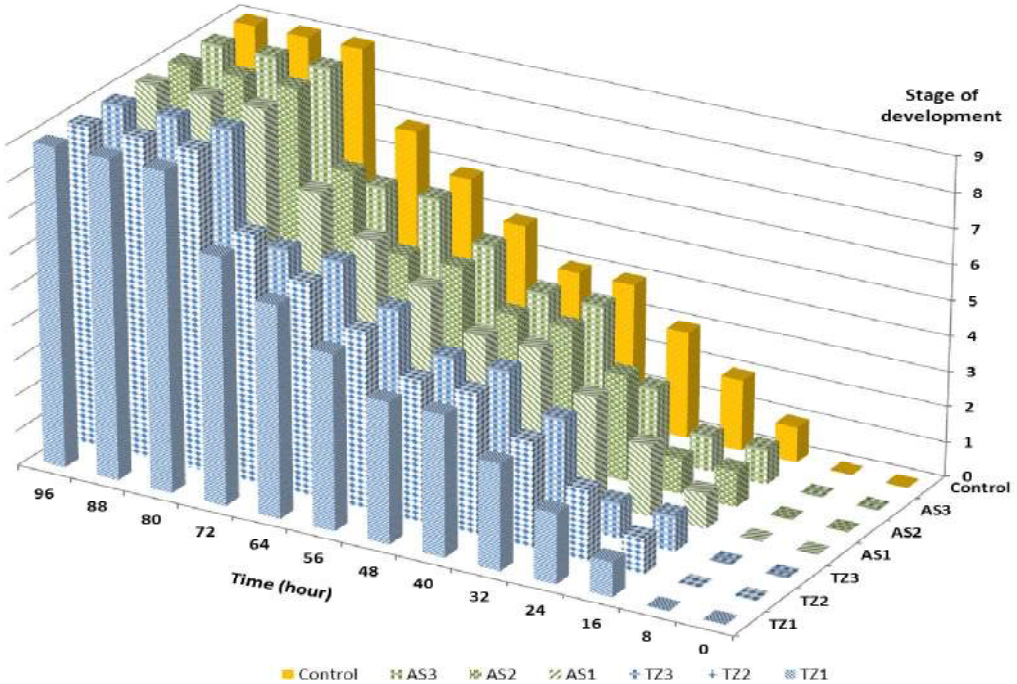
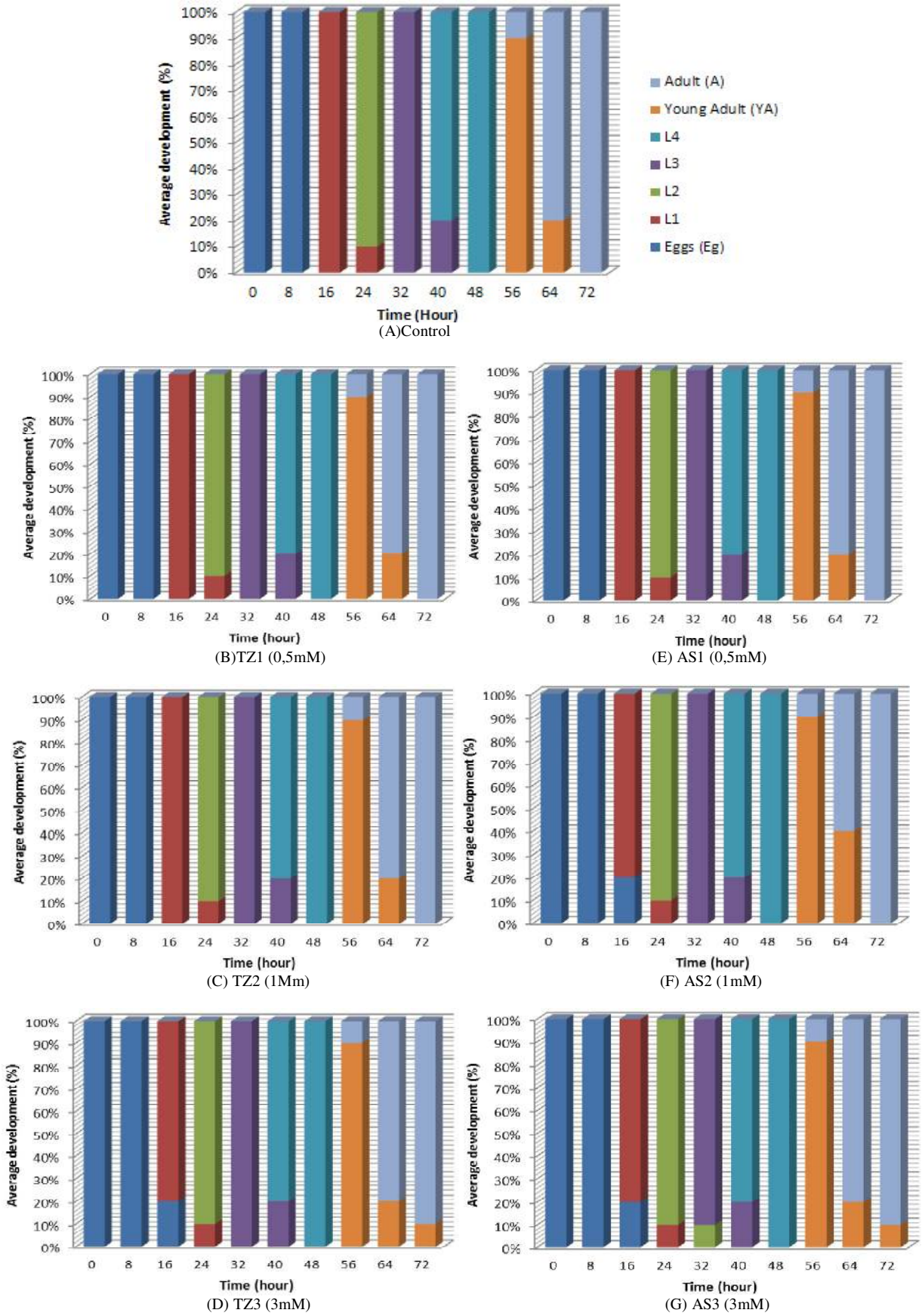


Fig.4: Assessment of nematode growth and development of *C. elegans* population at different stage of development (**0** = Eggs (Eg), **1** = L1 Larva, **2** = L2 Larva, **3** = L3 Larva, **4** = L4 Larva, **5** = Adult (A), **6** = (A) and (Eg), **7** = (A), (Eg) and L1, **9** = different stage (mixture)) during 96h with different concentration of Tartrazine (TZ1= 0,5mM, TZ2= 1mM and TZ3= 3mM) and Sulphanilic acid (AS1= 0,5mM, AS2= 1mM and AS3= 3mM).



(A)Control

(B)TZ1 (0,5mM)

(E) AS1 (0,5mM)

(C) TZ2 (1Mm)

(F) AS2 (1mM)

(D) TZ3 (3mM)

(G) AS3 (3mM)

Fig.5: Average of development of N2 population at the specified stage in different concentrations of Tartrazine (TZ) and Sulphanilic acid (AS).

Brine Shrimp toxicity assay

The Brine Shrimp test represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumor properties (McLaughlin, J. L. 1991).

In the present study the Brine Shrimp lethality of Tartrazine, which is a cheaper alternative to Saffron in morocco, and sulphanilic acid to Brine Shrimp was determined using the procedure of Meyer et al 1982.

Different concentrations of Tartrazine (10 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml, 80 µg/ml, 85 µg/ml, 90 µg/ml, 95 µg/ml, 100 µg/ml) were tested for their toxicity against Brine Shrimp using the Brine Shrimp lethality assay. The concentrations of Tartrazine did not show any significant toxicity against brine shrimp. Since in most cases toxicity is associated with pharmacological properties, it was deduced that Tartrazine has no significant activity.

The quantity of Sulphanilic acid which caused half of the Brine Shrimp to die within 24 h was ~82.3 µg/ml at the observation interval (Figure 6). Also data have shown that all Brine Shrimp died before 24 h only at the assay concentration levels of 100 µg/ml. Before 48 h and 72h all Brine Shrimp died at the assay concentration levels 90, 95, and 100 µg/ml, Figure 7 show the percentage of viable Brine Shrimp larvae exposed to the sulphanilic acid investigated in this study. In this assay, the positive control Colchicine showed significant toxicity; data have shown that 5 and 10 µ g / ml of Colchicine has a low inhibition, whereas 50 µg/ml has good inhibition of Brine Shrimp larvae (the percentage inhibition was 23%). This activity increases and reaches 60% at 100µ g / ml (Figure. 8).

KGN granulosa cell line Culture

Although primary-derived human granulosa cells are a good in vitro reproductive model to investigate the effects of toxins as they reflect the in vivo physiological state, granulosa-like cell lines are increasingly being used as a comparative tool. KGN are a human granulosa tumor cell line first developed and characterized by Nishi et al. (2001). Cells were obtained from a 63 year old woman with stage III ovarian cancer (Nishi, Y. et al. 2001). The KGN cells were exposed to all Tartrazine and Sulphanilic acid concentrations and were unaffected after 24 h of treatment exposure (Figure 9).

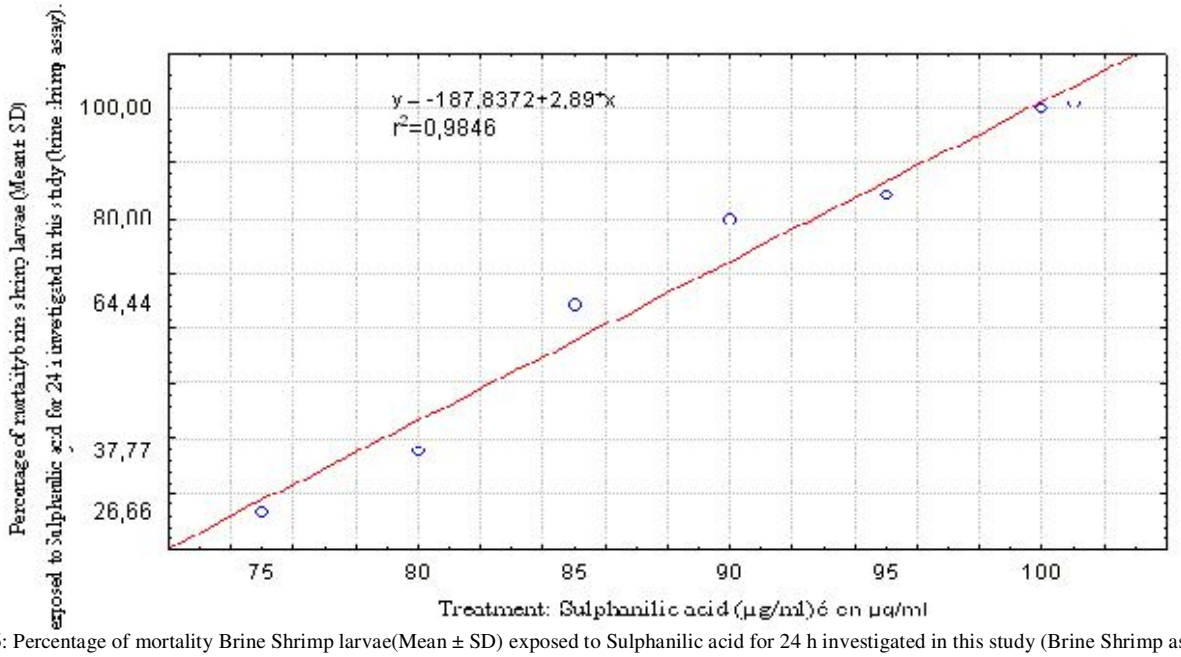


Fig. 6: Percentage of mortality Brine Shrimp larvae(Mean ± SD) exposed to Sulphanilic acid for 24 h investigated in this study (Brine Shrimp assay).

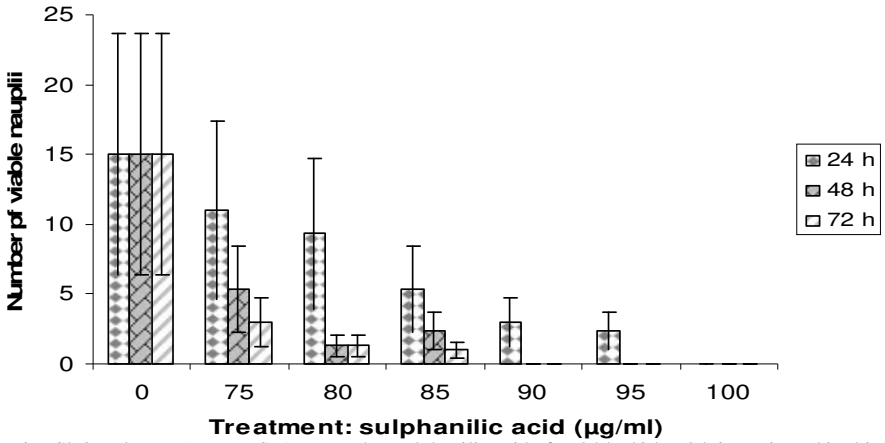


Fig. 7: Percentage of viable Brine Shrimp larvae (Mean ± SD) exposed to sulphanilic acid after 24 h, 48 h, 72 h investigated in this study (Brine Shrimp assay).

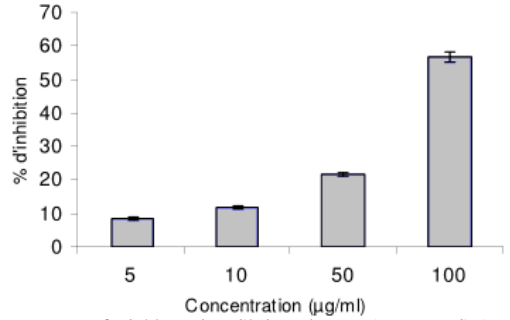


Fig. 8: Percentage of viable Brine Shrimp larvae (Mean ± SD) exposed to Colchicine after 24 h, investigated in this study (Brine Shrimp assay).

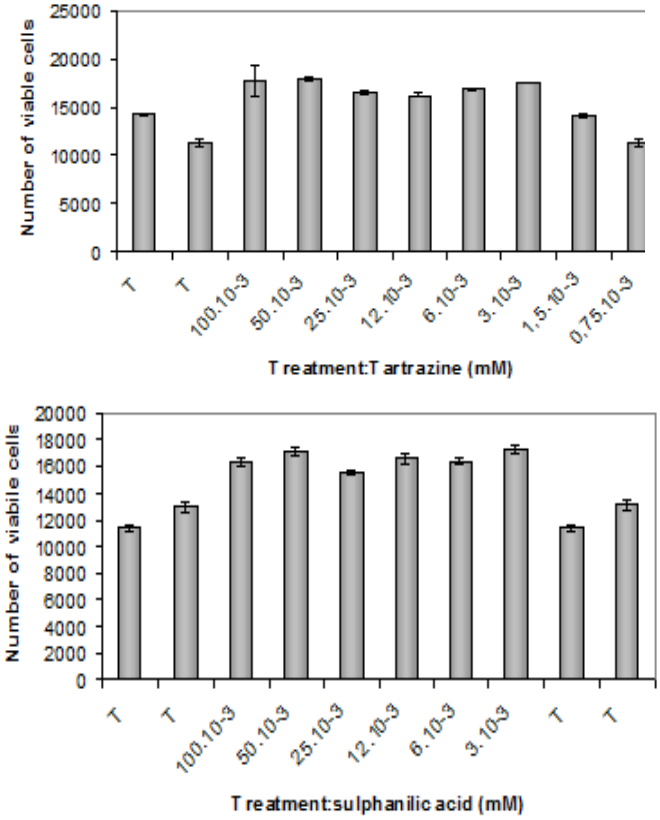


Fig. 9: Viability of KGN after Exposure to Tartrazine and sulphanilic acid.

CONCLUSION

The results presented in this paper show the toxicity of Tartrazine and Sulphanilic acid on, *C. elegans,* Brine Shrimp eggs and KGN Cell. It appears that Tartrazine and Sulphanilic acid do not have anti-nematode effect, but these products may have some effect on the cell cycle of the nematode (3mM for Tartrazine and 1mM for Sulphanilic acid),. Although Tartrazine did not show any significant toxicity against brine shrimp, Sulphanilic acid was mildly toxic (LC50 value (µg/ml) of ~82.3 µg/ml). Finally, the KGN cells were unaffected after 24 h of treatment exposure with Tartrazine and Sulphanilic acid.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

We are grateful to Dr Mohammed Bellaoui for help with worm studies and for critical reading of the manuscript. This research is partially funded by the CUD (Commission Universitaire pour le Développement).

REFERENCES

Anderson, J.E., Goetz, C.M., McLaughlin, J.L., Suffness, M.,. A blind comparison of simple bench-top bioassays and human tumor cell cytotocicities as antitumor prescreens. Phytochemical Analysis. 1991; 2: 107–111.

Antoshechkin, I., and Sternberg, P. W. The versatile worm: Genetic and genomic resources for Caenorhabditis elegans research. Nat. Rev. Genet. 2007; 8: 518–532.

Borzelleca, JF. and Hallagan JB. Chronic toxicity/carcinogenicity studies of FD & C yellow nº 5 (tartrazine) in rats. Food. Chem. Toxicol. 1988; 26(3): 179-87.

Brenner, S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77: 71–94.

Collins TFX., Black TN., Brown LH. and Bulhack P. Study of the teratogenic potencial of FD & C Yellow nº 5 when given by gavage to rats. Food Chem. Toxic. 1990; 28(2): 821-827.

Collins TFX., Black TN., Bulhack P. and O'donnell, MW. Study of the teratogenic potencial of FD & C Yellow nº 5 when given in drinking-water. Food Chem. Toxic. 1992; 30 (4): 263-268.

Freshney, R.I. 2005. Culture of Animal Cells: A Manual of Basic Technique; John Wiley & Sons: Hoboken, NJ, USA. 346-348, 359-373.

G.L. Anderson, W.A. Boyd, P.L. Williams. Assessment of sublethal endpoints for toxicity testing with the nematode C. elegans. Environ. Toxicol. Chem. 2001; 20: 833–838.

Hitchcock D.R. , S.E. Law, J. Wu, P.L. William. Determining toxicity trends in the ozonation of synthetic dye wastewaters using the nematode Caenorhabditis elegans Arch. Environ. Contam. Toxicol. 1998; 34: 259–264.

J Devlin and T J David. Tartrazine in atopic eczema. Arcjves of Disease in Cheldhood. 1992; 67: 706-711.

J.A. Humphrey, K.S. Hamming, C.M. Thacker, R.L. Scott, M.M. Sedensky, T.P. Snutch, P.G. Morgan, H.A. Nash ; A putative cation channel and its novel regulator: cross-species conservation of effects on general anesthesia. Curr. Biol. 2007; 17(7): 624–629.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1965. Specifications for the identity and purity of food additives and their toxicological evaluation: Food colors and some antimicrobials and antioxidants. Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 309.

JONES, R., RYAN, AJ. and WRIGHT, SE. The metabolism and excretion of tartrazine. Food Cosmet Toxicol. 1964; 2: 447.

M.J.MOSHI, E. INNOCENT, J.J. MAGADULA, D.F. OTIENO, A. WEISHEIT, P.K. MBABAZI and R.S.O.NONDO. Brine Shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. Tanzania Journal of Health Research. 2010; 12: 1.

MAEKAWA, A., MATSUOKA, C., ONODERA, H., TANIGAWA, H., FURUTA, K., KANNO, J. and JANG, JJ. Lack of Carcinogenecity of Tartrazine (FD & C YELLOW Nº 5) in the F344 Rat. Food Chem. Toxicol. 1987; 25 (12): 891-896.

McLaughlin, J. L. 1991. Crown gall tumors on potato discs and Brine Shrimp lethality: Two simple bioassays for higher plant screening and fractionation. In: Methods in Plant Biochemistry. 1-32.

Mehedi, S. Ainad-Tabet, N. Mokrane, S. Addou, C. Zaoui, O. Kheroua and D. Saidi. Reproductive Toxicology of Tartrazine (FD and C Yellow No. 5) in Swiss Albino Mice. American Journal of Pharmacology and Toxicology. 2009; 4 (4): 128-133.

Meyer N, Ferrigni NR, Putnam JE: Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982; 45:31-32.

Neuman, l., Elian, R., Nahum, H., Shaked, P. & Creter, D. The danger of 'yellow dyes' (tartrazine) to allergic subjects. Clin. Allergy. 1978; 8: 65.

Nishi, Y.; Yanase, T.; Mu, Y.; Oba, K.; Ichino, I.; Saito, M.; Nomura, M.; Mukasa, C.; Okabe, T.; Goto, K. Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. Endocrinology. 2001; 142: 437–444.

P. Flecknell. Replacement, reduction and refinement. ALTEX, 2002; 19 (2): 73–78.

P.G. Morgan, M.M. Sedensky. Mutations affecting sensitivity to ethanol in the nematode, Caenorhabditis elegans. Alcohol Clin. Exp. Res. 1995; 19 (6): 1423–1429.

PATTERSON, RM. and BUTLER, JS. Tartrazine induced chromosomal aberrations in mammalian cells. Food Chem. Toxicol. 1982; 20 (4): 461-5.

Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O. Brine Shrimp lethality activity of Thai medicinal plants in the family Meliaceae. Naresuan Univ J. 2004; 12:13-18.

R.D. Cole, G.L. Anderson, P.L. Williams. The nematode Caenorhabditis elegans as a model of organophosphate-induced mammalian neurotoxicity. Toxicol. Appl. Pharmacol. 2004; 194:248–256.

R.T. Peterson, R. Nass, W.A. Boyd, J.H. Freedman, K. Dong, T. Narahashi. Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology. 2008; 29 (3): 546–555.

Reach,. Commission of the European Communities. Proposal for a Regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH), establishing a European Chemicals Agency and amending Directive 199/45/EC and Regulation (EC) {on Persistent Organic Pollutants}. Brussels. 2003; 644.

Riddle, D. L., Blumenthal, T., Meyer, B. J., and Preiss, J. R. 1997. In: C. elegans II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ROXON J., RYAN, A. and WRIGHT, S. Enzymatic reduction of tartrazine by Proteus vulgaris from rats. Food Cosmet Toxicol.1967 b; 5: 645.

Siddiqui, E.J.; Shabbir, M.A.; Mikhailidis, D.P.; Mumtaz, F.H.; Thompson, C.S. The effect of serotonin and serotonin antagonists on bladder cancer cell proliferation. Br. J. Urol. Int. 2006; 97: 634–639.

Sprando R.L., Olejnik N., Cinar H.N., Ferguson M. A method to rank order water soluble compounds according to their toxicity using Caenorhabditis elegans, a Complex Object Parametric Analyzer and Sorter, and axenic liquid media . Food and Chemical Toxicology. 2009; 47(4): 722-728.

Stenius, B. S. M. & Lemola, M. Hypersensitivity to acetylsalicylic acid (ASA) and tartrazine in patients with asthma. Clin. Alleryy. 1976; 6: 119.

Stiernagle T., WormBook, ed. The C. elegans Research Community, WormBook, 2006; doi/10.1895/wormbook.1.101.1,http:// www.wormbook.org.

Sulston, J. and Hodgkin, J. 1988. In: The Nematode Caenorhabditis elegans, W.B. Wood, ed. (New York: Cold Spring Harbor Laboratory Press. 587.

WÜTHRICH, B. Adverse reactions to food additives. Ann. Allergy.1993;71(4): 379-84.

How to cite this article:

Imane Himri, Abdelkarim Guaadaoui, Faiza Souna, Mohammed Bouakka, Ahmed Melhaoui, Abdelkader Hakkou, Ennouamane Saalaoui., Toxicity Testing of Tartrazine using the Nematode *Caenorhabditis Elegans*, Brine Shrimp Larvae (*Artemia Salina*) and kgn Granulosa Cell Line. J App Pharm Sci, 2013; 3 (11): 051-058.