

**RESEARCH ARTICLE**

# Fluoxetine contamination in Dietary/Nutritional Supplements (un)bridges the quality of life for the youth to the elderly consumer.

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**Abstract**

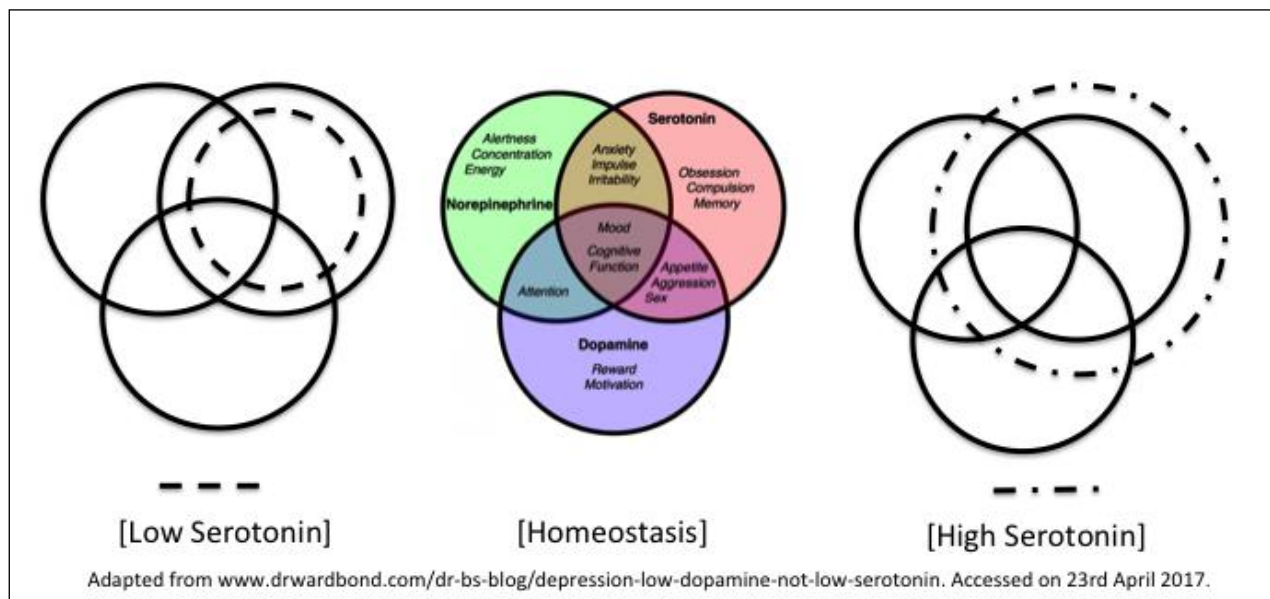
Dietary/Nutritional supplements have become popular in use by a spectrum of different consumers, including the elderly, those aged 65 and over, as a 'new' market. In the USA, their numbers have been increasing from 35 million in 2000, to a projected 69 million by 2030. Dopamine, serotonin, epinephrine neurotransmitter homeostasis is important in the 'healthy' physiological continuum of all ages. Imbalance in these neurotransmitters, over time, manifests in various 'disease' states, such as Obsessive Compulsive Disorder, Parkinson's Disease, and Schizophrenia. In context, dietary/nutritional supplements may contain contaminants/adulterants that could distort the 'normal' equilibrium of neurotransmitters. Therefore, for this investigation fluoxetine a Schedule 5 (South Africa) selective serotonin reuptake inhibitor (SSRI) drug was investigated. The extent of fluoxetine as contaminant/adulterant in dietary/nutritional supplements is not widely known. Further, fluoxetine prescriber caution for treatment (elderly), are adverse effects, particular CNS effects, such as nervousness, agitation, anxiety or excessive sedation, and insomnia. The aim of this study was to determine whether commercially available dietary/nutritional and traditional supplement products contained fluoxetine, even though the manufacturer may not declare this on the product label. A total of 138 dietary/nutritional supplements products formed part of the assessment. The products were laboratory analysed for fluoxetine, as part of an extensive multi-compound 'screen', using Tandem Liquid Chromatography Mass Spectrometry. The concentration of fluoxetine was then estimated via calibration curve standards that formed part of the extraction and analysis. The number of 'positives' for the tested products for fluoxetine in the overall sample was 54%, for South African produced products 67%, and, for imported products, bought in South Africa 56%. The median concentration estimate for fluoxetine in the products were, 3.9 µg/g for the overall sample, 5.2 µg/g for South African produced products, and 20.1 µg/g for imported products, bought in South Africa.

**Keywords:** Dietary/nutritional supplements, information labels and warnings, laboratory screen testing, antidepressants, children adolescence/youth, adults, elderly.

## Background/ Introduction

Dietary/Nutritional supplements have become popular in use by a spectrum of different consumers, including the elderly as a ‘new’ market. It is anticipated that the market would have reached US\$93.15 billion by the year 2015. A category of consumer that is often neglected are those aged 65 and over (the elderly). The probability that this age group is more susceptible to adverse effects, in the event of potential contamination/adulteration in dietary/nutritional supplements could occur at higher prevalence rates. Evidence from the Economic Research Service of United States Department of Agriculture based on the USA population, indicates that the number of elderly will increase from 35 million in 2000 to 69 million in 2030. These anticipated trends will contribute to the dietary/ nutritional supplement industry responding to new niche areas of consumers (*humans*) and consumption to that of the conventional marketing practices and marketing. In humans, the neurotransmitters dopamine, serotonin, norepinephrine play an important part, for required healthy

physiological homeostasis. Physiological disproportions of these neurotransmitters serve as indicator(s) in various ‘disease’ states, such as Obsessive Compulsive Disorder, Parkinson’s Disease, and Schizophrenia, and its related behaviour. Dietary/nutritional supplements products may contain contaminants/adulterants that could distort the ‘normal’ equilibrium of the neurotransmitters dopamine, serotonin, and norepinephrine, as presented in “Fig. 1”. The distortion could potentially contribute or exacerbate harmful short-and long-term health (*disease*) consequence and related behaviour patterns due to the irregular imbalance of neurotransmitter that may be created, as a result of consumption of such products. Also, insufficiencies in regulation of the industry, increase the risk of the dietary/nutritional supplements being contaminated, as has been shown in various studies of contamination, Van der Merwe et al (2004) [1] prohormones 67%, Gabriels et al (2015) [2] melamine 47%, and Cohen et al (2017) [3], recently showed four experimental stimulants found in sports and weight loss supplements.



**Figure 1.** Functional relationship of neurotransmitters dopamine, serotonin, and norepinephrine

The focus of this study is on Fluoxetine as an adulterant/contaminant in dietary/nutritional supplements, formed part of a broader laboratory ‘screen’ assessment. Fluoxetine was included due to its use clinically for over 25 years to treat major depression and other psychiatric disorders [4], and the potential presence as a ‘mood enhancer’ in dietary/nutritional supplements was explored. The importance for such assessment is due to the increased use of such products, and the increased awareness of illegal/prohibited substances within these products. [1, 5]. In context, special concern is warranted considering, the United States Food and Drug Administration (USFDA), has approved the use of Fluoxetine for use in children/adolescents age 7–17 years old [1,2,3] [5,6,7]. A subsequent report after the approval by the U.S. Department of Health and Human Services' Panel on Developmental Toxicity of Fluoxetine, pointed to sufficient evidence existing to conclude that fluoxetine exhibits developmental toxicity [5]. The dietary/nutritional supplements industry generally responds to increase in demand for products, thus making it attractive to ‘capture’ both adolescent and the elderly market, in addition to its current consumer base. Fluoxetine, a hydrophobic molecule (N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine) is a

selective serotonin reuptake inhibitor (SSRI) [8]. It is a potent psychotropic drug [9] with great anxiolytic and antidepressant efficacy in humans, in clinical setting [10,11], and varied findings in animal studies [11,12]. It has become increasingly clear that these agents not only are effective during the acute phase of therapy, but also prophylactic during the maintenance phase of therapy, that is beneficial in preventing the recurrence of depression [13,14].

A growing number of syndromes and disease as presented in Table 1, are treated with fluoxetine, from anxiety, to depression and obsessions, resulting in the rapidly rising, annual intake and the number of patients taking these drugs. Table 2 reflects the dose usage of Fluoxetine in selected studies, cell lines, mice, rats and patients. Table 3 reflects the potential consequence and cautions of antidepressant drug use (Fluoxetine), based on the selected range of studies.

This study, therefore investigated Fluoxetine a Schedule 5 (South Africa) selective serotonin reuptake inhibitor (SSRI), as an embedded undeclared drug in dietary/nutritional supplement products. The aim was to determine whether commercially available nutritional and traditional supplement products contained fluoxetine.

**Table 1.** Examples of Syndrome and Disease treatment with Fluoxetine

Syndrome or disease	Observation	Reference
Cancer related fatigue (CRF)	A complex syndrome associated with tiredness and depressed mood	15
Major depressive disorder (MDD)	Disabling psychiatric disorder that can affect one out of every five individuals, and 16% of adults in different points in life Role in longer-term prevention of recurrence remains unconfirmed	13,16,17
Asthenic syndrome (SCS)	Inherited neurodegenerative disease that causes mutations in the acetylcholine receptor (AChR) affecting neuromuscular transmission.	18
Epilepsy (established)	To manage anticonvulsant effects against the spontaneous seizure activity over the long-term	19
Persistent somatoform pain disorder (PSPD)	To manage patients with chronic pain that lasts for several months and limits a person's work, relationships, and other activities	20
Post-traumatic stress disorder (PTSD)	Trauma related incidences	11,12
Sexual dysfunction	Depressed patients may be complicated by adverse sexual side effects of antidepressant drugs, which may also lead to discontinuation of treatment	16
Intergenerational effects	The impact of therapy for early life stress related mental problems Consequence of chronic treatment increased (rather than reduced) anxiety in rats	11,22
Behavioural disorders in children	Despite little evidence regarding the safety and efficacy of the drug the use in children has grown rapidly	5

**Table 2.** Examples of Fluoxetine doses/use in selected studies

Dose	Use	Study type	Comment	Reference
10mg/kg per day	Intragastric administrations	Rats	Effects of maternal separation on contextual fear and anxiety-like behaviors in adult male rats of the first generation (F1), and also the transmission effects on the second generation (F2) generated from the F1 adult male rats	5
3mg/kg/day administered in drinking water	Injection of PBS or tumour cells.	Mice	Dose has shown to block depressive-like behaviour in mice without affecting the locomotor activity of healthy mice	15
5 mg/kg	Treatment	Mice Cell lines	Decreased plasma TNF $\alpha$ in mice injected with lipopolysaccharide Reduced TNF $\alpha$ expression in immune activated microglia and monocyte cell lines	15
7 or 18 mg/kg/day	Drug assignment and administration	Rats	Randomly assigned control, fluoxetine, reboxetine, and venlafaxine groups	22
10mg/kg/day for 7 days	Administered sub- acutely	Rats	The eventual metabolic impairment induced by the lithium-pilocarpine model of epilepsy	19
15 mg/kg/day	Chronic administration	Rats	Effects on liver injury via the measurement of liver enzymes, oxidative stress and histopathology- animal model of depression (chronic social isolation), and control	23
5 or 10 mg/kg	Treatment	Rats	Observed increased anxiety. Amitriptyline and Fluoxetine affected the traditional gross measures and also produced changes in incorrect transitions and regional distribution of grooming behaviour	24
10 mg/kg/day	Treatment	Aquatic invertebrates	Dose are toward the bottom of the range of plasma levels found in patients taking 20–80 mg/day Prozac (100–700 ng/ml)	25
18 mg/kg/day	Treatment	Territorial frog	Dose (approximately 560 ng/ml) they are toward the high end of this range	27

**Table 3.** Potential consequence and caution of Fluoxetine use

Consequence	Caution	Reference
Associated with changes in physiological process	Such sleep architecture and wakefulness, digestion, arousal, stress, and mood	26, 27
Drug- induced toxicity effects	Makes the liver's functional integrity the vulnerable target organ, due to antidepressant and antipsychotic drug metabolism Contraindication is renal failure and cautions for use are in hepatic or renal impairment, epilepsy, diabetes, and the elderly	22,23
Extensive biotransformation to the active metabolite nor-fluoxetine	Generates acceptable outcomes in mood and anxiety disorders that may result in hepatic toxicity and undesirable side effects	22, 23,28
In mouse model studies, observed changes	Changes such as steatosis (fatty change) and hepatocyte enlargement	28
Most prescribed psychotropic medication	Excreted by humans as 11% parent compound and 7% nor-fluoxetine (NFLX), both of which are pharmacologically active	28
Excretion by patients of these compounds when disposed of into wastewater systems	Has an eco-toxicological impact on aquatic environment, as these compounds bio-concentrate (accumulate in fish tissues), most notably in the brain. Other human medicines detected in sewage treatment plant effluents, surface waters, seawaters, groundwater and some drinking waters	9,10,17, 22, 25,28
Reported effects from water concentrations ng/l to $\mu$ g/l (acute and chronic effects)	Affect reproduction, foraging, stress responses, and aquatic organism locomotion	15,17

## Methodology

The dietary/nutritional supplements products investigated were based on the combination of a preliminary assessment of

popular brands, and research budget availability at the time of study implementation. The products assessed (n = 138) were obtained from (i) direct

purchases from shops, pharmacies and outlets, (ii) directly from consumers, and (iii) from suppliers, manufacturers and distributors. The nutritional supplement formulation types consisted of powder (P) 39% (54), tablet ground to powder (P/T) 30% (42), powder in capsule (P/C) 22% (30), liquid in capsule (L/C) 6% (8), liquid (L) 3% (4). The products were laboratory analysed for fluoxetine using Tandem Liquid Chromatography Mass Spectrometry. This laboratory technique was selected because it offers the desired multiple chemical compound (drug) separation, selectivity, and sensitivity with appropriate detection capability. The approach was to optimize (*via infusion*) using the analytical instrumentation to detect on the Applied Biosystems Sciex API 3200 mass spectrometer for fluoxetine (*approximately 300 ng/ml*). The sample extraction for fluoxetine procedure formed part of a multiple screen, yielded the best efficiency (triplicate samples (n=3) with the organic solvent tertiary butyl methyl ether (TBME), compared to diethyl ether, and chlorobutane. The brief steps for the procedure, consisted, weighing of samples and mixing with methanol. Britton Robinson universal buffer (0.1 M, pH 11) was then added to all standards and samples, followed by adding of TBME. The standards and samples were then centrifuged at 5° C at a high speed (2500 rpm) for 5 minutes. This ensured good separation of the organic layer TBME from and the non-organic layer. Liquid nitrogen was then used to freeze the bottom non-organic layer. Standards and samples were then evaporated in a nitrogen purge concentrator. The dried standards and samples were then reconstituted with mobile phase which consisted of acetonitrile and formic acid, vortexed and then transferred to 96 well polypropylene plates. The 96 well plate containing the

standards and samples was then centrifuged at 2500 rpm. 20 µl was injected onto the HPLC column for analysis. The chromatography method utilized prior to MS/MS detection was performed using an Agilent 1200 series HPLC with a Phenomenex Gemini C18 NX (5µm, 110A, 50x2 mm) analytical column. The mobile phase consisted of acetonitrile: 0.1% formic acid (1:1, v/v) and was delivered at a constant flow rate of 0.5 ml/min. The column was kept in a column compartment at 35 °C. The retention time for fluoxetine was 4.53 minutes. For the analysis for Fluoxetine the transition(s) from 310.2 to 148 atomic mass units was used for the final analysis which offered best assessment, compared to the transition from 310.2 to 117.2 atomic mass units. Calibration curve was established using prepared serial dilution to establish concentrations starting from 1000 ng/ml to 1.953 ng/ml (n=3). For the assessment screen the Lower Limit of Quantification was 1.95 ng/ml (19 ng/g). Regression analysis was used to estimate the concentration of fluoxetine in the dietary /nutritional supplement products investigated.

## Results

Of the overall products acquired, 54% tested 'positive' for fluoxetine. The summarized data (Table 4) shows that a greater percentage of products (67%) produced and purchased in South Africa, compared to those nutritional supplements products (56%) imported and purchased in South Africa were contaminated with fluoxetine. Based on median concentration values supplements imported and purchased in South Africa had a higher level of fluoxetine contamination (20052 ng/g) compared to those produced and purchased in South Africa (5194 ng/g).



**Table 4.** Summary of Nutritional Supplement products investigated for Fluoxetine

		Overall samples acquired in South Africa (n=138)	Produced and purchased in South Africa (n=27)	Imported and purchased in South Africa (n=50)
<b>Qualitative Assessment Screen</b>	Total No.	75	18	28
	Positives (%)	54	67	56
<b>Estimate Concentration</b>	≥ LLOQ (ng/g) Low	361	555	552
	≥ LLOQ (ng/g) High	525639	431710	104309
	Median (ng/g)	3890	5194	20052

Table 5 shows that of overall samples investigated, 40% of products that had fluoxetine contamination were powder products. Products produced and purchased in South Africa having fluoxetine

contamination were highest (47%) in tablet formulation. Imported products purchased in South Africa that were contaminated with fluoxetine were highest (31%) in powder products.

**Table 5.** Nutritional Supplement formulation types investigated that tested 'positive' for Fluoxetine contamination

Formulation Types	Number of 'positives' for Fluoxetine (Samples acquired in South Africa)	Overall 'positives' for Fluoxetine (%)	Produced and purchased in South Africa (n=27)	Produced and purchased in South Africa (%)	Imported product purchased in South Africa n=50	Imported product purchased in South Africa (%)
<b>P</b>	29	<b>40</b>	2	12	22	<b>31</b>
<b>P/T</b>	16	22	8	<b>47</b>	1	4
<b>P/C</b>	18	25	5	29	4	14
<b>L/C</b>	7	10	2	12	1	4
<b>L</b>	2	3	0	0	0	0
<b>Total</b>	<b>72</b>	<b>100</b>	<b>17</b>	<b>100</b>	<b>28</b>	<b>100</b>

Table 6 provides an illustrative example of the Fluoxetine consumption at identified high, medium and low values.

**Table 6.** Illustrative examples of fluoxetine contamination levels and consumption

Description	High	Medium	Low
Concentration of Fluoxetine (ng/g)	525639	5194	361
Formulation Type	P	P	P
Indication of Fluoxetine content on label (Y/N)	No	No	No
Product consumption quantity *	35	35	35
Local/ International Product purchase South Africa	International	Local	Local
Total Fluoxetine consumption per day (ng)	18397365	181790	12635
Total Fluoxetine consumption per day (mg)	18.4	0.182	0.013
Actual mg/kg of Fluoxetine (Average body weight 65.667kg)	0.280	0.0028	0.0002

\* Estimate per day 32 to 43 grams per day [26].

## Discussion

The study shows (Table 4) that of the overall samples acquired in South Africa (n=138), 75 (54%) products tested 'positive' for fluoxetine as a contaminant. For those products purchased and produced in South Africa the number of 'positives' for fluoxetine (n=27) was 18 (67%), and imported products and purchased in South Africa (n=50) 29 (56%). None of the products identified as fluoxetine 'positive' had information on the label stating that fluoxetine may be present as a contaminant. Of further concern specifically, is that fluoxetine is a Schedule 5 drug (*South Africa*) and its presence in dietary/nutritional supplements products, warrants further enquiry. The supplement products often have several label information categories, specifically regarding Dangers and Warnings, Pseudo-Science, Disclaimers, and Claims [38], that may not be apparent to the consumer [38]. Consumers' health may be at chemical risk if non-approved ingredients are used or contamination occurs [32]. This finding of fluoxetine in products should therefore be of concern, due to potential adverse health events. The risk and consequence of fluoxetine may be in specific and/or multi-folded as presented;

### Behavioural alterations

The study by Xiong et al. [11] (Fluoxetine) showed that mice can act as 'silent' or asymptomatic carriers of specific behavioural alterations [11], in that certain traits can be transmitted and expressed by the progeny without being clearly expressed by parents themselves [6,11]. The study by Norden et al. [15] showed that treatment with 3 mg/kg/day of fluoxetine was sufficient to ameliorate depressive-like behaviour in tumour-bearing mice without changing mood-related behaviour in healthy control mice [15]. In work by, Erman [6],

Norden [15], Enginar [24], and Gray [22], done in rats, with high doses of Fluoxetine, showed reduced head dipping and rearing, genital grooming, and enduring effects on attention related behaviours [6,15,22,24]. A study by Lebrón-Milad et al. [33], conducted with healthy subjects, on acute treatment (SSRI's) showed increased recognition of fearful faces, indicating an increase in the processing of anxiety-related stimuli [33]. SSRI's could also change, social behaviours in vertebrates, including group affiliation, defensiveness, aggression, and territoriality (*clinically and experimentally*) as presented by Ten Eyck et al. [27]. These studies show the respective impact that could potentially be hereditary and dose dependent [27]. Therefore the presence of embedded fluoxetine (*undeclared*) in dietary/nutritional supplement products, based on the respective study findings, should raise suspicion.

### Gender difference and treatment (human and animals)

The epidemiological study by Lebrón-Milad et al. [33] also indicates that women have a higher prevalence and higher symptom duration for anxiety disorders and depression, yet research (*animal*) involving antidepressants is overwhelmingly predominated by male-only studies [33].

Research by Gray et al. [22] illustrated by clinical reports, show that men respond more favourably than women to fluoxetine prescribed for both depression and anxiety [22]. Further, Lebrón-Milad et al [33] showed that that acute administration of fluoxetine increased fear responses equally in males and females during extinction learning and extinction recall [33]. A contrary study (*clinical*) Go'mez et al [34] to that of Gray et al [22], suggest females responded and benefitted better to the action of SSRIs (*e.g. Fluoxetine*) to the

exposure of 10 mg/kg dose with little evidence of effects from higher and lower doses as stated by LaRoche et al [5] and Erman et al [6]. Males were impaired, following exposure to the 5 or 10 mg/kg doses of SSRI's, with little evidence for effects at the higher dose of 15 mg/kg [5,6]. These *differential therapeutic actions* would result in longer durations of drug action in females compared to males due to the dose-response function that is different for each gender (*sex*) [6,34].

### **Acute, chronic, and high dose effects**

Stemming from 'Paracelsus' famous notion that 'poison' is in everything, and nothing is without poison: the dose (*fluoxetine*) makes it either a poison or a remedy' [9] and thus has application to embedded contaminants/adulterants in dietary/nutritional supplements. Fluoxetine may not produce therapeutic effects acutely, but may trigger toxicity at high doses [9]. Kermorgant et al [29] study further shows that high dosages of Fluoxetine (120 mg/day for 5 weeks) significantly prolonged the cardiac autonomic control (QTc interval) through the disrupting mechanism of action on ventricular repolarization [29], in patients [18, 21]. This is based on a case report study by Szólics et al. [26], of a patient (28 years old male) with past medical history of depression had attempted suicide (*with 1.8 g fluoxetine*) prior to admission to emergency room (ER). Observation in the study shows the consequence of high doses (*attempted suicide*), even after gradual recovery and regaining consciousness, the patient still suffered from severe akinetic rigid syndrome, gait apraxia and cognitive disturbance, and remained socially dependent for about 1.5 months [26]. In an animal study (*in mice*) by Zlatkovic' et al. [23], showed that hepatic dysfunction was probably due to drug-induced damage in the cell membrane resulting in steatosis (*fatty*

*change*) and hepatocyte enlargement [23]. Contrary views by Sumpter et al [17] states that 'low-dose' effects (*concentrations*), can cause effects that higher doses/concentrations do not [17, 35]. The knowledge that the role of serotonin in behaviour as presented in Stewart et al. [9] can be assessed through acute treatment with Fluoxetine, makes it an important neuropharmacological tool [9]. Further, the altered expression of serotonin receptors, seen only chronically (*several weeks later*) [9], is a result of the long-term anxiolytic/antidepressant effects [9,33]. These findings are further corroborated through the accumulation potential of fluoxetine within various respective tissue types that points to, that doses may differ for the same anxiolytic effects, between various invertebrates. Lower vertebrates such as *fish*, and mammalian species, also differ physiologically and developmentally [9]. Whilst in this study (*our paper*), focus was only assessed for fluoxetine, however, the knowledge that fluoxetine bioconcentrates in fish tissue, both in the laboratory and in wild-caught fish (*wastewater after excretion by patients*) [29], with bioconcentration factors higher for nor-fluoxetine (NFLX) (*metabolite*), and in excess of 74 for fluoxetine (FLX) [10], warrants further investigation in dietary/nutritional supplements. The study by Mennigen et al [10] shows that concentrations of 1.6 ng/g FLX and 8.9 ng/g NFLX [10] have been identified in the brain. Thus embedded undeclared fluoxetine in nutritional supplements may contribute to altered serotonin levels and adversely modify the brain functioning of the consumers of dietary/nutritional supplement products.

### **Doses in children**

Greater concern becomes apparent considering the impact of '*doses in children*', where the safety and efficacy of



antidepressant use remains a controversial issue [1]. The study points to the issue of clinical relevance, referring to the human therapeutic dose of FLX being 1mg/kg/day [1], whilst the dose in the specific study [1] ranged from 5-15 mg/kg (*higher dose*). The relevance of the observation by LaRoche et al [1] is further important, considering the ease with which dietary/nutritional supplement products are accessible to the youth, as a lifestyle [27], and as a specific 'target' market.

### **Protective effect**

The study of Shiha et al. [19] shows that the mechanism of action for fluoxetine on the glutamatergic system could provide neuroprotection in a variety of excitotoxic-related pathologies, not only epilepsy [12, 19, 21]. This fast-signaling system is important for information processing in neuronal networks of the neocortex and hippocampus in particular. A study by Norden et al. [15] showed that fluoxetine confirmed to prevent increases in physical and electrical brain wave activities, but not sleep disturbance [15]. Thus embedded (*although undeclared*) fluoxetine in dietary/nutritional supplements may have a beneficial protective effect (*treating*) underlying behavioural and neurochemical responses based on omega -3 fatty acid deficiency [36], and in particular treatment [24] of disease and conditions associated with the elderly.

### **Sexual dysfunction and performance**

The study by Khazaie et al [16] indicates that SSRIs (*Fluoxetine*), can negatively affect the sexual response cycle, causing decrease in libido, impaired arousal, erectile dysfunction and absent or delayed orgasm [16]. Further, prevalence rates of up to 80% of SSRI-induced dysfunction have been reported, considering that the dysfunctions (*e.g. MDD patients*) [16]

results in marked interpersonal difficulties. Also that impairment, in desire/drive was more prevalent in women compared to the prevalence in men, being impaired in orgasm/arousal [16], with other results showing the opposite prevalence in men to that of women [16]. This contradictory finding could have implication in context of embedded fluoxetine in dietary/nutritional supplements, and in particular where these products are marketed to the consumers as '*gender non-specific*'. Therefore assessing the frequency and differences between different SSRIs [16] is important. The association may not be immediately apparent within the context of adulterated dietary/nutritional supplements, being a contributory factor to complications for a pre-existing sexual dysfunction. Potential discontinuation of treatment, therefore suggest that clinicians should assess sexual dysfunction both before and after prescribing antidepressant drugs [16], due to its bi-directional association [16] and potential impairment in orgasm/arousal [16]. The study by Mennigen et al. [10], although done in male fish, at environmentally relevant concentrations, and may be similar to the human reproductive system, shows that FLX disrupts reproductive physiology (*i.e. spermatogenesis*) [10]. By implication, fluoxetine embedded as an adulterant in dietary/nutritional supplements could have similar impact on spermatogenesis in humans. The presence and awareness potentially of embedded undeclared fluoxetine in dietary/nutritional supplements (*adulterant/contaminant*) should be an important assessment and consideration for clinicians and health care promoters in their prescribing practices.

### **Specific anxiety and depression in women**

A study by Faramarzia et al. [36] in Iranian infertile (1 year of unprotected intercourse without pregnancy) women showed the

prevalence of depression and anxiety was 30–40% [36]. The study [36] points to the possibility that the infertility may be accompanied by crises and emotional tensions, interpersonal problems, suppressed anger, frustration, feelings of inferiority, and unconscious feelings of guilt [36]. In these settings, cognitive behavioural therapy and effectiveness, is important in the resolution or decreasing depression and anxiety in infertile women [36]. This specific approach is often frowned upon in the specific cultural setting [36]. In context a 'positive' role for embedded undeclared fluoxetine in dietary/nutritional supplements may assist in addressing the cultural sensitivities, within the specific setting, and potentially alleviate the depression and anxiety.

### **Growth and development impact**

The study by Erman et al. [6] points to pharmacological manipulation of the serotonin system during postnatal development [6], that enduring behaviourally relevant alteration of the CNS can occur [6]. SSRI's studies in adult populations are primarily in clinical and basic research that examines the safety and efficacy, with few research studies on its use during development [5], in particular chronic effects. The study by Péry et al. [25] although done in daphnids, provides some insight on development, as it shows the impact of fluoxetine on newborn lengths, and more important that the second generation of exposed individuals showed more pronounced effects [25,29,30]. There is a real lack of long-term effect studies, in particular chronic data on the entire life cycle and investigation of multigenerational effects and impact [25,37]. These findings and context could have implications where mothers who consume dietary/nutritional supplements, that have embedded undeclared fluoxetine, breast feeds infant(s).

### **Impact on memory and vision**

Whilst drugs (*such as fluoxetine*) are routinely used for treating anxiety disorders as well as depression, there is still uncertainty of fluoxetine's efficacy and effects on other processes, such as memory [19, 22]. Chronic exposure can cause a long-term loss prevention of re-instatement of conditioned fear memory due to possible synaptic protein changes [19, 22]. A contrary study by Gray et al. [22] showed possible modification of memory, and lack of consistency in effects on memory by fluoxetine, in both depressed patients and non-depressed volunteers [22]. The study by Erman et al [6] showed that Fluoxetine influenced performance on visual tasks, designed to measure discrimination learning, sustained attention, inhibitory control, and reaction time [6]. The study [6] showed dose-dependent reduction in the performance of fluoxetine-treated males, whereas fluoxetine-treated females tended to improve over baseline [6]. These studies [6, 19, 22] provides evidence that effects could possibly be patient or consumer specific, and the need for dietary/nutritional supplements to be contra-indicated with accurate warning and declarations, until otherwise declared or shown with laboratory scientific evidence.

### **Limitations of study**

The study only investigated Fluoxetine, a Schedule 5 (South Africa) selective serotonin reuptake inhibitor (SSRI). For future research work and specific focus, it would be relevant for a laboratory screen that would include a range of SSRI's (*as parent compound and metabolites*) that may be embedded in dietary/nutritional supplements. In context of the methodological approach in this study, ion suppression or enhancement may/or would also contribute to varied concentration and

hence the concentration should be viewed as an estimate and not in absolute terms. The period of the study (*concluded in 2013*) and specific product analysed may not represent the current product range in the market. It therefore cannot be assumed that the status quo as presented in this study is the same or similar with respect to fluoxetine (*other adulterants*) or if the situation has worsened, as a result of the ongoing changes and marketing practices in the nutritional (*dietary*) supplement landscape. Ongoing research, hence needs to be accomplished, that will clarify and provide a possible legislative shift in regulation and policy, in the interest of the health and wellness of the consumer.

## Recommendations

An enquiry is warranted by the Medicine Control Council in South Africa, due to a Schedule 5 drug (Fluoxetine) being present in nutritional supplement products as ‘contaminant’ [2, 37]. To institute on-going independent laboratory, batch-to-batch analysis for fluoxetine and nor-fluoxetine, with periodization scrutiny to ascertain whether the presence is as ‘contaminant’ or intentionally designed to be part of product composition, and in the process determine the source of fluoxetine and nor-fluoxetine of contamination/adulteration. Further, governments should have the responsibility to equip appropriately, the law enforcement capabilities, that will alert timeously, via ‘*early warning consumer communication systems*’ of ‘*problem products*’, and its potential recall of these products, in a speedy and efficient manner.

In the absence and evaluation of controlled human clinical trials in context of fluoxetine as a contaminant in dietary/nutritional supplements, and improving clinical diagnosis of adverse health effects that may be associated with

consumption of dietary/nutritional supplements, patients and doctors’ reports, and case studies, should be (re)assessed, to appropriately align the findings of this study. Dietary/nutritional supplements products should therefore be scientifically tested, to ensure that side-effects (*adverse*), are not inaccurately attributed and diagnosed, and that may lead to the establishment of incorrect policies, and/or warnings related to these products.

## Future Research

The limitation of animal (*human*) models could be outweighed by potential benefits from using aquatic models (*e.g. zebra fish*). These are excellent and inexpensive tools for neuropharmacology, pharmacogenetics, neurotoxicology and environmental biology [9, 10, 17]. Aquatic models in the context of assessing of nutritional (*dietary*) supplements products for the screening of multiple pharmaceutical compounds (*including fluoxetine*) in the environment could become a useful ‘*research platform*’ [9, 17]. Such models would also be beneficial to compare the synaptic mechanisms (*physiological mode of action*) of currently used effective pharmacological and non-pharmacological enhancers, and by extension the impact of contaminated and/or adulterated dietary/ nutritional supplements on the same mechanism (*mixture of compounds*) [25]. The findings of such studies would also be useful for patients in general, due to the as SSRI’s (*fluoxetine*) medications potential that may precipitate suicidality and manic symptom in high-risk youth [36], and in healthy consumers to assess both short-term and long term impacts [25].

Further, studying the environmental impacts of pharmaceuticals (*chemicals*) is that a very large amount of clinical data is available, particularly on their

pharmacokinetics and mode of action [17]. Investigating the effects of long-term treatments (*consumption of embedded adulterants*) would be important to determine if there are discrepancies between the anxiogenic or anxiolytic profile of the drugs and their therapeutic '*nutritional supplement consumption*' uses [24].

The long-term exposure of low concentration [25] of fluoxetine is also a concern, as the drug interacts with growth and reproduction processes in some invertebrate species. The extent is such that second generation species (*e.g. in daphnids*) was more sensitive to fluoxetine than the first one, and the knowledge that FLX also disrupts critical components of male goldfish reproductive processes [10]. Future research could potentially induce socio-sexual stimulation effects of SSRIs (*fluoxetine*) on sperm release in the testis [10]. This level of exposure warrants ongoing research investigation and highlights the requirement for greater scrutiny in the context of dietary/ nutritional supplements having undeclared pharmaceuticals embedded in them [25].

Further, future laboratory research should include NFLX (*metabolite*) due to the chemical impact on physiological processes, resulting in accumulation in various tissue types. The large factor differences in concentration and effect could be more pronounced in NFLX than with FLX [10]. Laboratory screen identification for NFLX, and its presence in dietary/nutritional supplement products could further provide some evidence for potential source(s) of this specific contaminant.

Studies should also take in consideration the fact that dietary/nutritional supplements users [3] often use more than the prescribed dose. The management and acute treatment to full remission of MDD that would

prevent recurrence [13] should take in consideration the dietary/nutritional supplement consumption that may contain embedded undeclared SSRI's (*fluoxetine*) in it. These products could be consumed over a long duration (*prophylactically*) for which efficacy of antidepressants in maintenance clinical treatment longer than 1 year is scarce [13]. This could have added implication and adverse consequence, due to fluoxetine presence (*as in this study*) but also the possible combination of other antidepressants (*multiple SSRI contamination*) in the same dietary/nutritional supplement product [13, 39]. This combination could provide enhanced synergistic effect, and more so for patients receiving SSRI as clinical treatment [39].

Linked to the assertion of multiple SSRI contamination arises the need for actual dietary/nutritional dosage consumption studies, at prescribed and extended doses, and in both healthy and diseased subjects, and other specific cohorts, to determine the impact that embedded undeclared fluoxetine (*and other SSRI's*) may have on current clinical treatment policy practice. Research in this specific area could also probe the potential psychological and emotional distortions that may manifest due to commodification of consumption [40].

From a gender perspective, many studies researching the effects of SSRIs treatment for fear and anxiety have focused on males, and ignore the sex difference that may occur [33]. Having an understanding of sex differences (*including behaviour and reproduction*) [28] will help sex-specific therapies that will be more beneficial for women [33]. The importance of having improved insight of the possible embedded SSRIs (*fluoxetine*) and the impact this would have on gender specific consumption makes for an important case, why such products should be regulated and better controlled. In summary most of the future



research suggestions in context of fluoxetine (*and other SSRI's*) would need to be based on the acceptance and validation of the premise of the 'read-across hypothesis' [17]. This implies, that the mode-of-action of a drug will 'read-across' from humans to other organisms and vice-versa, leading to similar effects in the different organisms (*e.g. in humans and fish*).

## Conclusion

The number of '*positive*' tested products for fluoxetine in the overall sample was 54% (75), for South African produced products was 67 % (18), and for imported products, bought in South Africa was 56 % (28). The median concentration estimate for fluoxetine in the products were, 3.9µg/g for the overall sample, 5.2 µg/g for South African produced products, and 20.1µg/g for imported products, bought in South Africa (Table 4 to 6). Whilst the concentration levels of fluoxetine detected in the products are low overall (*as contaminant*), the number of products containing the contaminant should place dietary/ nutritional supplement products under greater scrutiny. Further, the fact that nor- fluoxetine (*as metabolite*) levels have been detected in other studies, in multi-factor fold higher levels than fluoxetine, warrants critical studies, due to chemical impact this may have physiologically. Rigorous monitoring and vigilance, by health safety authorities, for fluoxetine and metabolite as contaminant, and possibly other SSRI's in dietary/nutritional supplement products become obligatory. An enquiry is warranted by the Medicine Control Council in South Africa, due to a Schedule 5 drug (Fluoxetine) being undeclared and present in dietary/nutritional supplement. That ongoing independent laboratory, batch-to-batch analysis for fluoxetine and nor-fluoxetine is warranted.

That governments alert consumers promptly, via '*early warning consumer communication systems*' of '*problem products*', and its potential recall of these products, in a speedy and efficient manner. It is time that all supplements in South Africa should be regulated by the Medicines Control Council of South Africa.

## Competing interests

None of the authors have any competing interest.

## Authors' contributions

GG contributed to the design, sample preparation, data collection, data analyses, interpretation and presentation, the drafting and the main writing to the paper. ML contributed to the design, data analyses, interpretation and presentation and editing of paper. PS study design and editing of paper, LW analysis of samples and data analyses facilitation, YC interpretation, presentation and editing of paper.

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