Cannabinoids and the Urinary Bladder

Evangelia Bakali1,2* and Douglas G Tincello1,2

1University Hospitals of Leicester, NHS trust, Leicester, UK
2Reproductive Sciences Section, Department of Cancer Studies and Molecular Medicine, University of Leicester, UK

Abstract

The presence of the Endocannabinoid System (ECS) in the urinary bladder has led to speculation that endocannabinoid-signalling is involved in the signal transduction pathways regulating bladder relaxation and may be involved in pathophysiological processes of the bladder. On the basis of this evidence, it was postulated that the binding of endocannabinoids to the cannabinoid receptors (CB1, CB2) may result in relaxation of the urinary bladder during the filling phase. Dysregulation of the ECS in human bladder may be responsible for the aetiopathogenesis of Overactive Bladder Syndrome (OAB) and Detrusor Overactivity (DO).

Keywords: Cannabinoids; Endocannabinoids; Cannabinoid receptors; Endocannabinoid system

Introduction

Over the past decade, interest in the role of endocannabinoids in regulating many mammalian processes has increased and has been proposed to be involved in the signal transduction mechanism regulating micturition [1,2]. In a sub-analysis of a multicentre, randomized controlled trial of Cannabis in Multiple Sclerosis (CAMS) the effect of cannabinoids on reducing urge incontinence episodes without affecting voiding in patients with multiple sclerosis and Neurogenic Detrusor Overactivity (NDO) was tested [3]. 630 patients were randomized to receive an oral administration of the cannabis extract, Δ9-Tetrahydrocannabinol (THC) or matched placebo. Based on incontinence diaries there was a 25% reduction (p=0.005) in the cannabis extract group and THC showed a 19% reduction (p=0.039) in urinary incontinence episodes relative to placebo suggesting cannabis may modulate detrusor function [3]. This clinical effect of cannabis is supported by the localization and increased density of suburothelial CB1 nerve fibres in patients with idiopathic detrusor overactivity and painful bladder syndrome compared with controls (p= 0.0123 and p= 0.0013 respectively) [2]. However, there are several possible CB receptor isoforms and subtypes and their anatomical distribution, through which the Δ9-THC effect is mediated, remains unknown. Since Δ9-THC acts on the brain, improvement in urgency and urinary incontinence episodes observed in the CAMS study might be attributed to the effects of Δ9-THC at any point in the peripheral nervous system and/or in the micturition centres of the central nervous system.

Historical Review

Cannabis consists of the aerial, seeds and root parts of Cannabis sativa, which is an annual herb indigenous to central and western Asia and is cultivated in other tropical and temperate regions for fibre used to produce ropes and carpets [4]. There have been more than 60 cannabinoids identified in Cannabis extracts of which the most abundant compound which induces the majority of the psychotropic effects of cannabis, is Δ9-THC [5]. Other constituents include cannabiol, cannabidiol, cannabinoids, cannabichromene and the relative acids [5]. Cannabis has been mentioned in early Hindu and Chinese medicine and its use spread through Persia to Arabia at around the time of the 10th century [6]. The therapeutic effects of cannabinoids were studied in the early 19th Century Irish physician Sir William B. O’Shaughnessy, who demonstrated the potential treatment in a range of disorders including cholera, rheumatic diseases, delirium and infantile convulsions [7]. Historically cannabis has been used in obstetrics and gynaecology for the treatment of menstrual irregularity, dysmenorrhoea, hyperemesis gravidarum, childbirth, postpartum haemorrhage, menopausal symptoms and urinary symptoms [8]. More common therapeutic applications of cannabis include analgesia, migraine, muscle spasms, seizures, attenuation of nausea and vomiting of cancer chemotherapy, anti-rheumatic and antiinflammatory actions [9,10].

The pharmacological effects of cannabinoids are mediated by two types of G Protein-Coupled Receptors (GPCR) called CB1 and CB2. CB1 was first identified in 1988 and subsequently cloned from rat cerebral cortex in 1990 [10,11]. It is most widely expressed in central nervous system regions involved with pain transmission and is the most abundant GPCR in the brain [12]. The CB2 receptor was cloned from human promyelocytic leukaemia cells (HL-60 cells) in 1993 and is mainly expressed in immune tissues but is also expressed in low levels in the CNS in both microglia and some neurons [13,14]. The localization of CB1 receptors in immune tissues implies that some cannabinoid-induced immunosuppression involves a receptor-mediated process. The cannabinoid receptors are activated by natural ligands with arachidonyl ethanolamine (anandamide) being the first endogenous ligand to be isolated Anandamide mimics the effects of Δ9-THC by binding to CB1 receptors, but lacks the psychotropic effects probably because it is highly susceptible to enzymatic hydrolysis [10,15].

The Endocannabinoid System

The Endocannabinoid System (ECS) consists of the cannabinoid receptors, the endogenous ligands for the cannabinoid receptors, the enzymes involved in the synthesis and degradation of these ligands and the transport systems involved in the transfer of these ligands across the cell membrane.

Cannabinoid Receptors

There are currently three known cannabinoid receptors; CB1, CB2, G protein-coupled receptor 55 (GPR55), which are GPCRs activated...
by endocannabinoid ligands that are arachidonic acid-derived lipid mediators [16]. There are two principal signal transduction pathways involving the cannabinoid receptors; the Cyclic-adenosine monophosphate (cAMP) signal pathway and the phosphatidylinositol signal pathway, which are mediated by the various subunits of G-proteins [16]. Most GPCRs are capable of activating more than one Gα subtype, but they show a preference for one subtype over another [16]. The effector of the Gαq/11 pathway is phospholipase C-β (PLCβ), allosterically activates proteins called calmodulins, which in turn go on stimulated by G-proteins of the Gαs class and conversely, interaction Adenosine triphosphate (ATP) to cAMP [17]. This mechanism is by Ca2+, Mg2+, Co2+, Mn2+, Ba2+ and Sr2+ and other organic cations [21].

Endocannabinoids

After the cannabinoid receptors were identified as the molecular targets for Δ9-THC, natural compounds, which bind to these receptors, were discovered. This group of bioactive lipid signalling molecules was collectively referred to as endogenous cannabinoids or endocannabinoids. N-arachidonoylthanolamide (anandamide, AEA) was the first endogenous ligand identified for the cannabinoid receptors in 1992, following its isolation from porcine brain [20]. Since then, a number of bioactive lipid signalling molecules with differing affinities for the cannabinoid receptors have been identified. Additional endocannabinoids include, N-docosatetra-7,10,13,16-enylethanolamine, 2-arachidonoylglycerol (2-AG), 2-arachidonoylglycerol ether (noladin ether), O-arachidonoyl ethanolamine (virodhamine), N-dihomo-γ-linoenoyl ethanolamine, N-docosatetraenoyl ethanolamine, oleamide, N-Arachidonoyl Dopamine (NADA) and N-Oleoyl Dopamine (OLDA) (Figure 1). Potency determinations are complicated by the possibility of differential susceptibility of endogenous ligands to enzymatic conversion.

Biosynthesis and degradation of N-acylethanolamides

AEA synthesis involves a series of enzymatic reactions, the final stage of which involves the enzyme N-arachidonoylphosphatidylethanolamine specific phospholipase D (NAPE-PLD). NAPE-PLD can be stimulated by Ca2+, Mg2+, Co2+, Mn2+, Ba2+ and Sr2+ and other organic cations [21]. Whilst spermine, spermidine, and putrescine are also stimulatory [21]. Initial characterization of NAPE-PLD revealed the enzyme to be membrane associated and it lacks the ability to catalyze a transphosphatidylation reaction, which is a common feature of known PLDs [22]. NAPE-PLD is the first PLD-type phosphodiesterase which belongs to the metallo-β-lactamase family [23]. Unlike classical neurotransmitters and neuropeptides, its primary product, AEA is not stored in vesicles but synthesized and released “on demand” in response to physiological and pathological stimuli, hormones neurotransmitters and depolarizing agents from its direct biosynthetic precursor N-arachidonoylphosphatidylethanolamine (NAPE) a phospholipid commonly found in biological membranes [24,25]. Figure 2 shows an outline of the major pathways through which anandamide and 2-AG are produced and degraded.

Fatty Acid Amide Hydrolase (FAAH) is the enzyme primarily involved in the hydrolysis of AEA, but can also degrade other endocannabinoids. FAAH was first cloned and purified from rat liver.

Figure 1: Structure of endogenous cannabinoid agonists. The structure of the members of the endogenous cannabinoid lipid mediators (A) Anandamide (AEA),( B) 2-Arachidonoylglycerol (2AG), (C) Oleoylethanolamine (OEA), (D) Palmitoylethanolamine (PEA) and (E) virdhamine and (F) the exocannabinoid Δ²-Tetrahydrocannabinol (Δ²-THC).

Figure 2: Synthesis and degradation of endocannabinoids Major pathways for the synthesis and degradation of 2-AG and anandamide.
CB1 and CB2 receptors [26]. In addition to FAAH, AEA can also be degraded by Palmitoylethanolamide-Preferring Acid Amidase (PAA), cyclooxygenase-2, lipoxigenases and cytochrome P450 [27].

2-AG is the second member of the endogenous cannabinoid family to be identified, which binds to both CB1 and CB2 receptors with similar affinities to AEA, although 2-AG has a higher affinity for CB2 receptors than CB1 [12]. The synthesis of 2-AG depends on the conversion of 2-arachidonate-containing phosphoinositides to diacylglycerols and their subsequent transformation to 2-arachidonoylglycerol by action of two Diacylglycerol Lipase (DAGL) isozymes, DAGLa and DAGLβ. Following their synthesis and release, these endocannabinoids are removed from their sites of action by cellular uptake and degraded by their enzymes. 2-AG is mainly degraded by Monoacylglycerol Lipase (MAGL) but a small amount is also degraded by FAAH.

**Synthetic Ligands**

Cannabinoid agonists are classified by chemical structure into four main groups: classical, non-classical; aminoalkylindoles; and eicosanoids [28] (Figure 3). Classical cannabinoids are dibenzopyran derivatives and include Δ²-THC, while non-classical consists of a bicyclic and tricyclic analogue of Δ²-THC that lacks a pyran ring [28]. One major practical difficulty associated with cannabinoid research both in vivo and in vitro, is the high lipophilicity and low water solubility of most CB1 and CB2 receptor ligands as this necessitates the use of a non-aqueous vehicle such as ethanol, Dimethyl Sulphoxide (DMSO), polyvinylpyrrolidone, Tween 80, Cremophor, Emulphor, bovine serum albumin, or water soluble emulsion Tocrisolve 100, which is a mixture of soya oil, Pluronic F68 and water to get the compound of interest to the cell surface [29]. It also means that these compounds “stick” to equipment during treatment, which needs to be taken into consideration during experimental procedures.

**Cannabinoid receptor signaling**

Calcium acts as an intracellular messenger where it plays a key role in regulating basic cellular responses, such as migration and proliferation [30]. Under resting conditions, cytoplasmic calcium concentration is maintained at approximately 100 nM [30]. When stimulated, calcium enters the cell from extracellular stores via ion channels in the plasma membrane or is released from intracellular stores through channels and receptors in the endoplasmic reticulum [30]. These channels may be activated and modulated by second messengers including IP₃, which is produced by binding of ligands, such as ATP, to GPCRs [31].

The CB₁ receptor is a member of the rhodopsin subfamily of GPCRs [32]. There are three cytosolic loops and a putative fourth loop formed by palmitoylation at the juxtamembrane C-terminal region, which contribute to the activation of the G-proteins [33]. The proximal CB₁ receptor intracellular C-terminal domain is critical for G-protein coupling and the distal C-terminal tail domain modulates signal transduction [33]. Most cannabinoid effects are sensitive to Pertussis Toxin (PTX) implicating a CB₁ and CB₂ receptor coupling to a Gᵢ/o protein [34]. The binding of endocannabinoids and cannabinoids to CB₁ and CB₂ results in a decrease of intracellular cAMP levels and activation of mitogen-activated protein kinase through the coupled Gₛ proteins [34-36]. Cannabinoid-mediated inhibition of cAMP has been demonstrated in slices of rat hippocampus, striatum, cerebral cortex and cerebellum [36]. CB₂ can also stimulate the formation of cAMP through Gₛ under certain conditions [37]. It may also be that CB₁ receptors can exist as two distinct subpopulations, one coupled to Gₛ proteins and the other to Gₛ [38,39]. The level of cytosolic cAMP may then determine the activity of various ion channels as well as members of the ser/thr specific protein kinase A (PKA) family [32,40,41]. Thus cAMP is considered a second messenger and PKA a secondary effector.

In addition, activation of CB₁ receptor modulates ion channels through Gₛ proteins leading to the activation of A-type and inwardly rectifying potassium channels [42-45]. This is due to decreased phosphorylation of the channels, as protein kinase A activity is decreased due to cannabinoid induced inhibition of AC [45]. Thus cannabinoids increase the efflux of potassium. In addition, activation of CB₁ causes a cAMP-independent, but Gₛ-dependent inhibition of N-type and P/Q-type calcium channels and activation of inwardly rectifying potassium channel proteins (e.g. GIRK1, GIRK2), leading to a decrease calcium influx and increase in potassium efflux [42-44].

Similarly, CB₁, CB₂ receptors can modulate AC and MAP kinase activity, through their ability to couple to Gₛ proteins [46]. The MAP kinase pathway is a key signalling mechanism that regulates many cellular functions such as cell growth, transformation, differentiation, gene expression and apoptosis [47]. Activation of the MAP kinase pathway is associated with the activation of a tyrosine kinase-linked receptor which activates the intracellular G protein Ras and sets up a signaling cascade beginning with the activation of the ser/threonine kinase Raf (MAP kinase kinase) [32]. Raf activates MAP kinase kinase (MEK) leading to phosphorylation and activation of MAP kinase, which can phosphorylate various cytoplasmic and nuclear proteins [32]. CB₂ receptors have been shown to link positively to MAP kinase [48]. However, in contrast to CB₁, CB₂ receptor stimulation is believed not to modulate ion channel function as seen in AIT-20 cells transfected with CB₁ receptors and Xenopus oocytes transfected with CB₂ [39,50]. In addition, unlike CB₁ receptors, CB₂ receptors do not appear to couple to Gₛ, suggesting there is a difference between CB₁ and CB₂ receptor signalling [51].

There is evidence that GPR55 is a novel cannabinoid receptor that has a different signalling pathway to that of CB₁ and CB₂ [52,53]. GPR55 is also a rhodopsin-like GPCR, which has been implicated in diverse physiological and pathological processes such as inflammatory and neuropathic pain, bone development and cancer. However, GPR55 shares only low amino acid sequence identity with CB₁ (13.5%) and CB₂ (14.4%) and lacks the typical functional response elicited by these receptors [54]. Activation of the GPR55 receptor coupled to the Gₛ₃, RHoA, actin, phospholipase C pathway triggers the release of Ca²⁺ from IP₃-R-gated stores, which leads to increased intracellular Ca²⁺ [53] (Figure 4). GPR55 can be activated by Lysophosphatidylinositol (LPI), which is an agonist, which can be antagonized by CP55940 and cannabidiol.
Cannabis and the Urinary Bladder

Cannabinoid Receptor distribution in the urinary bladder

The effect of cannabis on DJ symptoms is probably mediated through a mechanism that depends on endocannabinoids [3]. The mechanism of this effect is far from clear and published data on the expression and functional sites of cannabinoid receptors in the bladder are contradictory. It is thought that endocannabinoids bind to CB1 and CB2, resulting in the relaxation of the detrusor muscle during the filling phase [55,56]. CB2 receptors are mainly found at the central and peripheral neuron terminals of the bladder, inhibiting neurotransmitter release [55]. Several studies have localized both cannabinoid receptors in the urinary bladder of humans rats mice and monkeys [2,55-60]. The localization of CB2 receptors has been described to be in the urothelium and nerve fibres of the suburothelium and in human and rat detrusor muscle [2,58,60]. However, another study did not detect the CB2 receptor in rat urothelium or nerve fibres but reported immunoreactivity for CB2 in these structures and in ganglion cells of the outflow region [1,55]. In addition, human bladder studies identifying the presence of gene transcripts by quantitative Polymerase Chain Reaction (qPCR) and tissue expression and localization by Immunohistochemistry (IHC), revealed a higher abundance of the CB2 receptor in the urothelium compared to the detrusor [57]. Similar results were found for CB2 but overall, receptor protein expression was much lower when compared to CB1 receptor protein expression [57].

Cannabinoid Receptor function in the urinary bladder

Studies have demonstrated that the activation of presynaptic CB1 and CB2 receptors inhibit electrically evoked contractions in isolated mammalian tissue when using THC and the non-selective CB receptor agonists CP55940, CP55244, JWH015, which corresponds to the localization of CB2 receptors in nerve fibres of the detrusor muscle [55,60-62]. In isolated mouse bladder, several cannabinoid receptor agonists, including WIN 55212-2, Δ9-THC and anandamide, inhibited electrically-evoked bladder contractions in a concentration dependent manner [61]. In the same study, it was shown that the inhibitory effect was not a post-synaptic effect since contractile responses to muscarinic or purinergic receptor agonists were unaffected by pre-treatment with Δ9-THC [61]. In rat detrusor muscle, cannabinor (a CB2 selective agonist) did not have any effects on nerve-induced contractions [1]. Similarly, in a study where human bladder muscle strips were used, there was no inhibitory effect of the non-selective CB agonist, WIN 55212-2, on Electrical Field Stimulation (EFS) evoked contraction [62].

In contrast, another study found an attenuation of EFS evoked human detrusor contraction in the presence of both CB1 (ACEA) and CB2 (GP1a) agonists [57]. These findings suggest cannabinoids act on pre-junctional nerve endings attenuating contractile responses. These data, however, must be interpreted with caution because quantification of the effect by GP1a or vehicle (dimethyl-sulfoxide) control experiments were not presented [57]. Supporting that cannabinoids act on pre-junctional nerve endings to attenuate a contractile response, Gratze et al. demonstrated co-localization of vesicular acetylcholine transporter protein (VACHt) nerve structures and CB2 immunoreactive terminal varicosities. They also showed inhibitory effects of CP55, 940 on nerve mediated contractions but not on carbachol induced contractions in detrusor preparations, suggesting a modulatory function of CB1 on cholinergic neurotransmission [55]. Similarly, cannabinor (a CB2 agonist) did not affect the contractile response in isolated rat detrusor tissue, suggesting that the action of the CB2 receptor is not directly involved in post-junctional regulation of smooth muscle contractility [1]. A recent study showed that both pure Cannabidiol (CBD) and Cannabis Sativa extract enriched with CBD also termed as “CBD Botanic Drug Substance” (CBD BDS), which are devoid of psychotropic activity, inhibited human and rat bladder contractility via a post-synaptic site of action [63].

The differences seen between the results of these studies may be due to inter-species differences in cannabinoid receptor expression and distribution, the effect of these receptors on the release of contractile transmitters and anatomical variations in bladder innervation. Inter-species differences in the neuroanatomy of the mammalian bladders are known to exist [62]. For example there are several parasympathetic ganglia in isolated bladder tissue from guinea pigs and humans while there are none in the urinary bladder of mice and rats [64,65].

Cystometric studies have shown an increase of the micturition threshold in rats receiving systemic cannabinoids in normal and inflamed conditions induced by acetic acid, cyclophosphamide or turpentine oil [66,67]. These effects were stronger when the cannabinoids were administered through a close-arterial route rather than systemically through the tail vein of the rat, supporting the hypothesis of a local regulatory role of the cannabinoid system in the micturition reflex [67]. The mechanism by which cannabinoid receptors could modulate this reflex is by the presence of CB2 receptors in the afferent nerve fibre endings located in the suburothelial layer, which is supported by in vitro studies where CB2 agonists reduce neuronal activity and attenuate bladder contractility as a result of electrical field stimulation in isolated mouse bladder strips [61,68]. In rats, anandamide, WIN 55212-2 (synthetic CB non-selective agonist), and Ajulemic acid (IP-751) (synthetic THC analogue), suppress normal bladder activity and the urinary frequency induced by bladder irritation suggesting the inhibitory effects are least in part mediated by CB2 receptors [66,67,69]. A recent study, showed that CB2 receptor mediated signals using a high affinity CB2 receptor selective agonist, cannabinor 3.0 mg/kg, increased the micturition intervals and volumes by 52% (p <0.05) and 96% (p<0.01), respectively, and increased threshold and flow pressures by 73% (p<0.01) and 49% (p<0.001), respectively, in conscious rats during cystometry [1]. It has not been clarified if these actions are related to CB2 receptors in the central nervous system, at peripheral sites in the lower urinary tract, or both. Furthermore, it is not known which of the two CB receptor subtypes is mainly responsible for the regulation of micturition in the different species.

Cannabinoid receptors as therapeutic targets

The most studied cannabinoid compound is Cannabidiol (CBD)
which exerts a number of pharmacologic effects such as analgesic, anti-inflammatory, antioxidant, and anti-tumoral [70]. It has been clinically evaluated for the treatment of anxiety, psychosis, and movement disorders and has been found to have a safe clinical profile [70]. CBD is the main component of Sativex, which also contains Δ²-THC, a cannabis-derived drug used for the treatment of pain and spasticity associated with multiple sclerosis. Sativex is licensed for this indication in patients with multiple sclerosis. In a clinical survey, administration of Δ²-THC improved nocturia and detrusor overactivity in patients with multiple sclerosis [71]. To date, a small number of open-label and placebo-controlled studies have demonstrated that oral administration of cannabinoids may alleviate OAB/DO symptoms as first line. Most of these studies have been carried out on patients with advanced multiple sclerosis using preparations containing Δ²-THC and/or CBD. One such study concluded that a single dose of a THC/CBD delivery system (GEP-001) may be beneficial in patients with multiple sclerosis [72,73]. Other cannabinoid receptor agonists are already used clinically to suppress nausea and vomiting provoked by anticancer drugs (nabilone) or to boost the appetite of AIDS patients but these have not been studied for their effects upon urinary symptoms [9].

However, the oral use of cannabinoids may induce undesirable CNS effects including hyperactivity, hypothermia and cataplexy, but may in turn improve OAB symptoms, which are known to be afferently mediated [3,74]. What remains unclear is whether the latter beneficial effects are centrally mediated or whether a local bladder component acting on the afferent bladder pathway, plays a significant role. There are no human data that exists which can answer this question. Data from animal studies support a local effect on bladder afferents where cannabinoid administration systemically and intravesically, improved parameters associated with OAB and DO [55,75].

In addition to using an intravesical route of administration for cannabinoid drugs in order to bypass the CNS effects associated with activation of CB1, the use of CB2 agonists and FAAH inhibitors is being explored and appears to be a promising route [76,77]. There is emerging evidence that activation of CB2 inhibits tissue inflammation and has analgesic properties [78-80]. The CB2 subtype is mainly expressed outside the CNS, as described earlier, so it can act as a potential endocannabinoid target where analgesic effects may be separated from psychototropic effects by activating the peripheral receptors. In addition, pharmacological targeting of the homeostasis of endogenous cannabinoids by manipulating the degradation enzymes, may also offer the possibility of avoiding the CNS side effects of exogenous cannabinoids. FAAH, an enzyme that specifically degrades anandamide has been localised in the urinary bladder [56,77,81]. Inhibition of FAAH activity with FAAH inhibitor Oleyol Ethyl Amide (OEtA), significantly increased inter-contraction intervals, micturition volume, bladder capacity and threshold pressure urodynamic parameters in rats which reflect sensory functions of micturition. These effects were prevented by a selective CB2 antagonist. Similarly, another FAAH inhibitor, URB597 has been found to have a functional role in the colon, where FAAH has been localized by reducing inflammation [77,82,83]. The use of a FAAH inhibitor needs to be explored further in the urinary bladder because it may be the way forward in treating OAB symptoms. However, the complexity of the endocannabinoid system at the tissue level may mean that we are still a long way from obtaining a clinically useful compound for treatment.

The Future

Modulation of the endocannabinoid system is currently being investigated for a wide range of potential therapeutic applications including smoking cessation, treatment of obesity, epilepsy and other CNS related conditions. Similarly, the presence of the endocannabinoid system in the urinary bladder has led to speculation that endocannabinoid-signalling is involved in the signal transduction pathways regulating bladder relaxation and may be involved in pathophysiological processes of the bladder. This role of the endocannabinoids in the lower urinary tract supports their therapeutic potential in conditions of OAB and DO, whereas evidence already exists for their role in bladder inflammation [2,59,75,84]. There are still a number of unanswered questions in the understanding of cannabinoid pharmacology in the urinary bladder. Clearly, further research is required to investigate the role of cannabinoid receptors and their exogenous modulators on bladder control prior to embarking on a clinical trial involving cannabinoids and healthy volunteers with OAB. The inhibitory effects of CB2 and the effect of FAAH inhibitors on lower urinary tract control should be the focus of future studies.

Acknowledgments

This work was funded by Wellbeing of Women Training fellowship, UK.

References


Submit your next manuscript and get advantages of OMICS Group submissions

**Unique features:**
- User friendly/feasible website-translation of your paper to 50 world’s leading languages
- Audio Version of published paper
- Digital articles to share and explore

**Special features:**
- 250 Open Access Journals
- 20,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing in PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: [http://www.omicsonline.org/submission/](http://www.omicsonline.org/submission/)